CHAPTER V

LOCAL AND SYSTEMIC ALTERATIONS IN PROTEIN CONTENT AND TRANSAMINASES DURING TAIL REGENERATION IN THE SCINCID LIZARD, MABUYA CARINATA

Transaminases or aminotransferases are a group of enzymes widely distributed in living systems bringing about transfer of amino groups to α-keto acids generating the corresponding amino acids. Though there are many specific transaminases bringing about transfer of amino groups from specific amino acids, two of the most commonly studied ones - the glutamate-pyruvate transaminase (Alanine aminotransferase - GPT : EC 2.6.1.2) and glutamate-oxaloacetate-transaminase (Aspartate aminotransferase - GOT : EC 2.6.1.1) are reportedly capable of acting on almost all amino acids. This broad spectrum activity of these two aminotransferases have earned them the status of major transaminases of animal tissues. GPT and GOT by their strategic positioning in the metabolic pathway, at the level of pyruvate and oxaloacetate formations are pivotal in linking the metabolism of carbohydrates and lipids with that of proteins. The transamination reaction catalysed by these two enzymes thus serves as the first and last chemical events respectively, during the synthesis and degradation of amino acids. Though these two enzymes have obvious significance in protein metabolism, most of the studies todate on GPT and GOT are done only with a clinical bias. Increased levels of these two enzymes in liver, heart and serum are usually correlated with protein catabolism and certain specific clinical conditions (Wroblewski and LaDue, 1955, 1956; DeRitis et al., 1956; Waldman and Borman, 1959; Sacks and Lanchantin, 1960). Barring a few studies (Felig, 1975; Muralimohan and Sasirababu, 1976), there are hardly any attempts made in correlating the role of these enzymes in alterations of protein metabolism that may be associated with normal physiological phenomena of living systems.

Proteins and their metabolism do have crucial significance in vertebrate reparative regeneration, a process which essentially involves localised reactivation of developmental events. Studies on lacertilian tail regeneration have indicated marked fluxes in the metabolism of lipids and carbohydrates (Shah and Ramachandran, 1970, 1973, 1976; Shah et al., 1977 b, 1981). Besides, some of the past studies on vertebrate regeneration have also shown significant alteration in protein content both at the site of regeneration as well as in the liver (Schmidt, 1968; Ramachandran et al., 1980 b). Since there is a paucity of information on transaminases in normal physiological processes and as proteins do play an important role in regeneration, in the present study an analysis of total protein content together with assay of GPT and GOT have been undertaken in the tail of *Mabuya carinata* during its regeneration. Further, as systemic factors are suspected to be involved (as per our previous studies) in the metabolic alterations associated with tail regeneration in *M. carinata*, these investigations have also been extended to the two visceral organs *viz.*, liver and skeletal muscle, as well as serum.

MATERIALS AND METHODS

Healthy Mabuyas of both sexes, weighing around 20-24 gms, obtained from Hyderabad, India, were acclimated to the laboratory conditions on a diet of insects. Autotomy was performed by pinching off the tail at about 1.5-2.0 cms distal to vent. The animals were sacrificed on fixed intervals of 3, 5, 7, 10, 12, 15, 25, 40 and 60 days post-autotomy along with normal animals with intact tails. Blood was collected by cardiac puncture, in a test tube and after stirring gently with a glass rod was centrifuged at 10000 X g, at 4°C, for 10 mins. The pale yellow coloured serum was decanted carefully and used with suitable dilutions. Liver and skeletal muscle were removed quickly along with the regenerating or normal tail, as the case may be and homogenized in ice-cold redistilled water. A 2% homogenate was prepared for liver and skeletal muscle whereas a 4% homogenate was found satisfactory in the case of tail tissue. The crude homogenate as well as the diluted serum were used for assaying quantitatively the amount of protein by the method described by Lowry et al. (1951). Activities of Glutamate-pyruvate and Glutamate-oxaloacetate transaminases (GPT and GOT) were assayed by making use of dl-alanine and L+-aspartate respectively as the substrate following the method of Bergmeyer and Bernt (1965). The amount of protein was expressed as mg/100 mg of fresh tissue weight in the case of liver, muscle and tail and gm/100 ml in the case of serum. The activity of both the transaminases was expressed as µ M glutamate formed/mg protein/min.

For each day and each tissue and serum specified, a total of five to seven determinations were made. The mean and standard deviation were obtained and students' 't'-test was used to determine statistical significance.

RESULTS

Total Protein Content : Total protein content is noted to show an increase in the tail regenerate right from the 5th day post-autotomy till about the 25th day. Statistically significant increase could be accredited to 10th, 12th, 15th and 25th days of tail regeneration which correspond to the differentiation phase of regeneration. Systemic levels of protein too are noticeably increased with the serum depicting an elevated level right from 3rd day uptill the

Table 1. Quantitative levels of Protein content in the regenerate, liver, skeletal muscle and serum during tail regeneration in M. carinata.

Periods of tail regeneration in days	TAIL	LIVER	MUSCLE	SERUM
N	9.6 ±1.2	16.6 ±1.19	14.2 ±1.36	1.03 ±0.06
3	9.2 ±1.27	17.2 ±0.97	16.2 ±2.18	1.31* ±0.12
5	10.8 ±1.33	20.4@ ±1.11	14.4 ±0.99	1.57*@ ±0.12
7	11.8 ±1.42	22.01@@ ±1.37	14.6 ±1,35	1.74*@ ±0.18
10	12.8* ±1.11	23.6*@ ±1.38	16.2 ±2.61	1.91*@ ±0.15
12	14.2⁑ ±1.75	23.6*@ ±1.35	15.8 ±2.31	1.75*@ ±0.16
15	13.6@ ±1.32	25.6*@ ±1.97	18.4* ±1.69	1.72*@ ±0.14
25	13.01* ±1.65	20.4* ±1.56	16.2 ±2.88	±1.52*@ ±0.13
40	10.2 ±1.6	16.8 ±1.41	17.2 ±1.44	1.42*@ ±0.09
60	10.8 ±1.59	17.41 ±1.15	15.4 ±1.94	1.07 ±0.07

* $P < 0.01$; ⁑ $P < 0.005$; @ $P < 0.0025$;
@@ P 0.001; *@ $P < 0.0005$
N - Normal (Pre-autotomy level).

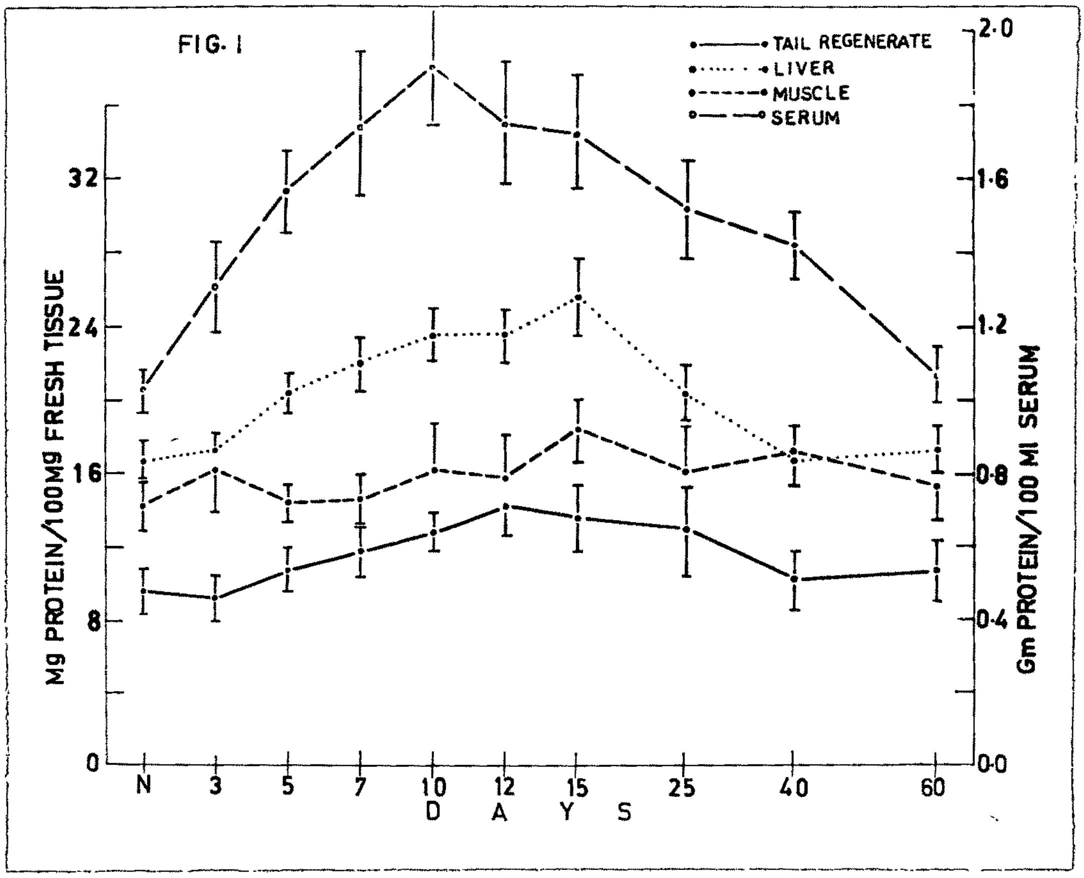

Fig. 1. Changes in protein content in the regenerate, liver, muscle and serum during tail regeneration in *M. carinata*.

40th day of regeneration. Concurrently, hepatic protein content too registered a constant increase immediately subsequent to tail autotomy till about the 25th day of regeneration with statistical significance being restricted to 5th to 25th days post-autotomy. Muscle protein content, however, registered statistically insignificant fluctuations with a minimal significant increase only on the 15th day. These changes in protein content are recorded in Table 1.

GPT and GOT : The changes in the activity levels of these two transaminases in liver, skeletal muscle, serum and tail regenerate during regeneration are represented in Table 2 and Figs. 2 and 3. From these representations it becomes evident that in the regenerate, whereas GPT activity maintained a normal level during the first five days post-autotomy and then remained subnormal from 7th till 40th day, GOT activity showed a tremendous increase on 3rd day post-autotomy and then remained in the normal range more or less from 10th day onwards till the completion of regeneration except for a significant subnormal level on the 40th day. Similarly in liver, whereas GPT activity showed increased levels during the first 5 days post-autotomy with more or less subnormal levels during the remaining periods of regeneration, GOT activity remained statistically unaltered except for a short phase of significant subnormal level between 7th and 10th days post-autotomy. Most significant positive alteration in GPT and

Table 2. Quantitative levels of GPT and GOT in the regenerate, liver, skeletal muscle and serum during tail regeneration in M. carinata.

(Values are expressed as µM glutamate formed/mg protein/min.)

Periods of tail regeneration in days	GLUTAMATE-PYRUVATE TRANSAMINASE				GLUTAMATE-OXALOACETATE TRANSAMINASE			
	TAIL	LIVER	MUSCLE	SERUM	TAIL	LIVER	MUSCLE	SERUM
N	0.365 ±0.039	1.616 ±0.195	0.203 ±0.021	0.078 ±0.005	1.81 ±0.15	4.48 ±0.215	2.17 ±0.195	0.552 ±0.047
3	0.366 ±0.034	1.96 * ±0.27	0.727*@ ±0.075	0.106 ±0.009	2.96*@ ±0.38	4.31 ±0.26	3.44 *@ ±0.32	0.228*@ ±0.02
5	0.329* ±0.05	2.02 *@ ±0.302	0.691*@ ±0.036	0.063 ±0.0075	2.04 ±0.27	4.56 ±0.312	5.38 *@ ±0.467	0.53 ±0.046
7	0.183*@ ±0.022	1.22 *@ ±0.181	0.339*@ ±0.0246	0.045 ±0.001	2.25* ±0.18	4.07 * ±0.239	4.95 *@ ±0.5	0.37 ** ±0.08
10	0.189*@ ±0.046	1.05 *@ ±0.088	0.337*@ ±0.042	0.049 ±0.006	1.48 ** ±0.101	3.68 @@ ±0.28	6.09 *@ ±0.432	0.35 *@ *0.025
12	0.111*@ ±0.025	1.47 @ ±0.12	0.317 ±0.016	0.0303 ±0.0026	1.33 ** ±0.15	4.12 ±0.36	6.79 *@ ±0.203	0.24 *@ ±0.09
15	0.123*@ ±0.037	1.74 ±0.167	0.274 ±0.036	0.027 ±0.005	1.84 ±0.22	4.82 ±0.47	7.93 *@ ±0.5	0.183*@ ±0.049
25	0.078*@ ±0.015	1.29 *@ ±0.081	0.587*@ ±0.73	0.085 ±0.005	1.36 * ±0.16	4.61 ±0.43	5.15 *@ ±0.411	0.197*@ ±0.059
40	0.121*@ ±0.021	0.928*@ ±0.081	0.964 ±0.111	0.19* ±0.008	0.78 *@ ±0.113	4.24 ±0.37	2.44 ±0.324	0.463 ±0.141
60	0.275*@ ±0.03	1.541 ±0.113	0.583*@ ±0.026	0.12 ±0.016	1.36 @@ ±0.14	3.45*@ ±0.33	1.89 ±0.148	0.782*@ ±0.086

* P < 0.01; ** P < 0.005; @ P < 0.0025; @@ P < 0.001; *@ P < 0.0005

N - Normal (Pre-autotomy state)

FIG. 2

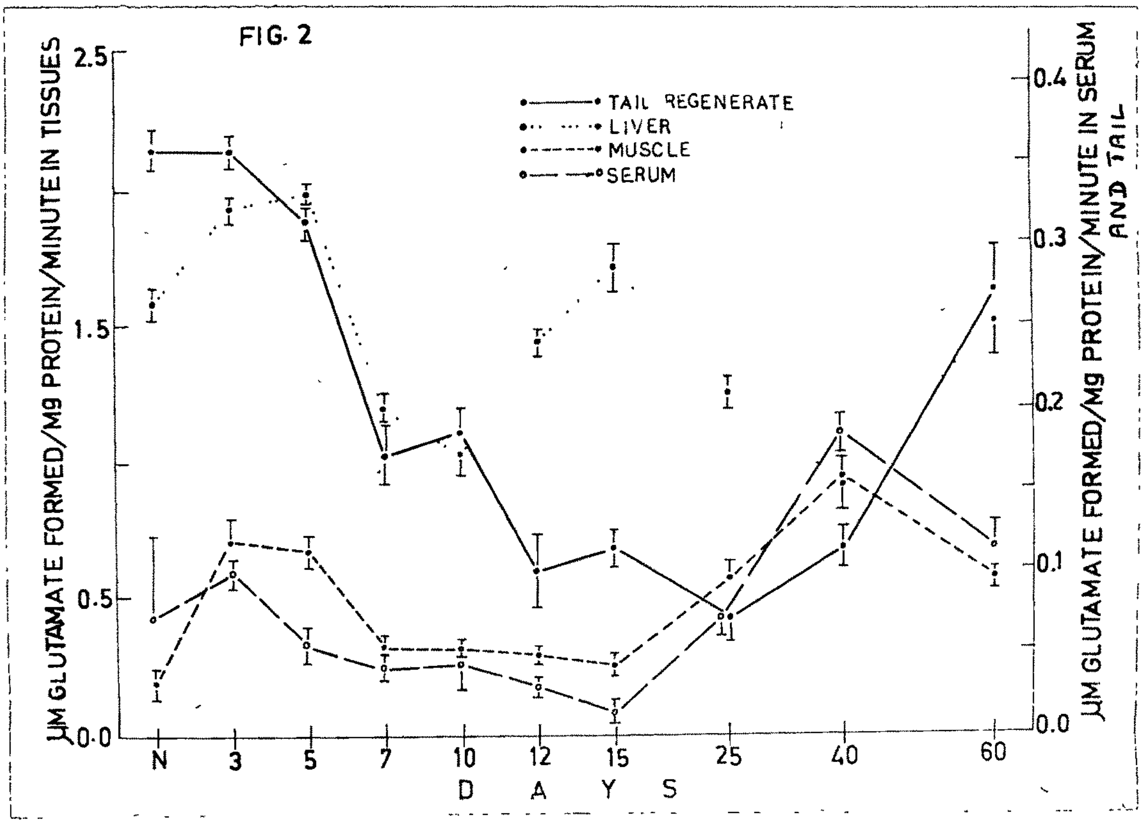

Fig. 2. Changes in glutamate-pyruvate transaminase (GPT) activity in the regenerate, liver, muscle and Serum during tail regeneration in M. carinata.

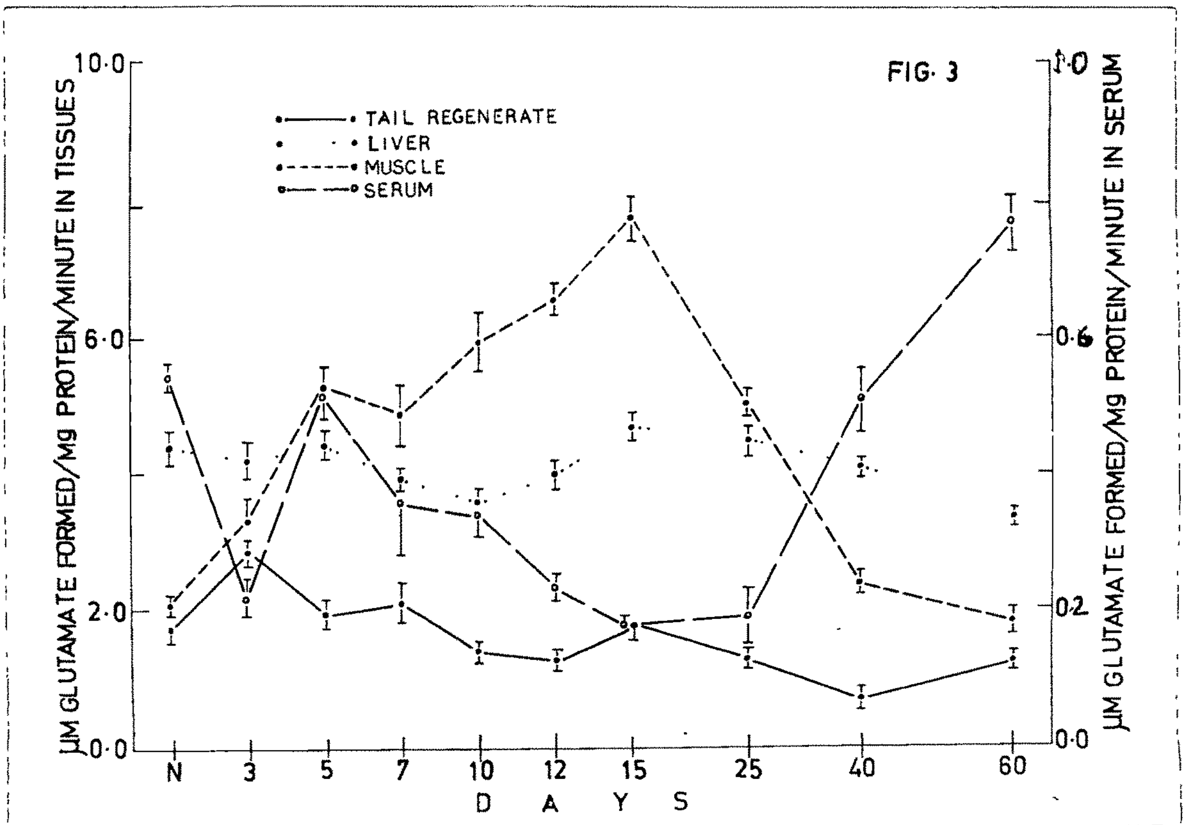

Fig. 3. Changes in glutamate-oxaloacetate transaminase (GOT) activity in the regenerate, liver, muscle and serum during tail regeneration in *M. carinata*.

GOT activity was seen only in the skeletal muscle. Significantly elevated level of GOT activity could be visualized right from the time of autotomy with a maximal increase of 265% on the 15th day. Above 100% increased levels were seen during 5th, 7th, 10th and 25th days of tail regeneration while on 3rd and 12th days the increase was about 58% and 75% respectively. On the other hand, GPT activity registered an above 200% increase during the first five days after which it came down gradually to about 34% by about 15th day through 7th, 10th and 12th days during which the enzyme level was at an average about 60% above normal. There was once again a tremendous increase in GPT activity by about 25th day (187%) which ultimately reached a record maximal increase of 373% on 40th day to show a decline by 60th day. Finally, the results also indicate the maintainence of subnormal levels of GPT and GOT in serum all throughout tail regeneration except for an increased above normal level of GPT only during the 40th day and GOT during 40th and 60th days of regeneration in a descending order.

DISCUSSION

From the results outlined above as per the Tables 1 and 2 and Figs. 1-3, the most obvious fact that emerges is the prevalence of an all pervading protein anabolic influence in the body of M. carinata during its tail regeneration. Since regeneration essentially entails the formation of new tissues, an increment in protein content in loco is very much in order. Apart from the present report in which an increased protein content in the regenerate is shown as the feature till about 25 days of regeneration, similar increase in protein content during amphibian limb regeneration too has been reported (Schmidt, 1968). A recall of the data recorded herein (Table 1, Fig. 1) shows increasing protein content in the tail regenerate from about 5th day onwards with statistically significant increase being achieved between 10th and 25th days of regeneration. This in loco increment in protein content is well paralleled by the rate of growth of the tail regenerate observed during this period (Chapter I). Apparently above normal level of protein content is recorded in the ascending order during preblastemic, blastemic and differentiation phases of regeneration. Apart from the many structural and house keeping proteins which could be purported to be formed anew in a developing system, the continuing increase mentioned as above might also possibly be due to the elaboration of certain specific luxury proteins which could specifically promote the regeneration associated events of dedifferentiation and differentiation. Pertinently, based on the electrophoretic separation of soluble proteins from limb regenerates of amphibians, Schmidt (1968) had also tentatively hinted at the possibility of formation of such promoter proteins during regeneration. Previous study of Ramachandran et al. (1980 b) has reported a concurrent increase in hepatic protein level too during identical phases of tail regeneration as part of the systemic response. The present work which is a sequel to the above observation mainly intended to explore the pattern of protein metabolism occurring in loco as well as systemically, has yielded evidences in favour of a positive nitrogen balance in liver, muscle and serum along with that of the regenerate (see Table 1 and Fig. 1). However, the maintainence of normal to subnormal levels of GPT and GOT in the tail all throughout its regeneration indicate the inability of the regenerating system to spare the metabolic intermediates towards amino acid biosynthesis. Nevertheless, the short living spurt in GOT activity obtained on the 3rd day post-autotomy might be correlated with protein catabolism expected to occur due to the injury induced tissue demolition and autolysis. In spite of the increase in protein content, hepatic transaminases too remained subnormal almost all throughout regeneration except for the slight increase in GPT activity on the 3rd and 5th days which may be correlated with the initiation of active glycogenolysis and the sudden elevation of pyruvate concentration within (Chapter IV). The most positive response in this respect was shown by the skeletal muscle transaminases. Both the transaminases showed tremendous stage specific elevation: with GPT depicting above 250% elevation initially during first five days and later during the 40th and 60th days of regeneration.

On the other hand, GOT depicted similar maximal levels between 7th and 25th days of post-autotomy. Interestingly the elevations in muscle GPT and GOT outlined above are found to be in parallel correspondence with the changes in A and B LDH subunits respectively (Chapter IV). Glycogen depletion from the muscle noted to occur during the first 15 days of tail regeneration was inferred to meet other metabolic requirements other than that for energy production <u>in loco</u> especially as muscle glycogen cannot serve as a direct source for blood glucose. This observation coupled with the present one on transaminases together with the correspondence noted between GPT and A type LDH subunits on one hand and GOT and B type LDH subunits on the other hand highlight the adaptive metabolic strategy employed by this lizard in meeting the requirements of regeneration. It may be safely presumed that during the first week of tail regeneration whence anaerobic breakdown of muscle glycogen is predominant, the increasing pool of pyruvate is being transaminated by GPT to alanine and thence to the alanine family of amino acids. Similarly during the progressive phases of regeneration when aerobic oxidation of glycogen is well established as denoted by the increase in B type LDH subunits and LDH 1 and 2 isozymic bands (Chapter IV), the oxaloacetate molecules that are generated through TCA cycle are being effectively transaminated by GOT to yield aspartate and thence the asparatate family of amino acids. Pertinent to quote here is the report of Shah *et al*. (1979 b) wherein they have shown *in loco* elevated content of many amino acids during tail regeneration in *H. flaviviridis*. Transportation of part of the amino acids (that are being so generated in skeletal muscle from its carbohydrate source) to liver for eleboration of specific proteins also can be visualized. In fact, the earlier report of Ramachandran *et al*. (1980 b) has hinted the possibility of elaboration of certain regeneration specific proteins within the hepatic tissue. Further they have also tentatively surmised the possibility of mobilisation from other source under the influence of cortisone as one of the factors for the observed increase in the hepatic protein content as per the reports of Munro (1964). It is of relevance to recall here the recent review of Felig (1975) on amino acid metabolism wherein he has suggested the operation of a glucose-alanine cycle by which muscle glucose derived pyruvate is transminated by GPT to alanine and transported to liver. Apart from their significance in forming many other amino acids, alanine and aspartate generated by the skeletal muscle during tail regeneration in *M. carinata* might also be accredited adaptive roles in gluconeogenesis, nitrogen and energy metabolisms as well as formation of pyrimidines and RNA synthesis (Sheid and Roth, 1965; Knox and Greengard, 1965; Felig, 1975), all of which could be well associated with the process of regeneration. Though both GPT and GOT mediated amino acid biogenesis seems to be inoperative in both liver and the regenerate, endogenous amino acid formation in both these tissues via glutamate cannot be overruled as very high GDH activity has been observed by Ramachandran et al. (unpublished). In fact a two to three fold increase in glutamate throughout regeneration has been shown by Shah et al. (1979 b) in their studies on free amino acids during tail regeneration. Other possible sources of hepatic proteins during regeneration have been reviewed in an earlier publication by Ramachandran et al. (1980 b). Increased level of protein and subnormal levels of GPT and GOT in serum during the early half of regeneration indicate the presence of proteins either in transit and/or carrier proteins. Above normal levels of GPT and GOT during terminal phases of regeneration might be correlated with the decreasing content of proteins in serum.

Finally it may be appropriate to mention here that Knox and Greengard (1965) in their extensive review on hormonal regulation have cited the depressive roles of thyroxine and growth hormone on hepatic GPT and GOT. As both these hormones are purported to be involved in the regenerative mechanics, the currently obtained fall in hepatic and tail GPT and GOT might be self explanatory. Another correlation cited by

Knox and Greengard (1965) that finds validity in our studies is the stimulatory influence of cortisone on transaminases especially hepatic GPT. This when coupled with the presently recorded increase in GPT activity in liver during the first 5 days post-autotomy and the discussed role of cortisone in regeneration (Ramachandran _et al._, 1980 b) gains credence.

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