CHAPTER 12

LEVELS OF INORGANIC PHOSPHORUS DURING TAIL REGENERATION IN NORMAL, SHAM OPERATED AND GONADECTOMISED GEKKONID LIZARDS, HEMIDACTYLUS FLAVIVIRIDIS

Inorganic phosphorus is present in varying quantities in diverse cells. While the total phosphorus content of a particular cell is relatively constant, its distribution among different intracellular compounds is in constant flux as it is incorporated into various phosphate esters which are formed in the course of carbohydrate and lipid metabolisms. Its involvement in metabolic activity is extremely rapid in kidney, slower in liver and slowest in muscle (White et al., 1968). Injection of insulin has the effect of reducing the serum inorganic phosphorus (Varley, 1963; Harper et al., 1979). In diabetes mellitus, there is a lower concentration of inorganic phosphorus in the serum (Harper, et al., 1979). During menopause, many women develop a negative calcium and phosphorus balance leading to a type of osteoporosis (under mineralization of bone) (West et al., 1955). The part played by sex hormones in regulating Ca and P levels and the metabolism of these elements in such individuals has been studied especially by Albright and co-workers (Reifenstein et al., 1947). They have

demonstrated that negative balances are markedly improved by the administration of estrogen or androgen. Regeneration being a dynamic process involving many anabolic transformations and varying energy requirements, the content of free inorganic phosphorus could be of crucial significance in energy metabolism as well as biogenesis of phospho compounds. The present study, thereby, attempts an evaluation of free inorganic phosphorus content during various phases of tail regeneration in the regenerate and liver of normal, sham operated and gonadectomised lizards, Hemidactylus flaviviridis.

MATERIALS AND METHODS

Adult H. flaviviridis collected from the local animal dealer were kept in the laboratory for a fortnight prior to experimentation. The animals were kept on a diet of insects. The animals were divided into three groups as mentioned in the earlier chapters, i.e. normal intact, sham operated (SGX) and gonadectomised (GX) groups. The liver and tail tissues were collected immediately, after sacrifising the animals at fixed time intervals of 3,5,7,10,15,25,40 and 60 days post-autotomy, weighed and the inorganic phosphorus content measured according to the method of Gomori (1942).

RESULTS

Hepatic free phosphorus content of intact controls during post-autotomy period showed an increase from the pre-autotomy level by the third day, followed by a gradual and continuous fall through 5th, 7th, 10th and 15th days. This was followed by a slight increase on the 25th day and another fall on the 40th day. This low level was maintained thereafter. The SGX lizards too showed a similar and parallel pattern of change in the phosphorus contents of the liver. The gonadectomised animals showed a decreased level prior to autotomy followed by a slight increase on the 3rd day and a gradual fall on 5th and 7th days. Thereafter, there was more or less steady increase till the 25th day to reach the maximal level with a small drop in between 10th and 15th days. This was followed by a sharp fall on the 40th day which then tended to show a slight increase during 40th to 60th days of regeneration.

Tail phosphorus content in SGX was noted to the lowest in the pre-autotomic condition. All the three groups of animals showed a more or less similar pattern of changes till the 10th day. The normal intact animals showed an increase on the 3rd day followed by a fall on day 5th. An increase was noted again on the 7th day followed by a fall

Table 1. Levels of hepatic phosphorus content (mg/100 mg fresh tissue) during tail regeneration in normal, sham operated and gonadectomised lizards, H. flaviviridis

Periods of regeneration in days	N	3	5	7	10	15	25	40	60
Normal intact (IC)	0.0801 ±0.0022	0.128 ±0.014 0.001@	0.112 ±0.008	0.069 ±0.005	0.060 ±0.011	0.0381 ±0.004	0.0627 ±0.013	0.0499 ±0.018	0.0517 ±0.015
Sham operated (SGX)	0.087 ±0.011 0.0025@	0.1196 ±0.023	0.0446 ±0.001	0.0605 ±0.0012	0.0641 ±0.0028	0.0450 ±0.0085	0.049 ±0.003	0.042 ±0.0043	0.055 ±0.018
Gonadecto-mised (GX)	0.067 ±0.017 0.001@ 0.0025*	0.0794 ±0.0154	0.0716 ±0.001	0.0553 ±0.026 0.001*	0.075 ±0.009	0.061 ± 0.0201	0.103 ±0.039 0.0025*	0.0453 ±0.0077	0.0517 ±0.005

± S. D.

@ P values obtained in comparison to normal intact lizards

* P values obtained in comparison to sham operated lizards

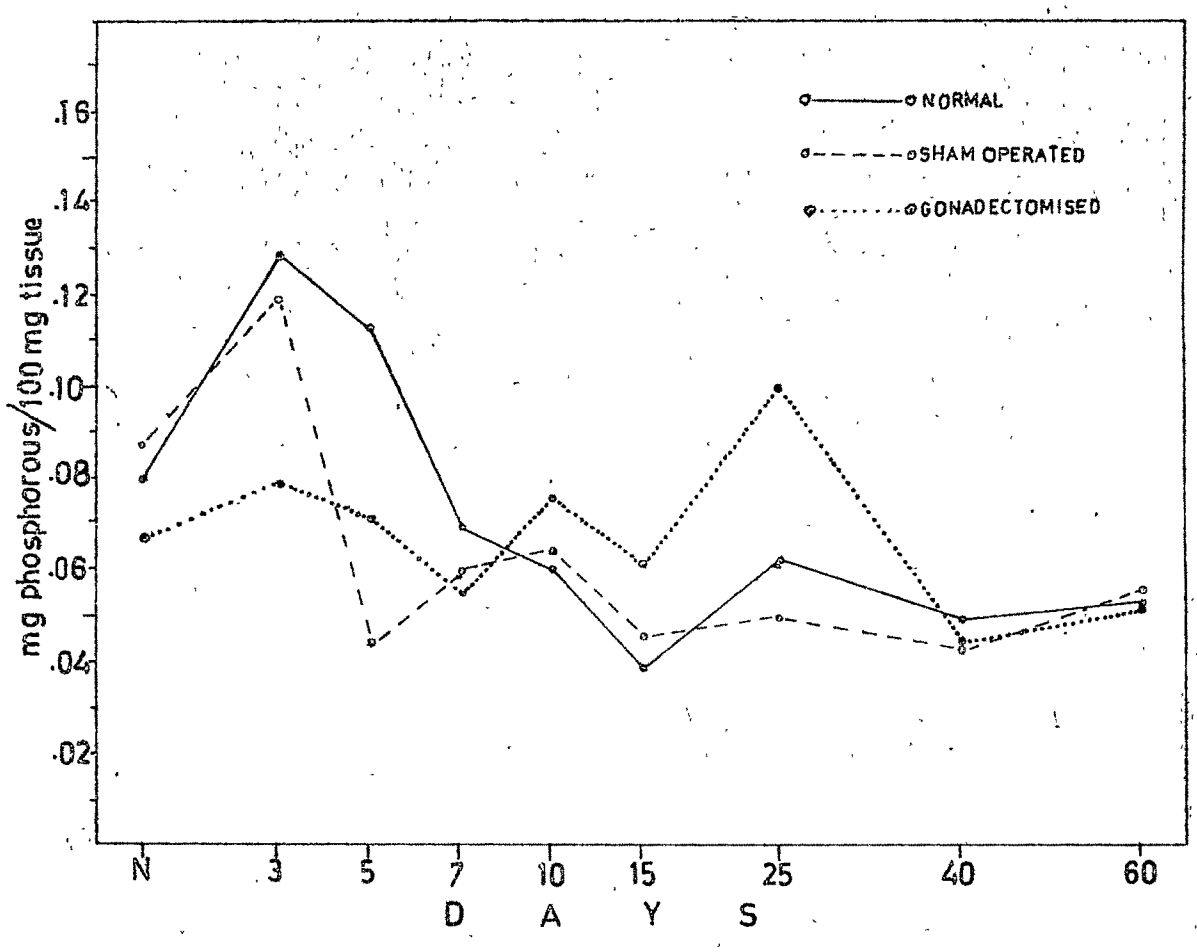

Fig. 1 : Graphic representation of the levels of inorganic phosphorus in the liver during tail regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis

Table 2. Levels of phosphorus in the tail (mg/100 mg fresh tissue) during its regeneration in normal, sham operated and gonadectomised lizards, H. flaviviridis

Periods of regeneration in days	N	3	5	7	10	15	25	40	60
Normal intact (NC)	0.236 ±0.074	0.302 ±0.121 0.0025@	0.131 ±0.081	0.261 ±0.056	0.0801 ±0.024	0.089 ±0.0183	0.0829 ±0.0069	0.0892 ±0.019	0.108 ±0.04
Sham operated (SGX)	0.143 ±0.027 0.001@	0.114 ±0.034 0.005*	0.0977 ±0.017	0.2125 ±0.05	0.0503 ±0.018	0.0509 ±0.035	0.068 ±0.020	0.085 ±0.008	0.0633 ±0.009
Gonadectomised (GX)	0.202 ±0.009 0.0025@ 0.001*	0.201 ±0.03	0.101 ±0.018	0.34 ±0.14 0.0005*	0.132 ±0.038	0.0786 ±0.028 0.0025*	0.109 ±0.072 0.001*	0.184 ±0.063	0.082 ±0.0074

± S. D.

@ P values obtained in comparison to normal intact lizards.

* P values obtained in comparison to sham operated lizards.

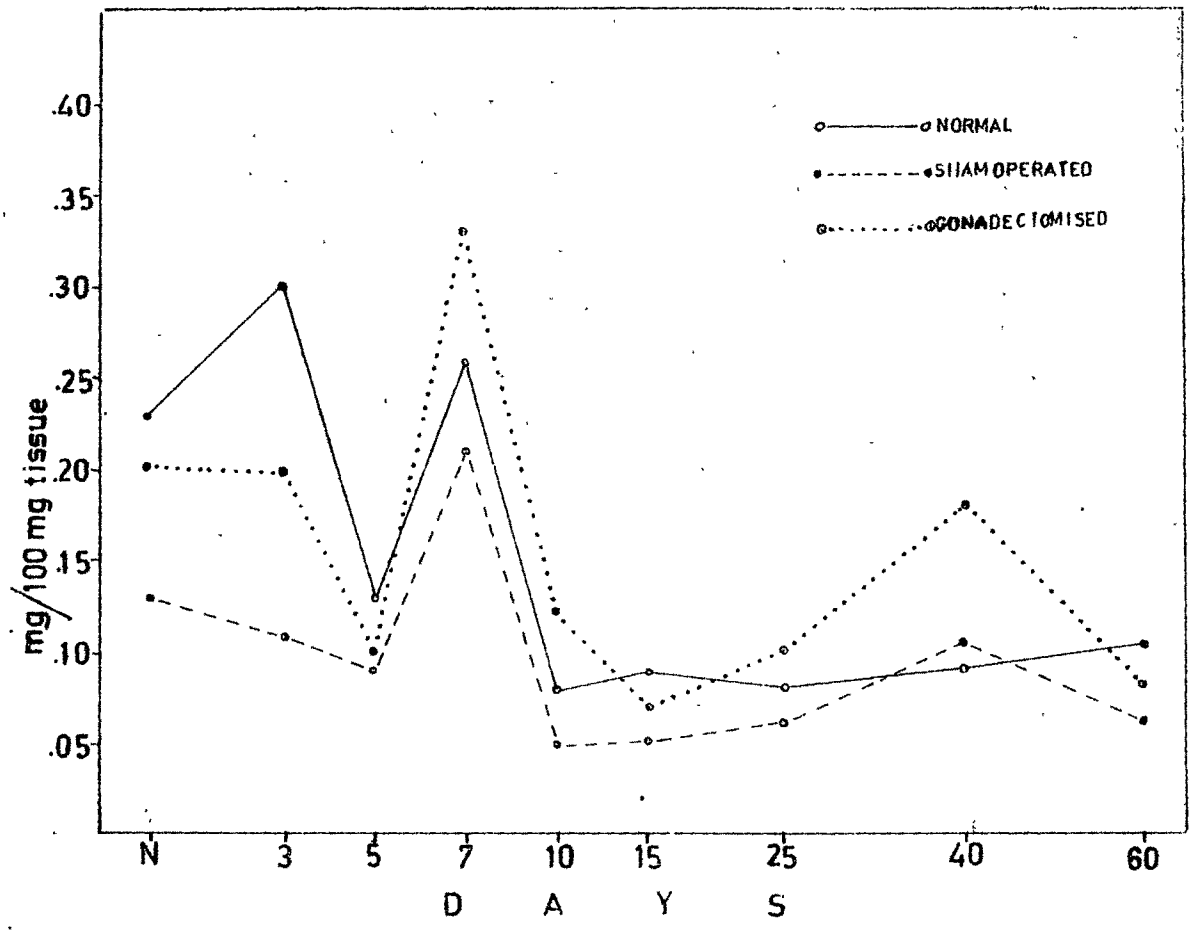

Fig. 2 : Graphic representation of the levels of inorganic phosphorus in the tail during its regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis

by the 10th day. This subnormal level was, thereafter, maintained till the 60th day with little fluctuation. The SGX lizards also depicted a decrease on the 5th day. Thereafter, there was an increase to a peak level on the 7th day and on the 10th day the level was more or less same as that on the 5th day. Thereonwards, gradual increase was the feature on 15th, 25th, and 40th days, which by the 60th day dropped to a level comparable to that of the normal intact controls. GX lizards also registered a noticeable fall in their caudal phosphorus content on the 5th day post-autotomy. Thereafter, an increase on the 7th day led to the attainment of the highest level which then gradually decreased till the 15th day. Thereonwards, there was gradual increase on 25th and 40th days followed by a drop to the control levels by 60th day. The changes outlined above are represented in figures 1 and 2 and tables 1 and 2.

DISCUSSION

Phosphorus metabolism is extremely rapid in kidney, slower in liver and slowest in muscle (White et al., 1968). Comparatively higher metabolic status of liver is indicated by higher phosphorus content and lower content in tail (which is principally composed of muscular tissue) in the normal lizards. Gonadectomy appears to induce a negative

balance in liver, indicating this organ to be more influenced by gonadal steroids. However, surgical stress seems to affect the muscle in a significant way as denoted by the very much reduced phosphorus content in the tail of SGX lizards. In fact, gonadectomy tends to induce a nullifying effect on the surgery induced caudal phosphorus depletion. These differential responses probably suggest a positive and negative influence of gonadal steroids in muscle and liver respectively. The demonstration of Reifenstein et al. (1947) of a marked improvement of negative balance of phosphorus by the administration of gonadal steroids seems to hold good only for the hepatic tissue in lizards. In general, the changes in the free phosphorus content of the tail and liver were more or less identical in all the three groups of lizards all throughout regeneration except for difference in the level more specifically in GX lizards. Moreover, this decrease in inorganic phosphorus along with the reported decrease in K^+ content coupled with the increase in Na^+ content (Shah and Hiradhar, 1974) could be considered to provide an anaerobic environment favourable for the formation of a blastema. Similar low levels of phosphorus in the liver too, during the blastema and post-blastemic periods, indicate systemic utilization of metabolites as well as changing energy equilibrium in response to the needs of regeneration.

Sub-normal levels of phosphorus during the progressive phase of regeneration in the regenerate presumably suggest increased conversion of inorganic phosphorus into organic phosphate compounds.

The present results also suggest the relative insensitivity of regeneration associated phosphorus metabolism to gonadal steroids, as gonadectomy did not produce any deviation in the pattern of changes in caudal and hepatic phosphorus content. However, during the blastema phase in the regenerate, and during the post-blastemic phase in both the regenerate and liver, the increment in phosphorus content had been more significant. This might indicate a favourable disposition that might be afforded by the absence of gonadal hormones towards a regeneration specific factor for elevations in the phosphorus pool. In this light, the high level of caudal phosphorus observed during the 7th day in GX lizards may some how be thought to bear correlation with the observed faster rate of regenerative growth in these lizards between the 7th and 25th days post-autotomy. Further, the significant elevation in inorganic phosphorus noted to occur between the 25th and 40th days also seem to have some relation with the earlier reported retardation in the rate of regenerative growth during this period in GX lizards. The reports of increased phosphorus

content in metabolically inactivated gastrocnemius and sartorious muscles (Kirzan et al., 1962; Fainshmidt, 1939; Ferdman, 1960; Allik, 1963) may be relevant in the present context as caudal tissue is principally constituted of muscles.

Seventh day which roughly corresponds to blastema phase is clearly marked by increased content of caudal phosphorus with a more pronounced one in GX. Apparently this would denote a preparatory build up of phosphate for the metabolism of nucleic acids as well as the biosynthesis of macromolecular in the dividing and differentiating blastemic cells. This is well evidenced by its significant depletion by 15th day and the maintainence of the low level, thereafter, till the end of regeneration.