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Effects of Hypophysectomy and Pituitary Extract Treatment on Blood and Liver Metabolites of a Catfish, *Clarias batrachus*

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Abstract Complete hypophysectomy in male catfish *Clarias batrachus* elicits a sudden hyperglycemia, after two days of the operation. Along with a parallel rise in the alkaline phosphatase activity of the whole blood and the liver, a simultaneous fall was observed in the blood calcium, serum protein and liver glycogen and cholesterol contents. The sham operated fish did not show such alterations of significance. All the parameters rise quickly following injections of pituitary extract obtained from the male of *C. bactrachus*.

Hypophysectomy is known to influence various aspects of metabolic activities in fishes (Pickford, 1957). Hormones secreted by the pituitary mainly act through some other target organs, even then immediate effects of hypophysectomy in relation to regulation of blood calcium level, enzyme activity, protein levels etc. have given us interesting results (Fontaine, 1956; Olivereau and Baraduc, 1965; Ogawa, 1968; Pang et al., 1971, 1973). A good amount of information has already been compiled on the role of different hormones produced by the pituitary, and following hypophysectomy by Ball and Baker (1969). But still no important work has been done on these lines in any Indian freshwater teleosts with special relation to the hypophysectomy and its effects on blood and liver metabolites. This paper briefly presents some changes in the biochemical composition of the blood and the liver in a freshwater clariid cat fish *Clarias batrachus*, following hypophysectomy and pituitary extract treatment.

Material and methods

Live specimens of *Clarias batrachus* were obtained from the local fish market at Moradabad, brought and acclimatized to laboratory conditions for about a month, as reported elsewhere previously (Joshi, 1978). Hypophysectomy was carried out on 75 male specimens, following the method described by Belsare et al. (1970). Out of 75 specimens, 52 survived and the rest succumbed to the operation. The pituitaries obtained from these fish were observed under a microscope to ascertain that it had been taken out completely, then homogenate prepared and preserved in deep freeze until used. The pituitary extracts were prepared in 0.6% saline solution. The volume of the homogenate prepared was so adjusted as to give a final volume of 0.1 ml of whole pituitary extract. Simultaneously sham operations were carried out on 40 male specimens, out of which eight succumbed to the operation.

The fish were divided in three large aquaria, the first contained hypophysectomized fish, second contained sham operated fishes and the third contained normal healthy specimens of *Clarias batrachus* to serve as control. Fish were sacrificed on the 2nd, 4th, 8th, and 15th days, after hypophysectomy. On each of these days two specimens from the control group, eight from the hypophysectomized group and eight from the sham group were sacrificed. From 8th day onward 10 specimens from hypophysectomized group were given intramuscular injections of pituitary extract in the caudal peduncle region at the rate of 0.001 ml of extract per g body weight on alternate days until the 14th day. Out of these 10 fish, eight were sacreficed on the 15th day of the experiment. Thus eight specimens from the control group and 32 specimens, each from the hypophysectomized and sham groups were sacrificed. Values obtained on each turn, for various parameters, are pooled separately. Estimations were made on whole blood glucose, calcium, protein and alkaline phosphatase activity, and on the liver glycogen, alkaline phosphatase activity and cholesterol contents, following the standard methods as given by Oser

(1965). Care was taken to handle the fish so as to cause minimum stress, and within minimum time possible, 3～5 minutes, required tissues i.e. blood and liver were collected. Methods used in handling a fish were the same as described by Ikeda et al. (1975) and Joshi (1978). All parameters observed here were estimated from the whole blood of the *C. batrachus* except the protein contents, which were estimated from the serum of the fish.

Results

Results obtained for various parameters on two tissues reveal conspicuous changes in their biochemical compositions (Table 1) following hypophysectomy and pituitary extract treatment. After two days of hypophysectomy and the sham operation the fishes showed a highly significant ($P<0.001$) hyperglycemia. The values ($x \pm SD$) being 112.7 ± 13.4 mg/dl and 105.2 ± 13.0 mg/dl in the hypophysectomized and the sham fishes, respectively, against the control value of 62.5 ± 6.8 mg/dl (Table 1). During the next two days the hyperglycemia subsided nearly to the control level. From the fourth day onward the hypophysectomized fish revealed a significant hypoglycemic status, being 47.6 ± 5.3 mg/dl on the 15th day. Almost no change occurred in the glycemic level of the sham fish during the same period. The hypophysectomized fishes which were given pituitary extract injections turned normoglycemic, during a short span of seven days, the value being 64.2 ± 4.9 mg/dl on the 15th day in these fishes.

The whole blood calcium level in the hypophysectomized and in the sham operated fishes depleted significantly ($P<0.01$) as compared to the control value of 10.2 ± 2.1 mg/dl (Table 1).

Table 1. Alteration in some biochemical components of blood and liver of *Clarias batrachus*, following hypophysectomy and pituitary extract treatment. All values are means ±SD for 8 observations each. H, hypophysectomized fish; S, sham operated fish; H+P, hypophysectomized and treated with pituitary extract.

Status and time	Blood				Liver		
	Glucose mg/dl	Calcium mg/dl	Protein g/dl	Alk. phos. KAU/dl	Glycogen g/100g	Alk. phos. KAU/100g	Cholesterol mg/dl
Control	62.5	10.2	3.6	5.2	3.42	4.8	460.0
	±6.8	±2.1	±1.1	±1.4	±0.95	±0.8	±25.0
Post 2 days:							
H	112.7	9.0	3.3	5.7	3.00	5.6	418.5
	±13.4	±1.8	±1.6	±1.8	±1.1	±1.1	±37.8
S	105.2	9.6	4.0	5.6	3.28	5.4	471.3
	±13.0	±1.8	±1.3	±1.5	±0.86	±1.1	±30.4
Post 4 days:							
H	61.3	8.6	3.2	4.9	2.76	4.3	409.0
	± 7.3	±2.3	±0.9	±0.9	±0.82	±0.6	±30.4
S	65.5	9.4	3.8	5.3	3.25	5.0	462.3
	± 5.2	±1.7	±1.1	±1.2	±0.65	±1.0	16.6±
Post 8 days:							
H	53.8	7.6	3.0	3.7	2.58	3.1	376.5
	± 8.2	±1.8	±0.9	±0.8	±0.39	±0.2	±13.4
S	60.0	9.7	3.4	5.0	3.51	4.6	436.7
	± 4.7	±2.0	±1.1	±1.1	±0.78	±0.4	±28.2
Post 15 days:							
H	47.6	7.2	3.1	3.7	2.81	3.3	332.3
	± 5.3	±1.3	±0.7	±1.0	±1.72	±0.5	±14.4
S	63.8	9.8	3.7	5.3	3.46	4.4	446.2
	± 4.7	±2.1	±0.9	±0.9	±0.76	±0.9	±26.8
H+P	64.2	10.0	4.0	5.1	3.03	4.7	401.2
	± 4.9	±2.4	±1.1	±1.8	±0.98	±1.0	±31.2

The hypocalcemia was more conspicuous in the hypophysectomized fish, than in the sham fish. On the subsequent days of observations the hypocalcemia became more pronounced being 7.2 ± 1.3 mg/dl in the hypophysectomized fish, whereas the value remained almost static in the sham operated fishes by the concluding day of the experiment, whereas the fishes treated with the pituitary extract recovered from the hypoalcemic status (Table 1) to a level of 10.0 ± 2.4 mg/dl.

The protein contents of the serum in the sham operated fishes revealed almost the same pattern of alteration as the whole blood glucose contents, whereas the protein level fell continuously in the hypophysectomized fish until the 15th day. The fishes treated with the pituitary gland extracts showed improved protein value of the serum, being 4.0 ± 1.1 g/dl (Table 1) and to the hypophysectomized fish value of 3.1 ± 0.7 g/dl on the 15th day.

The alkaline phosphatase activity of the whole blood also altered in the same manner as the blood glucose level, and even showed a sudden rise following pituitary extract injection in the hypophysectomized fishes (Table 1).

The glycogen contents of the liver depleted during the whole course of 15 days of the experiment in the hypophysectomized fishes ($P<0.001$) whereas little fluctuations occurred in the sham group ($P<0.01$). Little rise in the liver glycogen contents was also noted following the pituitary extract injections (Table 1).

The alkaline phosphatase activity of the liver had the same mode of fluctuation as that of the blood enzyme level (Table 1).

The cholesterol content of the liver fell conspicuously in the hypophysectomized fish after two days of the operation, but a sudden rise in cholesterol level in the sham group persisted until the 4th day of the experiment (Table 1). However, the cholesterol value depleted significantly ($P<0.01$) in the hypophysectomized fish as compared with the control value of 460.0 ± 25.0 mg/dl by the concluding day of the experiment. In the sham group, fall in cholesterol level was just negligible by the 15th day. Interestingly, the pituitary extract injections elevated the cholesterol contents of the liver significantly ($P<0.01$), when compared to the value for the hypophysectomized fishes.

Discussion

Pickford (1957) and Kayes (1979) pointed out that hypophysectomy leads to the cessation of growth, length and weight in the fish *Fundulus heteroclitus* and *Ictalurus melas* respectively, whereas Crim and Peter (1978) noted in the fish *Salmo salar* that the hypophysectomy arrests the maturation of the male gonads. This clearly means inhibition of general body metabolism through common as well as specific pathways. Kayes (1977, 1979), on the other hand, noted that hypophysectomy in the black bullhead *Ictalurus melas* retards the function of the somatotrophs. Still further, it has been observed by Kayes (1977) that beef growth hormone replacement therapy led to the restoration of rapid growth in the same species. Keeping in view these earlier results, our observations clearly show that pituitary is certainly involved not only in the regulation of the blood calcium contents but also in that of the blood glucose and a few other metabolites of the liver like the glycogen and the cholesterol. Though the involvement of the pituitary may be indirect, our results strongly show that pituitary may not only be acting through target glands, but may also be involved, itself, directly. This allows us to infer that when various secretions of the pituitary are in blood circulation, some sort of feedback mechanism is established for the maintenance of the general homoeostasis, as may be reflected by the varying needs of different tissues and the relative concentration of certain metabolites in the blood (Joshi, 1980).

The role of pituitary is further substantiated by the rapid elevation or recoupment of all biochemical parameters, studied here, in the blood and in the liver of the fish *Clarias batrachus,* following pituitary extract treatment, of the hypophysectomized fishes. Our results are also in conformity to those of the earlier observations of Pang et al. (1971) and Kayes (1977) on *Fundulus heteroclitus* and *Ictalurus melas,* respectively. The influence of hypophysectomy on the liver cholesterol content is not available on any other species, so far. However, from the present observations it appears that even the lipid metabolism is intricately related to pituitary gland hormones.

A sudden rise in the blood and liver alkaline phosphatase activity and in the blood glucose

level noted in the sham as well as in the hypophysectomized fishes clearly suggests that the rise in these two parameters with simultaneous fall in liver glycogen content is mainly due to rapid mobilization of these moieties after the operation. All other parameters showed almost an immediate fall. This may be again due to two reasons; the first due to hypophysectomy and the second due to rapid utilization of substances like calcium, protein and glycogen by the tissues undergoing healing following the operation. It is hoped that the fish would have recuperated from operational stress within two to four days, as can be inferred from the glycemic alterations in the blood, on one hand, and a parallel fall in the alkaline phosphatase contents during the same time.

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Clarias batrachus の血液と肝臓の成分に対する脳下垂体除去と脳下垂体エキス処理の効果

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ヒレナマズ *Clarias batrachus* の♂で脳下垂体を除去すると2日後に急速に血糖上昇を来した．これと平行して，全血と肝臓のアルカリフォスファターゼ活性が上昇し，血液カルシウム，血清蛋白，肝臓グリコーゲンとコレステロール量は低下した．擬手術魚ではこれらの成分には有意な変化はみられなかった．本魚種♂から得た脳下垂体エキスを脳下垂体除去魚に注射すると，すべての成分は急速に増加した．