α-naphthaleneacetic acid, kinetin and yeast extract to induce callusing in bulb explants.

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CHANGES IN CARBOHYDRATE METABOLISM ASSOCIATED WITH ATROPHY AND ELECTRICAL STIMULATION IN THE GASTROCNEMIUS MUSCLE OF *RANA HEXADACTYLA* (LESSON)

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ELECTRICAL stimulation has been shown to induce exercise in muscle[1]. Chronic exposure (10 days) of the muscle to exercise through electrical stimulation seems to improve muscular performance[2]. Sciatectomy and disuse of the muscle was widely known to lead to atrophy and muscle wasting leading to impaired structural and functional organization[3]. In the present study, the impact of electrical stimulation of muscle carbohydrate metabolism of denervation atrophied muscle of frog was analysed, since carbohydrate metabolism is believed to be associated with muscle contractile kinetics.

Male specimens of *Rana hexadactyla* (Lesson) 30 ± 2 g were employed in the present study.

The frogs were unilaterally denervated under aseptic conditions. The sciatic nerve supplying the shank was separated and the nerve (about 2 cm length) was cut at the posterior part of the thigh. Sham-operated normal animals were also maintained as control specimens (C). Ten days after sciatectomy, the gastrocnemius muscle was significantly atrophied. Hence, a period of 10 days after sciatectomy was selected for testing the impact of electrical stimulation.

A special chamber was designed to restrain the animals during electrical stimulations. The denervated gastrocnemius muscles of sciatectomized frogs after 10 days of sciatectomy were stimulated for 30 min daily for 10 days using an electrical stimulator (INCO/CSIO, Ambala, India). Biphasic pulses (5 V; 100 msec duration and 2 C/s) were applied to stimulate the muscles. Another batch of denervated animals was maintained as denervated control (DC).

After appropriate periods, all the three groups of animals viz. normal, sham-operated control (C), denervated control (DC) and denervated stimulated (DS) were double-pithed, gastrocnemii were isolated and chilled rapidly by keeping in freezing mixture. The muscles were used for biochemical estimations.

The levels of glycogen[4], phosphorylase activity (a,b and ab)[5], glucose[6], aldolase activity[7], lactic acid[8], pyruvate[9], the activity levels of SDH, MDH and LDH[10] and G-6-PDH[11] were estimated.

Table 1 presents data on the impact of electrical stimulations on carbohydrate metabolism.

i) Denervated control (DC) muscle:

After 10 days of sciatectomy, the muscle glycogen (+32%) and glucose (+28%) contents increased significantly. The activity levels of glycogen-phosphorylase and aldolase representing glycogenolysis and glycolysis were inhibited significantly. The accumulated carbohydrate reserves observed in the DC muscle can be attributed to their decreased mobilization through glycogenolytic and glycolytic pathways. Despite decreased glycogenolysis and glycolysis, the DC muscle showed increased lactate and decreased pyruvate contents. The NAD-LDH activity, which represents the mobilization of lactate into TCA cycle, was decreased leading to the accumulation of lactate and decreased pyruvate contents. This decreased mobilization of lactate could be responsible for the observed inhibition in the activity levels of TCA cycle enzymes viz. SDH and MDH. Even the G-6-PDH activity was also decreased in the DC muscle, indicating the decreased level of operation of HMP shunt. Thus the

Table 1 *Levels of glycogen, phosphorylase activity (a, b & ab), glucose, aldolase activity, lactate, pyruvate, LDH, SDH, MDH & G-6-PDH activities, in control (C), denervated (DC) and denervated stimulated (DS) muscles. Each value represents the mean of six individual observations*

	Control muscle (C)	C vs DC	Denervated muscle (DC)	DC vs DS	Denervated stimulated muscle (DS)
Glycogen (mg/g wet wt)	4.28 ± 0.39	32.47 $P < 0.001$	5.67 ± 0.22	−23.45 $P < 0.001$	4.34 ± 0.46
Phosphorylase *a* activity (μmol of Pi formed/mg protein/h)	17.16 ± 0.60	−22.84 $P < 0.001$	13.24 ± 0.50	20.24 $P < 0.001$	15.92 ± 0.22
Phosphorylase *b* activity (μmol of Pi formed/mg protein/h)	22.54 ± 0.84	12.24 $P < 0.001$	25.30 ± 0.050	−16.79 $P < 0.001$	21.05 ± 0.93
Phosphorylase ab activity (μmol of Pi formed/mg protein/h)	39.70 ± 0.91	− 2.92 $P < 0.01$	38.54 ± 0.97	− 4.07 $P < 0.001$	36.97 ± 0.95
Glucose (mg/g wet wt)	2.22 ± 0.12	28.01 $P < 0.001$	2.97 ± 0.69	−19.86 $P < 0.001$	2.38 ± 0.18
Aldolase activity (μmol of FDP cleaved/mg protein/h)	12.86 ± 0.61	−31.72 $P < 0.001$	8.78 ± 0.58	48.86 $P < 0.001$	13.07 ± 0.48
Lactate (mg/g wet wt)	3.03 ± 0.15	33.66 $P < 0.001$	4.05 ± 0.46	−21.48 $P < 0.001$	3.18 ± 0.16
Pyruvate (μmol/g wet wt)	2.16 ± 0.14	−16.20 $P < 0.001$	1.81 ± 0.14	32.04 $P < 0.001$	2.39 ± 0.22
LDH activity (μmol of formazan formed/mg protein/h)	0.128 ± 0.0013	−14.06 $P < 0.001$	0.110 ± 0.001	11.81 $P < 0.001$	0.123 ± 0.001
SDH activity (μmol of formazan formed/mg protein/h)	1.21 ± 0.01	−16.52 $P < 0.001$	1.01 ± 0.02	27.72 $P < 0.001$	1.29 ± 0.01
MDH activity (μmol of formazan formed/mg protein/h)	0.897 ± 0.036	−23.07 $P < 0.001$	0.69 ± 0.004	20.28 $P < 0.001$	0.83 ± 0.002
G-6-PDH activity (μmol of formazan formed/mg protein/h)	1.64 ± 0.01	−35.36 $P < 0.001$	1.06 ± 0.02	57.54 $P < 0.001$	1.67 ± 0.016

Mean ± S.D.; P denotes the level of statistical significance.

DC muscle exhibited decreased mobilization of carbohydrate reserves through anaerobic and aerobic pathways and also HMP shunt. This decreased utilization of carbohydrate reserves is the result of muscular disuse due to sciatectomy.

ii) Denervated stimulated (DS) muscle:

The DS muscle, on the contrary, showed depletion of both glycogen (−23%) and glucose (−17%) contents. The activity levels of phosphorylase and aldolase were significantly elevated, leading to the mobilization of carbohydrate reserves through hexose diphosphate pathway. The activity level of G-6-PDH was also increased in DS muscle, mobilizing the carbohydrate reserves through the hexose monophosphate pathway also. The decreased lactate content and increased pyruvate with enhanced activity level of LDH in the DS muscle reflect the increased mobilization of lactate into TCA cycle. Enhanced activity levels of TCA cycle enzymes, SDH and MDH observed in DS muscle support such a possibility. The induced muscular function in the DS muscle thus mobilized carbohydrate reserves. On the contrary, in the DC muscle, the muscular disuse due to sciatectomy, resulted in the accumlation of carbohydrate reserves.

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STUDIES ON YELLOW GRUB DISEASE OF FRESHWATER FISH *CHANNA PUNCTATUS* (BLOCH)

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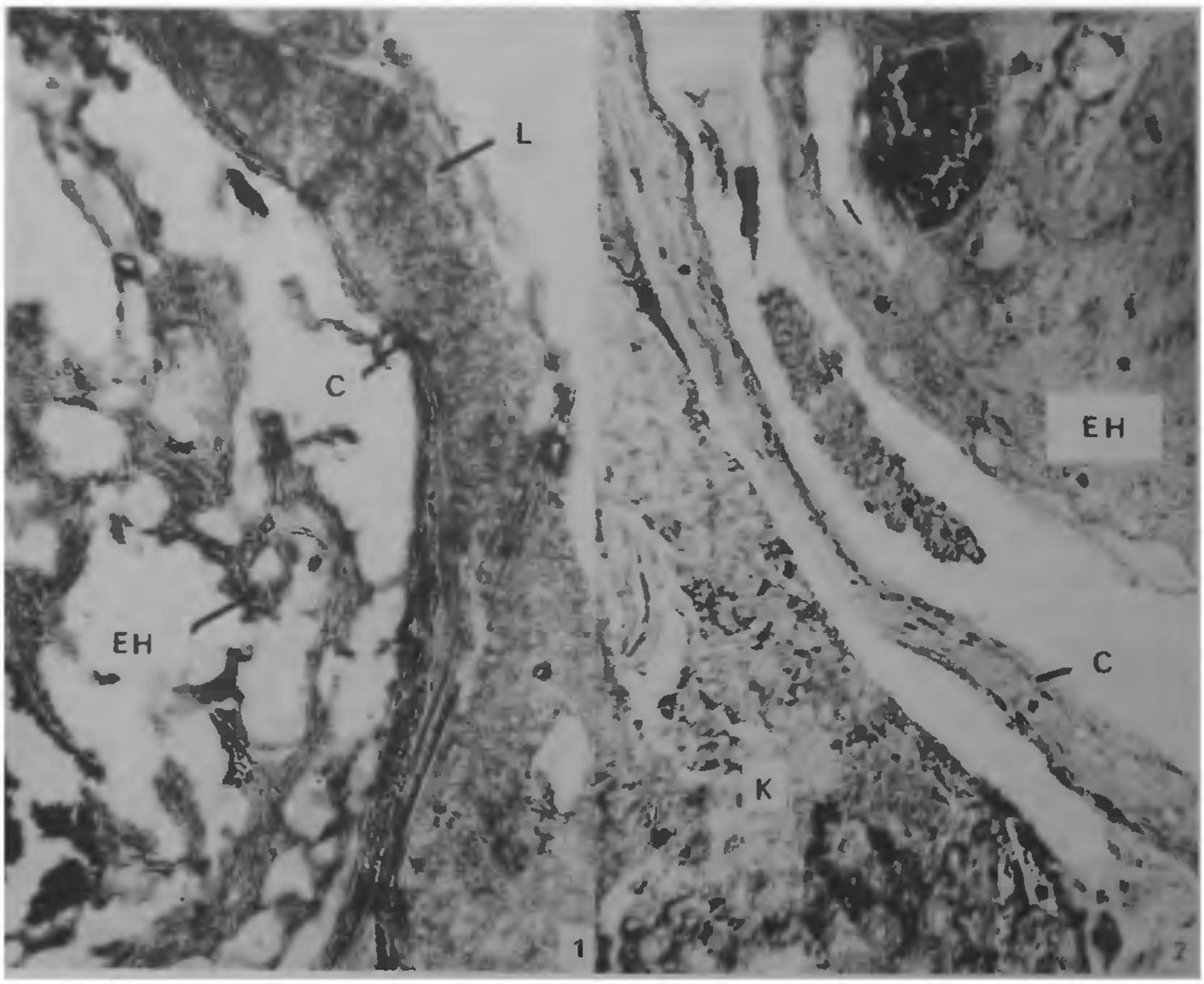
PATHOGENICITY in yellow grub disease of *Channa punctatus* (Bloch) has been studied during March 1984 to February 1986. A total of 485 (216 male and 269 female) *C. punctatus* were examined. On an average 54.27% and 3.86% male 58.34% and 4.93% female fish were infected respectively by the metacercaria of *Euclinostomum heterostomum* and *Clinostomum giganticum*. The maximum intensity of infection was observed from April to August and the minimum in December and January. Concurrent infection by *E. heterostomum* and *C. giganticum* was observed in 26.25% male and 23.15% female *C. punctatus*.

The metacercaria of *E. heterostomum* were localized in the liver, kidney and spleen whereas



Figures 1 and 2. (×100). **1.** Section of liver of *C. punctatus* showing encapsulated metacercaria of *E. heterostomum* (EH). H&E. **2.** Section of kidney of *C. punctatus* showing encapsulated *E. heterostomum* (EH). H&E [L, Liver; C, Capsule wall; K, Kidney].