ON THE OCCURRENCE OF SUCCINIC DEHYDROGENASE AND ITS NEURAL REGULATION IN THE BONE TISSUE OF FROG *RANA HEXADACTYLA*

BONE is considered to be a comparatively inert tissue as the intercellular substance far exceeds the cell material. Very little is known about the metabolism of bone tissue. In recent years increasing attention is being paid to the biochemistry of bone formation. Doskocil[1], demonstrated the influence of peripheral nerve interruption upon the growth of tibia and meta-tarsus in rat. Though a number of studies has been made on the enzyme systems with special reference to Kreb's cycle in the cartilage chondrocytes[2–7], scant attention has been paid to the presence of oxidative enzymes in the osteoblast cells in bone[8–10]. Hence an attempt was made to find out the level of activity of the oxidative enzyme, succinic debydrogenase (SDH, E.C. 1.3.99.1), in bone cell and its neural regulation, by the *in vivo* interruption of nerve supply to the bone tissue of the tibiofibula of frog, *Rana hexadactyla*. Changes in protein level and dry weight of the bone were also studied.

Medium sized frogs were fed daily with cockroaches and earthworms. After 3 or 4 days of acclimatization, the sciatic nerve of the right hind limb was excised, by removing two centimeters of the nerve.

After 30 days, the frogs were double pithed, the tibiofibula of both the denervated and contra-lateral limbs were excised, isolated in cold and the attached muscle tissue was carefully removed. The bone was cut open and the marrow was removed completely and washed 3 or 4 times in Amphibian Ringer.

The bone thus isolated in cold was quickly weighed and homogenised in 0·25 M sucrose solution. The homogenate was centrifuged for ten minutes and the supernarant, equivalent to 100 mg of tissue was used for the enzyme assay. SDH was estimated by the modified method of Nachlas *et al.*[12 13]. The reaction mixture was incubated for two hours. SDH was also estimated in the tibiofibula of normal frogs. A set of tibiofibulae was exposed to 10% trichloro-acetic acid (TCA), for two minutes to remove any cell debris of the associated muscle and marrow and the enzyme activity was estimated after washing in the Amphibian Ringer for 4 times. Protein content was estimated by the method of Lowry, *et al*[14]. Another set of tibiofibulae was dried in a hot air oven for one week and the weights were recorded.

The results (Table I) indicate the measurable level of SDH activity in bone tissue. The TCA washing of the bone, did not virtually alter the enzyme activity (Table I). Though a number of investigations has been carried out in the cartillage cells[2,6], the bone tissue did not receive attention with reference to the SDH activity, in spite of a few studies on the structural aspects of mitochondria[8,9].

TABLE I

Succinic dehydrogenase activity in the bone tissue of tibiofibulae of normal and denervated Rana hexadactyla

Enzyme activity expressed in μmoles/mg protein/hr.

Normal Frog			Denervated Frog		
TCA treated	TCA untreated	't' test	Control	Denervated	't' test
0·059±0·023	0·062±0·029	N.S.	0·097±0·041	0·057±0·034	P<0·05

• Each value is mean ± S.D. of 6 individual observations.

Available experimental evidence indicates that the motor nerve exerts a 'trophic influence' on skeletal muscle other than that of neuromuscular transmission and the resulting muscle activity[15,16]. Similar function of the nerve on the growth of tibia and meta-tarsus of rat was reported by Doskocil[1]. No such influences of the netve were shown to be present on the level of SDH activiry in the bone tissue. Analysis of the protein content and dry weight of the tibiofibulae of the contralateral control and denervated limbs showed an increase in the protein content and a decrease in the dry weight of the bone on denervation. In earlier studies, the muscle weight was found to decrease after transection of the nerve[13,17] similar to the decrease in bone weight, observed in present experiment. On the contrary the protein content increased in the denervated limb bone. In spite of an increase in the

TABLE II

Bone weight and protein level of the control and denervated tibiofibulae of Rana hexadactyla

	Control	Denervated	't' test
Bone weight (dry wieght in gms)	0·262±0·032	0·207±0·037	$P < 0.05$
Protein content (mg/gm wet weight)	6·042±2·331	11·292±4·818	$P < 0.05$

* Each value is mean ± S.D. of 6 individual observations.

protein level, the SDH activity has decreased in the denervated limb bone (Table I). It may be due to greater synthesis of the nonspecific proteins and proteins of glycolysis with a retarded synthesis of specific proteins like SDH, *etc.*, as it happens in the denervated muscle[18].

The results of the present experiment on the tibiofibulae of denervated frog indicate that the interruption of sciatic nerve significantly alters the bone weight, protein content and the oxidative enzyme activity (Tables I and II), which shows that the intact nerve exercises a control over the enzyme activity in bone and also regulates bone weight. We believe that this is the first evidence of the neural regulation of SDH activity in bone tissue. It may be mentioned that the immobilization of the denervated limb may be one of the causative factors in developing the denervation changes since similar changes were found to be important factors in the establishment of denervation muscle atrophy[19]. It is interesting to note that similar induced atrophy in muscle by the sciatic nerve section results in a decrement in SDH activity[11], indicating a neural regulation of the oxidative enzyme in muscle and bone.

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PREHISTORIC REMAINS OF THE GREY SEAL, *HELICHOERUS GRYPUS* FABRICIUS 1791, FROM HARAPPA, PAKISTAN

WITH the object of reviewing the old collection of animal remains from Harappa (Prashad[1]), we examined a number of undetermined specimens of very fragile nature. Among them was a tooth belonging to the Grey Seal *Helichoerus grypus* Fabricius which is very interesting from the point of view of its present restricted distribution in the arctic and temperate seas far away from Harappa. The animal inhabits in the Arctic region and in the colder seas in and around Europe, U.S.S.R., and North America (Ellerman and Morrison-Scott[2]).

Its presence in the collection is quite astonishing and interesting. It is reminiscent of the fact that the Harappan people had some sort of relationship or trade connection with far-off foreign countries.

Material. No. 10212; Mound F; Trench 1.
Square M 12/9; depth 9′ 10″–10′ 6″.

One upper molar; a few fragments of the skull,