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## MYONECROSIS IN SWISS ALBINO MICE FOLLOWING XYLOTOX ADMINISTRATION. CHANGES IN ALKALINE AND ACID PHOSPHATASES

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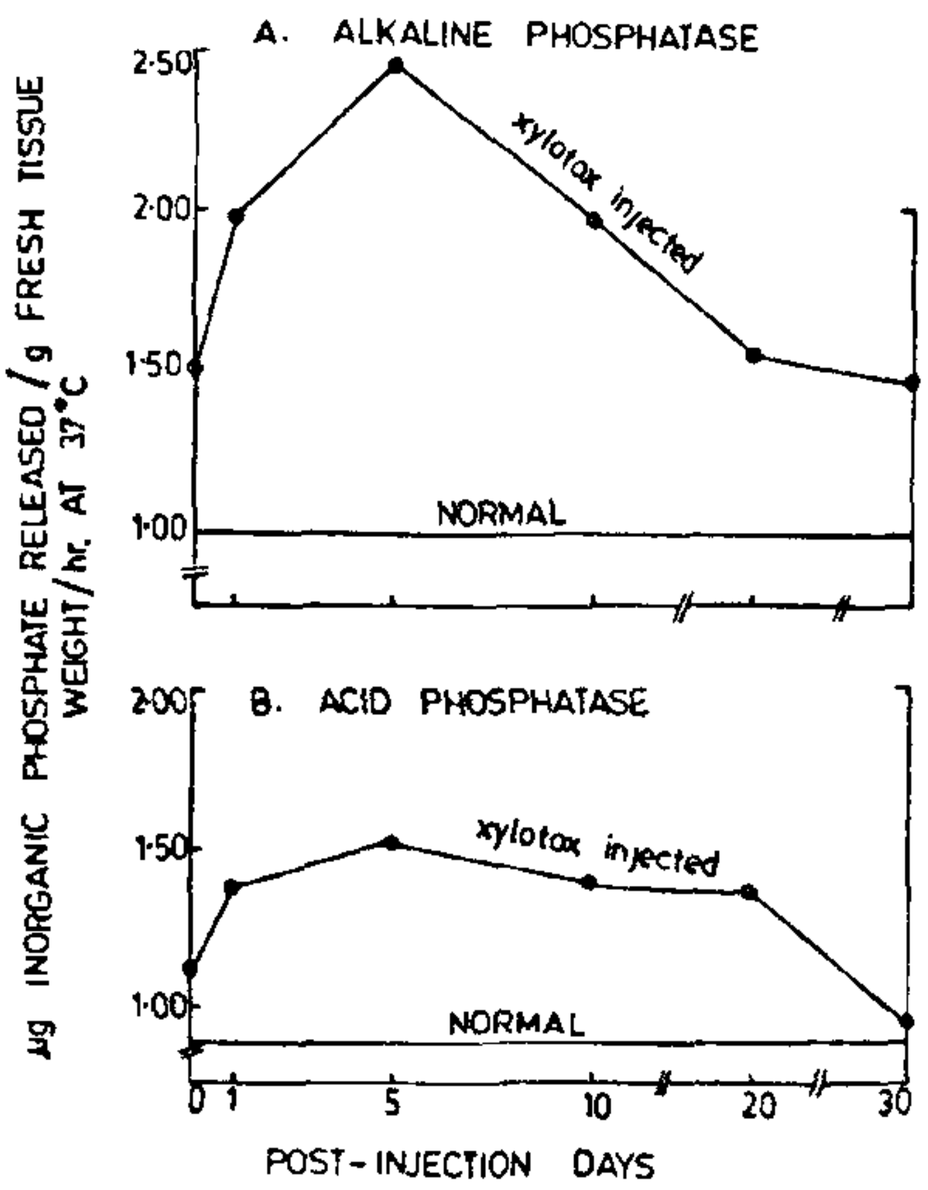
INTRAMUSCULAR injections of a variety of local anesthetics used in clinical practices produce widespread myofibrillar degeneration[1–4] followed by regeneration[1,5,6]. This degeneration/regeneration process has been well documented in a number of histopathological studies. Myonecrotic effects which persist for few days include incorporation of polymorphonuclear leukocytes and macrophages at degenerative sites and also displacement of myonuclei in some fibers. Regeneration reverses this process[6]. However, biochemical events i.e. reactions in initiation and progression of this myofibrillar degeneration and regeneration have not been characterized. But for the scant reports on correlation between serum enzyme levels and histopathological disturbances following local anesthetic administration[4], no one has ever been interested in following the sequence of enzymic changes during this process especially those enzymes with a lytic function in the affected muscle fibers. We report in this communication the status of two lytic enzymes viz acid and alkaline phosphatases working at widely different pH optima, during myofibrillar degeneration/regeneration following a local anesthetic adminstration. The phosphatases have been shown to have an intimate association with a number of muscle diseases including neuromuscular disorders[7–11].

Five repeated doses of 50 µl of 0.2%(v/v) xylotox (lignocaine hydrochloride) at 8 hr intervals were administered in the *gastrocnemius* muscle of Swiss albino mice. A total of 36 animals were injected the local anesthetic and three mice sacrificed every time (0, 1, 5, 10, 20, 30 days following last injection) for the quantitative estimation of each of the phosphatases in the target muscle[12,13]. Striking variations in the levels of both acid and alkaline phosphatases resulted within hours after the last injection (0 day). Peak values in enzyme levels were recorded between the 5th and 10th day which remained considerably elevated till the 20th day (figures A and B). Beyond this post-injection period, the enzymic contents, however, witnessed a decline towards the normal levels. This pattern of variation in lytic enzymes is absolutely in parity with our earlier report[6] that the maximal myofibrillar degeneration under identical experimental conditions occurred around 15-20 days following xylotox administration.




**Figures A and B.** Alterations in the levels of alkaline and acid phosphatases in xylotox injected *gastrocnemius* muscle. Early elevation in the enzyme levels is characteristic. 0 day indicates 2 hr following xylotox administration.

Beyond 20th day, the process of regeneration sets in[6] which is well characterized in the present study by a progressive fall in lytic enzyme concentrations. Elevated levels of hydrolytic enzymes have also been reported in a number of muscle diseases including those of neuromuscular disorders. Alkaline phosphatase has been characteristically reported to show a proportionate elevation with the onset and progression of fibrosis during neuromuscular disorders[9,11]. Acid hydrolases also exhibit increase in their levels in diseased muscle[8,10]. The progressive morphological distintegration of muscle fibers following xylotox administration as such, results from stimulated levels of both acid and alkaline phosphatases. Phosphatases thus serve as biochemical markers of myonecrosis following local anesthetic administration. The increased levels of lytic enzymes result from accumulated macrophages and polymorphonuclear leukocytes in the affected muscle fibers. These cells contain lytic enzymes in them. It can be concluded that synthesis and subsequent accumulation of both acid and alkaline phosphatases act as a prelude to myofibrillar degeneration following local anesthetic injections. Regeneration finally ensures when the lytic enzymes have returned or are tending to return to the normal value.

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## PRODUCTION OF CELLULASES FROM *CURVULARIA LUNATA**

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RECENTLY much attention has been paid to the utilization of cellulosic materials as renewable resources of energy, chemicals and food. Most of the studies are concerned with enzymatic hydrolysis of the polysaccharide to glucose[1]. Cellulolytic enzymes produced by the members of the *Trichoderma* have been used extensively in these studies, because they produce the most potent enzyme system. In our pursuit to isolate cellulytic fungi from soil, we have assayed several fungi for cellulases, of which *Curvularia lunata*, a potent cellulase producing organism, has been reported here.

*C. lunata* isolated from soil, was maintained on PDA slants. Production of enzymes was studied in 500 ml Erlenmeyer flasks containing 250 ml of modified basic salts medium[2] containing 50 g of substrate (cellulose, lactose and glucose) per litre at pH 5. The medium was inoculated from one-week-old PDA slants and incubated on a rotary shaker at room temperature (28–30°C). After eight days, the cultures were filtered and the filtrate analysed for enzyme activity.

Mycelial dry weight was determined by filtering the samples through preweighed filter papers, rinsing thrice with water, and drying at 80°C for 24 hr. Enzyme activities, such as filter paper activity (FPA), carboxymethylcellulase (sodium salt) activity and cellobiase activity were measured by the methods described earlier[3]. Arsenomolybdate method[4] was used to estimate the substrate level as well as liberated reducing sugars in terms of glucose. Activities are expressed in International Units (IU) as micromoles of equivalent glucose produced per ml per min. Extracellular protein was estimated by Lowry's[5] method without precipitation, using bovine serum albumin as the standard.

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