of 5.16% and the vehicle injected controls did not exhibit large deviations from this value during the 4-day period. However, a statistically significant decrease in the mitotic index ($p<0.05$) was observed in the marrow of hamsters receiving 1000 mg/kg of Δ^9-THC in samples examined up to 24 h post-treatment. The values returned to control levels in those examined at 48 and 96 h after treatment. A much smaller dose of 10 mg/kg of Δ^9-THC also produced a significant drop in the mitotic indices of marrow at corresponding times, but the effect was less marked than in samples receiving the larger dose.

There has been conflicting evidence as to whether or not *Cannabis* or its derivatives cause chromosomal damage. In the present study, pure Δ^9-THC did not cause any significant effects on chromosome structure or karyotype stability in the marrow of hamsters receiving even a very large dose of 1000 mg/kg of Δ^9-THC. However, the possibility of minute undetected chromosomal aberrations or point mutations cannot be completely excluded. It is possible that the aberrations observed by some investigators[4,5,7,8] may have been caused by impurities or other cannabinoids present in the preparations used. Our findings on the reduction of mitotic indices after Δ^9-THC treatment in vivo are consistent with previous reports which employed exposure to marihuana smoke[9], *Cannabis* resin[6] or Δ^8- and Δ^9-THC[11] in in vitro systems. The reduced mitotic activity in the bone marrow could have significant effects on the pattern of proliferation and differentiation of hematopoietic cells.

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Alkaline phosphatase activity in normal and denervated skeletal muscle

R.K. Malhotra, S. Dhingra and S.S. Katoch

Department of Bio-Sciences, Himachal Pradesh University, Summer Hill, Simla-171005 (India), 25 January 1978

Summary. Changes in the specific activity of alkaline phosphatase in the normal and denervated skeletal muscle have been studied both histochemically as well as biochemically for a maximum period of 8 weeks of its postnatal development. In the normal muscle, a heterogenous population of fibres with respect to the enzyme distribution is observed. Relatively higher levels of enzyme in the denervated muscle and also the proliferation of extrafibrillar connective tissue in the diseased muscle show its specific association with the lytic processes.

The role of acid phosphatase in different myopathies including various neuromuscular disorders has been a subject of extensive investigation[1-5], and very little attention has been paid to alkaline phosphatase which is an equally important enzyme functioning at a different pH optimum. Kar and Pearson[6] have associated the proliferation of noncontractile connective tissue with higher levels of alkaline phosphatase activity in the diseased human muscle and have assigned a lytic role to this enzyme. On the other hand, Dubowitz[7] rules out the presence of this enzyme in normal muscle, whereas Pennington[8] does not consider the possibility of the 2 enzymes functioning at very different pH optima in the same cell at one time. In light of these conflicting views, it was considered desirable to study the sequential changes in the levels of this enzyme during the growth of normal as well as denervated skeletal muscle since both lysis and an increase in the noncontractile connective tissue are observed in the denervated muscle. The present investigation has been carried out on the gastrocnemii muscles of chick and includes quantitative estimation of the enzyme at weekly intervals for a maximum period of 8 weeks, in the normal as well as denervated muscle. Histochemical localization of the enzyme has been made to determine the sites of activity change during different stages of muscle growth.

Material and methods: Male chicks of *Gallus domesticus* (white Leghorn variety) were procured from Government Poultry Farm at Simla (India). The animals were divided into 2 groups and maintained under normal laboratory hygienic conditions. 1 group served as controls, whereas members of the other group were subjected to unilateral sciatectomy on the 5th day of their postnatal life. Denervation procedure is described elsewhere[9]. Autopsies were performed at weekly intervals for a maximum of 8 weeks after denervation and the 3 gastrocnemii viz; pars externa, media and interna were excised immediately and processed as follows. The enzyme was localized histochemically by Gomori's technique[10] at pH 8.9 with minor modifications using unfixed, fresh-frozen air-dried sections and employing sodium-B-glycerophosphate as the substrate. The quantitative estimation of the enzyme was made as by Weil and Russel[11] and Fiske and Subbarow[12]. Optical density was read in Carl Zeiss VSU-2 spectrophotometer at 600 nm. Standard curve was plotted using different concentrations of KH_2PO_4.

Results. The positive fibre staining in all the 3 gastrocnemii at pH 8.9 confirms the presence of alkaline phosphatase in the normal muscle, and a distinct heterogeneity in the fibre population[13] is observed (figure 1). The narrow red fibres are richer in the enzyme concentration than the white type.

The gastrocnemius medius exhibits a continuous increase in the intensity of enzyme localization upto 4-week stage (figure 2). The fibre differentiation on the basis of alkaline phosphatase localization is however lost in all the 3 muscles towards the 8-week stage, and a homogeneous population of fibres with low but uniform enzyme activity is observed.

The denervated muscles lose fibre heterogeneity immediately following denervation (figure 3) and there is a predominant rise in the alkaline phosphatase content of almost all the muscle fibres. During 3–4-week periods following denervation, a large number of muscle fibres exhibit foci of degenerations. These degenerating areas are devoid of any enzyme reaction, though the major part of each muscle fibre exhibits dense localization of the enzyme (figure 4). A number of hypertrophied fibres showing central degenerations and conspicuous subsarcolemmal accumulation of enzyme activity are also well marked (figures 5 and 6). Another anatomical observation recorded during the post-denervation period, is a continuous increase in the extrafibrillar connective tissue accompanied by a gradual decline in the enzymic levels within this non-contractile element (figures 4–6).

The quantitative data obtained for alkaline phosphatase activity in the normal and denervated muscles is presented in figure 7 and the table. The specific activity of alkaline phosphatase in the normal muscles is maximum at 1-week

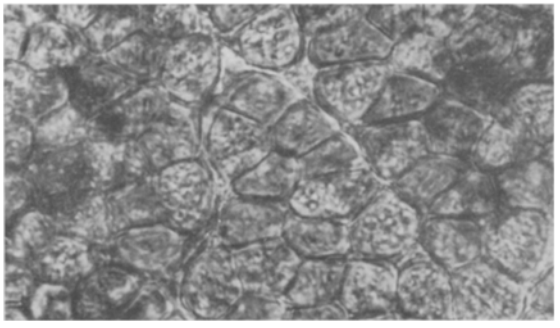

Fig. 1. Transverse section of gastrocnemius pars interna 1 week old showing a heterogenous fibre population and also a relatively higher concentration of enzyme in the narrow red fibres. × 400.

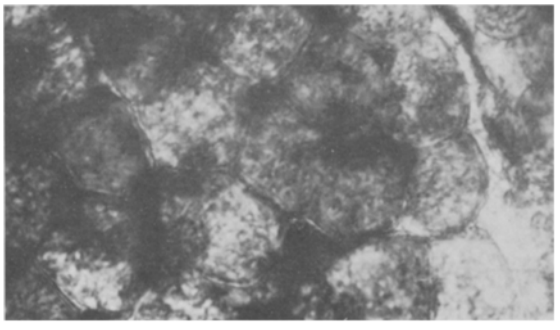

Fig. 2. Transverse section of pars media at 4-week stage revealing a high alkaline phosphatase content in all the muscle fibres. A poor fibre heterogeneity in some parts of the section is still maintained. × 525.

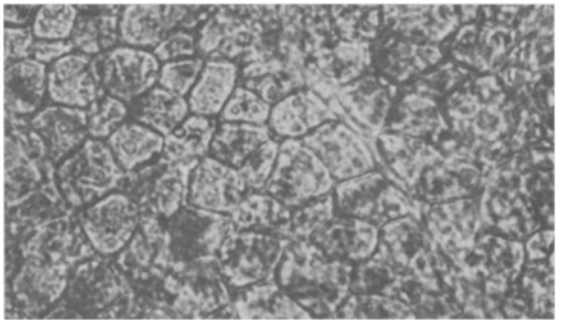

Fig. 3. Denervated pars externa at 3-week stage. Although the fibre heterogeneity is completely lost, the fibres continue to exhibit elevated enzyme content. × 430.

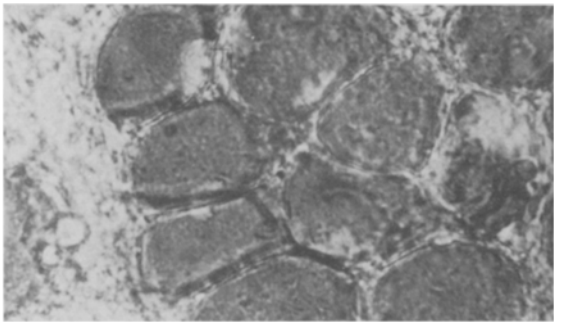

Fig. 4. Denervated pars interna at 3-week stage depicting enzyme-free foci of degeneration in most of the fibres and also an appreciably high enzyme concentration in rest of the intrafibrillar spaces. Also, note the increased noncontractile element in the extrafibrillar spaces. × 675.

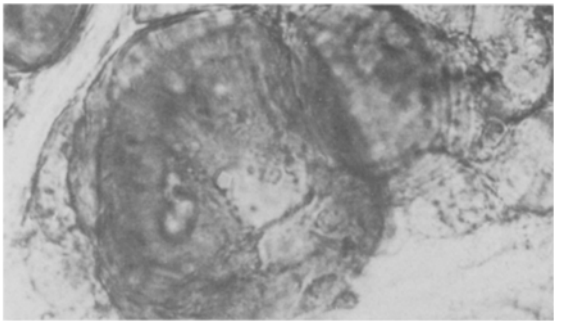

Fig. 5. Transverse section of pars externa 8 weeks following denervation. Section reveals a large hypertrophied fibre with a central enzyme and myofibrils-free area. Extrafibrillar spaces continue to exhibit higher noncontractile tissue with almost negligible enzymic concentration. × 1600.

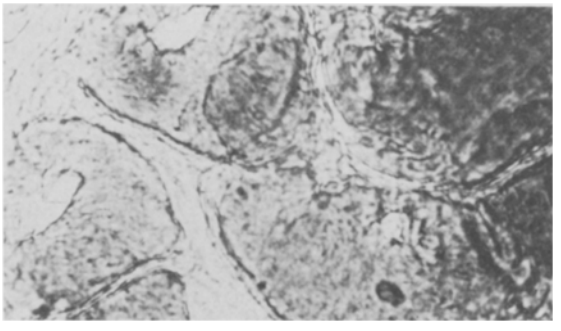

Fig. 6. Denervated pars interna at 8-week stage. The section shows an accumulation of enzyme in the subsarcolemmal regions while central foci of degeneration are devoid of any enzyme content. Mild phosphatase activity in the extrafibrillar regions is noticed. × 1000.

stage beyond which a continuous but gradual decline is witnessed, except for pars interna that shows this decline upto 5-week stage followed by a gradual rise. The denervated muscles exhibit relatively higher alkaline phosphatase activity at all the post-denervation periods but particularly on 1 and 4 weeks after denervation. While the variation in the enzyme content in normal and denervated muscle is maximum in pars media, the other 2 gastrocnemii demonstrate relatively little variation.

Discussion. The observations on the histochemical localization of alkaline phosphatase in the normal muscles establish the presence of a heterogenous population of fibres with respect to the distribution of this enzyme. In contrast to the low enzyme contents recorded in mammalian muscle[14], the strong enzyme localization in chick skeletal muscle points towards some specific role of alkaline phosphatase in the post-embryonic differentiation of muscle fibres[15]. The gradual decline in the intensity of enzyme localization and the loss of fibre heterogeneity towards 8-week stage, is in conformity with the quantitative data showing a gradual loss in the enzymic levels of muscles under investigation. The strong alkaline phosphatase reaction in the pars media at 4-week stage, however, does not correspond to the fall in enzyme content recorded biochemically at this stage.

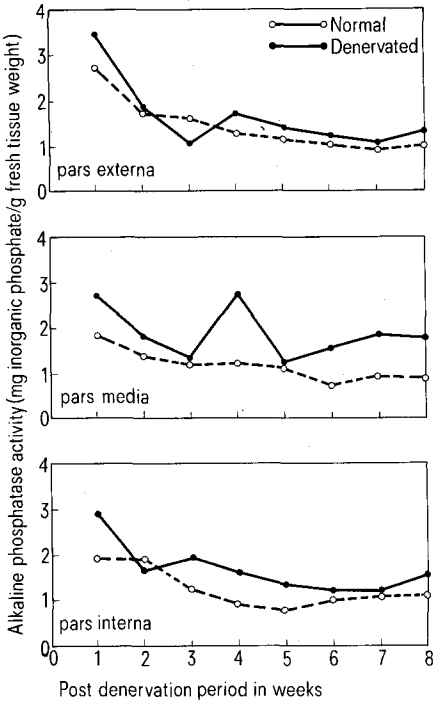

Fig. 7. Relative concentration of alkaline phosphatase in the 3 muscles at different stages of postnatal development. Whereas pars externa and media show elevated levels of enzyme on 1- and 4-week stages, a similar rise in pars interna is, however, witnessed at 1- and 3-week stages.

Both histochemical and biochemical observations reveal an increase in the enzymic levels as a result of denervation. The abolition of neural influence as a result of denervation, leads to the early loss of fibre heterogeneity with respect to alkaline phosphatase and as such, establishes the presence of trophic factor(s) which maintains the differentiating pattern in skeletal muscle[9,16,17]. It is therefore logical to believe that the trophic factor(s) tend to control and regulate the alkaline phosphatase levels in the normally growing muscle. Since the denervated muscles continue to maintain higher alkaline phosphatase levels than the normal muscles during the entire period of investigation, it can be surmised that the chick skeletal muscle lacks the intrinsic control[9,18] for the maintenance and regulation of alkaline phosphatase activity.

The present observations show the occurrence of lytic processes taking place in the fibres and immense proliferation of the noncontractile connective tissue as a result of muscle denervation. Since the foci of degenerations exhibit a total absence of the enzyme, it is implied that the progress of fibrolytic process is coupled with the gradual loss of alkaline phosphatase, resulting in the formation of enzyme-free and myofibrils-free areas within the muscle fibre. Similarily, proliferation of the extrafibrillar tissue is accompanied by a gradual loss of the enzyme in the interfibrillar regions. As such, there appears to be a definite relationship between the alkaline phosphatase levels, fibrolysis and the proliferation of the extrafibrillar tissue, as suggested by Kar and Pearson[6]. Since the bulk of evidence for the lytic processes available at present, is in favour of acid hydrolases[1,3,4], it is difficult to assign a specific role to alkaline phosphatase with respect to the above-mentioned pathological conditions in the denervated skeletal muscle. Further work on alkaline phosphatase activity in the normal and

Alkaline phosphatase activity in mg. inorganic phosphate per gram fresh tissue weight per 1 h incubation at 37 °C ± SD

Muscle	Postdenervation period in weeks 1	2	3	4	5	6	7	8
A) Normal								
M. gastrocnemius pars externa	2.726± 0.383	1.738± 0.521	1.644± 0.218	1.298± 0.428	1.163± 0.328	1.042± 0.314	0.937± 0.128	1.010± 0.296
M. gastrocnemius pars media	1.892± 0.481	1.444± 0.463	1.240± 0.254	1.219± 0.303	1.132± 0.301	0.775± 0.148	0.963± 0.223	0.882± 0.248
M. gastrocnemius pars interna	1.997± 0.463	1.963± 0.628	1.254± 0.402	0.997± 0.414	0.852± 0.298	1.022± 0.248	1.151± 0.328	1.132± 0.328
B) Denervated								
M. gastrocnemius pars externa	3.495± 0.728	1.877± 0.618	1.002± 0.278	1.756± 0.487	1.457± 0.498	1.225± 0.386	1.036± 0.336	1.365± 0.428
M. gastrocnemius pars media	2.767± 0.683	1.872± 0.598	1.365± 0.321	2.772± 0.663	1.268± 0.398	1.553± 0.423	1.844± 0.558	1.754± 0.384
M. gastrocnemius pars interna	2.950± 0.498	1.699± 0.513	1.989± 0.419	1.667± 0.453	1.367± 0.528	1.268± 0.448	1.162± 0.506	1.573± 0.478

diseased muscle is envisaged before its mode of action is fully understood.

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Effect of amputation and limb regeneration on the pars distalis of the newt, *Notophthalmus viridescens*[1]

D. Louise Barber and S.R. Scadding

Department of Zoology, University of Guelph, Guelph (Ontario, Canada N1G 2W1), 6 March 1978

Summary. A study of the pituitary of the newt, *Notophthalmus viridescens*, during limb regeneration indicated no observable changes in histology or ultrastructure of any of the cells of the pars distalis when compared with the pars distalis of unamputated control newts.

That the pituitary gland is essential for limb regeneration in the adult newt, has been confirmed many times since the initial observation by Schotté[2]. Subsequently, workers have suggested that specific pituitary hormones are the necessary factors which are required for limb regeneration. ACTH[3], growth hormone[4,5], and prolactin[6], have all been proposed as the specific pituitary hormone that is necessary to support limb regeneration. Other investigators[7,8], have claimed that there is no specific requirement for any one hormone but that the pituitary is necessary to provide the appropriate hormonal milieu in which regeneration could occur. It has been demonstrated that in some cases where there is a large increase in requirements for a specific hormone, that there are immediate cytologically detectable changes in the pituitary. Shiino and Rennels[9] have demonstrated an increase in exocytosis of secretion granules in growth hormone cells of the rat pituitary following partial hepatectomy. DeVolcanes and Weatherhead[10] have shown degranulation of pars intermedia cells 8 h following transfer of *Xenopus laevis* from a white to a black background. If, then, there is a sudden release of a pituitary hormone during salamander limb regeneration, as some authors have suggested, then we might expect to see observable changes in pituitary cells. Consequently, this study was undertaken to see if there were any histologically or ultrastructurally observable changes in the pituitary of the newt following limb amputation.

Methods. Limbs of 37 newts, *Notophthalmus viridescens* were amputated and fixed at intervals from day 1 to day 28 following amputation of the limb and compared to control unamputated animals. This covers the period in which the pituitary is reported to be essential for limb regeneration[11]. For light microscopy, the brain with attached pituitary was fixed in 10% buffered formalin, embedded in methacrylate plastic, sectioned sagittally at 2 µm, and stained with Herlant's polychrome stains[12]. For electron microscopy, pituitaries were fixed in 3% glutaraldehyde followed by 1% osmium tetroxide, embedded in Epon, and stained with uranyl acetate and lead citrate.

Results and discussion. Methacrylate plastic embedding proved to be superior to paraffin embedding for observation of pituitary cells, since the thinner sections allowed better visualization of cytoplasmic granules. The 2 acidophils and 2 basophils previously reported for newt pituitary could be recognized, and had the same distribution as that previously reported[13–15]. The cell types, numbers, locations within the pars distalis, and ultrastructure were compared in amputated and unamputated control newts, and the observations were the same. No differences could be detected in the pituitary between regenerating and control newts. No evidence could be found to support hypersecretion of any particular hormone. Our results support the conclusion of Vethemany-Globus and Liversage[8], that the pituitary does not provide a specific factor for regeneration, but that the pituitary is necessary because it contributes to the environmental mileau that supports growth and differentiation of the blastema cells of the regenerating limb.

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