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DEVELOPMENTAL PATTERNS OF GLYCOLYTIC ENZYMES IN REGENERATING SKELETAL MUSCLE AFTER AUTOGENOUS FREE GRAFTING*

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SUMMARY

Extensor digitorum longus muscles of rats were removed and injected with a solution of Marcaine plus hyaluronidase After incubation in Marcaine solution for 10 min, the muscles were grafted into their original beds The grafts and the contralateral control muscles were removed from the rats at 0, 1–5, 7, 11, 36, and 69 days postoperatively The muscles were then frozen in dry ice and isopentane and subsequently homogenized and centrifuged The supernatant was analyzed for a number of enzymes, the regenerative patterns of which can be classified into 3 groups (1) early increase in activity hexokinase, glucose-6-phosphate dehydrogenase, (2) early decrease in activity with failure to recover to control levels phosphorylase, phosphofructokinase, α-glycerophosphate dehydrogenase, and (3) early decrease followed by return to control levels lactate dehydrogenase, pyruvate kinase, creatine phosphokinase, adenylate kinase These patterns are not identical to those reported for embryogenesis of muscle The data are discussed with regard to correlative histological studies of muscle regeneration

INTRODUCTION

The regeneration of mammalian skeletal muscle follows a characteristic sequence of events After injury, the sarcoplasm of the muscle fiber begins to degenerate The principal changes are swelling and internal alterations of mitochondria, distortion of myofilamentous organization, particularly in the area of the Z lines, and the appear-

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ance of abnormal membranous structures (Reznik 1973, Neerunjun and Dubowitz 1974a, Mastaglia, Dawkins and Papadimitriou 1975) Regeneration is heralded by activation of nuclei beneath the basement membrane of the muscle fiber, as indicated in autoradiographic studies by nuclear incorporation of labelled thymidine and uridine (Reznik 1968, Carlson 1972, Snow 1977a,b) Before these activated myoblastic nuclei develop further, sarcolysis of the original muscle fibers must occur In mammalian muscle sarcolysis is accomplished primarily by the activities of phagocytic cells, the majority of which appear to be derived from the blood The myoblastic cells then undergo a characteristic sequence of steps in cytodifferentiation, first into myotubes and later into cross-striated muscle fibers After neuromuscular contacts are established, the initially homogeneous population of regenerating muscle fibers undergoes a final differentiation into distinct groups, which have contractile and histochemical properties related to their innervation

The timing, and to some extent, the sequence of early events in the degenerative-regenerative process depends upon the degree to which the blood supply to the muscle is affected by the initial traumatic event In some experimental models the blood supply around the damaged muscle fibers is relatively little affected, and degeneration and early regeneration of muscle are rapid In other models the damaged muscle is initially divorced from a direct blood supply, often for several days This prolongs the degenerative phase and delays the onset of regeneration in the damaged muscle because the sarcolytic phase does not occur until a local blood supply is re-established

Biochemical studies on early phases of muscle regeneration have been hampered by lack of homogeneity of the material to be analyzed In some experimental models, damaged and undamaged muscle fibers cannot be readily separated In other models large areas of degenerating original muscle fibers are found alongside regions containing a highly asynchronous population of regenerating muscle fibers, with several different developmental stages represented simultaneously (Carlson 1972)

Recently, Marcaine, a highly myotoxic local anesthetic agent (Benoit and Belt 1970, Jirmanova and Thesleff 1972), has proven to be of considerable use in the analysis of muscle regeneration (Max and Rifenberick 1975, Max and Albuquerque 1975, Hall-Craggs and Singh-Seyan 1975) Employing a series of intramuscular injections of Marcaine plus hyaluronidase extending over 3 days, Hall-Craggs (1974) obtained essentially complete degeneration and regeneration of the tibialis anterior muscle in rats, although similar treatment of the rat extensor digitorum longus muscle allowed survival of significant numbers of muscle fibers (Carlson, unpublished observations) The combination of Marcaine treatment plus free grafting of the extensor digitorum longus muscle in the rat produces a model in which virtually all the original muscle fibers are destroyed and replaced by a largely homogeneous population of regenerating muscle fibers with a minimal increase in the formation of new connective tissue (Carlson 1976) This model seems ideally suited for biochemical studies of skeletal muscle regeneration

In the present study, we examined two major questions concerning muscle regeneration The first is whether regenerating muscle is identical to embryonic muscle with regard to biochemical aspects of differentiation, the second concerns the source

of metabolic energy which permits survival of degenerating fibers and supports subsequent regeneration Previous work from this laboratory, employing the model of Marcaine-induced muscle regeneration described by Hall-Craggs (1974), suggests that glycolysis, rather than oxidative metabolism, is a likely candidate for this role (Rifenberick, Koski and Max 1975, Wagner, Max, Grollman and Koski 1976) Thus, we studied developmental patterns of a number of enzymes involved in glucose metabolism and compared our results with published enzyme patterns of muscle developing in vivo and in vitro Among the enzymes studied are the regulatory enzymes phosphorylase, hexokinase, phosphofructokinase, pyruvate kinase, and glucose-6-phosphate dehydrogenase Lactate dehydrogenase and α-glycerophosphate dehydrogenase were included because of their respective roles in anaerobic metabolism of muscle and in oxidation of extramitochondrial NADH Creatine kinase and adenylate kinase were measured because of their well-known functions in the energy metabolism of muscle, and because they have been extensively studied in developing systems These data have been presented in preliminary form (Wagner, Carlson and Max 1976)

MATERIALS AND METHODS

This study was carried out on 66 male Sprague–Dawley rats (175–200 g) obtained from Spartan Farms, Haslett, Michigan Other materials and their sources were NADH, $NADP^+$, ATP, phosphoenolpyruvate, 5′-AMP, Tris–HCl, glycogen, fructose-6-phosphate, mercaptoethanol, aldolase, α-glycerophosphate dehydrogenase, triosephosphate isomerase, dihydroxyacetone phosphate, dithiothreitol, glucose-6-phosphate dehydrogenase, hexokinase, glucose-1-phosphate, glucose-6-phosphate, lactate dehydrogenase, phosphocreatine, histidine, hyaluronidase (Sigma), glucose, $MgCl_2$, KCl, KF, toluene (Fisher Scientific), [U-^{14}C]glucose-1-phosphate (Amersham Searle), PPO, POPOP (New England Nuclear), Tenbroeck tissue homogenizers (Kontes Glass Company), Marcaine (Bupivacaine) (Winthrop Laboratories)

Treatment of muscles

During all surgical procedures, rats were anesthetized with ether Immediately upon removal, the right extensor digitorum longus muscle was injected with as much Marcaine solution (0 75% Marcaine in 0 9% NaCl with 300 units of hyaluronidase per ml) as it could hold (usually 100–200 μl) The muscle was then soaked in Marcaine solution for 10 min to ensure exposure of peripheral muscle fibers to the damaging effects of the anesthetic agent The muscle was immediately grafted back into its own bed and sutured to its proximal and distal tendons No attempt was made to re-establish nervous or vascular continuity between the graft and the host In each animal the left extensor digitorum longus muscle was untouched and served as a normal control At selected post-operative intervals (0, 1–5, 7, 11, 36 and 69 days), both the graft and its contralateral intact counterpart were removed from each of 6–7 rats The muscles were weighed and immediately immersed in a mixture of dry ice and isopentane They were kept continuously frozen on dry ice during the 2-day shipping period from

Michigan to Maryland Immediately upon arrival, muscles were minced with scissors in ice-cold 0 05 M Tris–HCl, pH 7 6, containing 0 20 mM dithiothreitol (DTT) The finely minced muscles were then homogenized (1 10, w/v) by hand, using a Tenbroeck homogenizer The homogenate was centrifuged at 18,000 × g for 20 min at 4 °C Enzymes were assayed in the resulting supernatants

Histological analysis

Grafted muscles from a parallel series of rats were examined at each of the times when muscles were removed for biochemical analysis These grafts were fixed in Bouin's solution for histological study, and were then cut at 7 μm and stained in Ehrlich's hematoxylin and eosin

Enzyme assays

The following enzymes were assayed at 25 °C in a reaction volume of 1 0 ml by monitoring the change in NADH or NADPH absorbance at 340 nm using a Beckman model 25 spectrophotometer hexokinase (2 7 1 1) (Uyeda and Racker 1965), phosphofructokinase (2 7 1 1) (Uyeda and Racker 1965, Paetkau and Lardy 1967), cytoplasmic α-glycerophosphate dehydrogenase (1 1 1 8) (Holloszy and Oscai 1969), pyruvate kinase (2 7 1 40) (Shonk and Boxer 1964) Lactate dehydrogenase (1 1 1 27) was assayed in the direction of pyruvate synthesis, employing 190 mM hydrazine sulfate buffer, pH 9 7, 4 0 mM NAD, and 7 5 mM lactate Adenylate kinase (2 7 4 3) and creatine kinase (2 7 3 2) were assayed in a medium containing 25 mM Tris–HCl, pH 8 1, 1 0 mM $MgCl_2$, 0 5 mM DTT, 0 5 mM NADP, 1 0 mM glucose, 0 5 mM ADP, 1 0 unit of hexokinase, and 1 0 unit of glucose-6-phosphate dehydrogenase To measure creatine kinase activity 5 0 mM 5′-AMP was added to inhibit adenylate kinase (Smith 1972) after which 10 0 mM phosphocreatine was added Glucose-6-phosphate dehydrogenase (1 1 1 49) was assayed in a medium containing 50 mM Tris–HCl, pH 8 1, 3 0 mM glucose-6-phosphate and 0 5 mM NADP Total glycogen phosphorylase (2 4 1 1) was determined in the direction of glycogen synthesis in a

TABLE 1

ENZYME ACTIVITIES OF CONTROL MUSCLES

Enzymes were assayed as described in the text

Enzyme	Specific activity (nmol/min/mg protein)
Phosphorylase	973 6 ± 67 1
Hexokinase	13 3 ± 0 9
Phosphofructokinase	688 4 ± 40 8
Pyruvate kinase	3584 9 ± 267 6
Lactate dehydrogenase	2385 1 ± 196 3
Glucose-6-phosphate dehydrogenase	2 2 ± 0 2
α-Glycerophosphate dehydrogenase	641 5 ± 59 5
Adenylate kinase	5724 1 ± 442 9
Creatine kinase	4291 1 ± 106 1

reaction medium containing 1 0% (w/v) glycogen and 10 0 mM [U-^{14}C]glucose-1-phosphate as substrates plus 2 0 mM 5′-AMP and 100 mM KF The reaction was started with the addition of supernatant and stopped by spotting 80 μl of the reaction mixture on Whatman 31 ET filter paper and carrying the papers through the ethanol washing procedure of Thomas, Schlender and Larner (1968) (B I Brown, personal communication) The filter papers were then placed in counting vials containing liquifluor and counted in a Beckman LS 235 liquid scintillation counter Protein was determined by the method of Lowry, Rosebrough, Farr and Randall (1951)

Expression of data

Under our assay conditions, enzyme activities were linear with respect to time and protein concentration Enzyme specific activities were calculated as nmol/min/mg protein and then expressed as average percentage of contralateral control ± S E M Control values are given in Table 1

RESULTS

The wet weights of muscle grafts provided a good indication of both the course of regenerative activity within the graft and the overall physiological environment of which the graft is a part (Fig 1) During the first day or two after grafting, the muscle transplant and the surrounding tissues were markedly edematous Although degenerative changes were obvious in the muscle fibers throughout the graft, sarcolysis was minimal except in a few muscle fibers at the extreme periphery (Fig 2) The com-

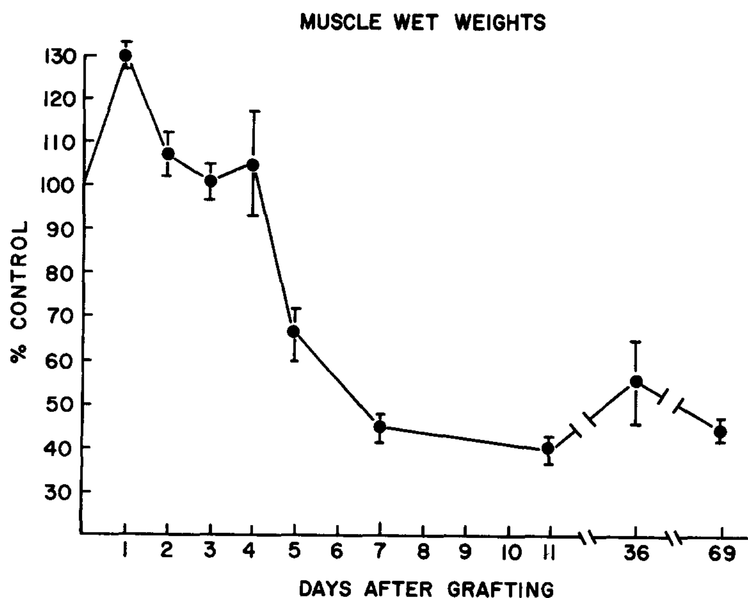


Fig 1 Fresh weights of rat extensor digitorum longus muscles after Marcaine treatment and orthotopic free grafting Values are means ± S E M Numbers in parentheses are numbers of rats Experimental procedures are described in the text

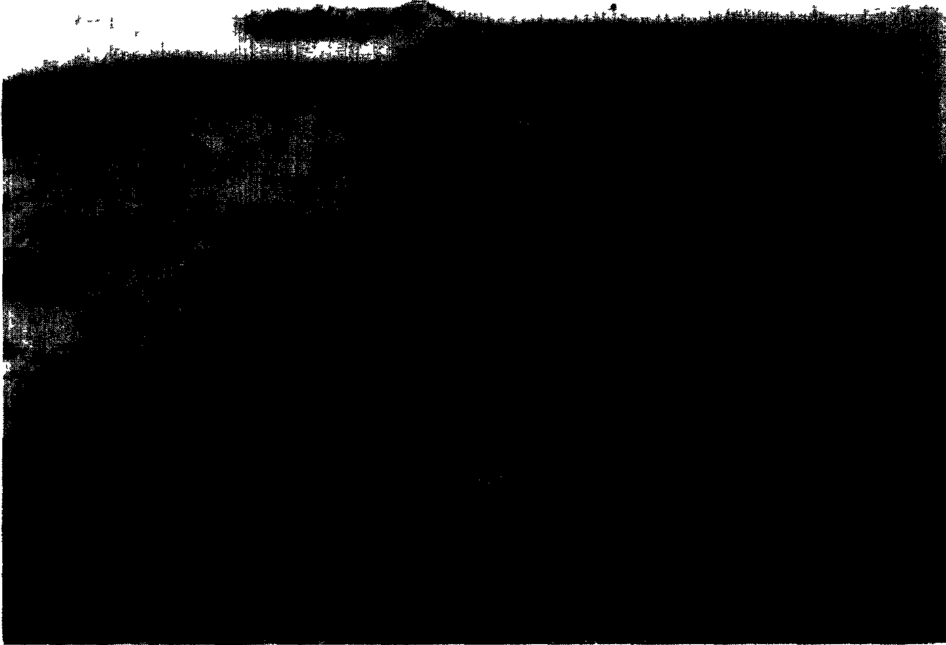


Fig 2 Cross-section through a one-day graft of Marcaine-treated muscle Fragmentation has begun in some of the peripheral muscle fibers H and E, 100

bination of acute postoperative edema plus lack of fragmentation of most of the degenerating fibers within the graft caused the grafts to be heavier than normal contralateral muscles during the early postoperative days (Fig 1)

On the first day after grafting, the muscle was completely divorced from a blood supply and contained a homogeneous population of ischemic mature muscle fibers During the second and third postoperative days the degenerating muscle fibers at the periphery of the graft underwent fragmentation Individual myoblastic cells became activated beneath the basement membranes of the degenerated original muscle fibers The ischemic muscle fibers in the central part of the graft (approximately 80% of the total number) had not yet undergone fragmentation

By the fourth day a well-defined centripetal gradient of regeneration and degeneration had been set up Surviving muscle fibers rarely persisted (mean of 2 2 surviving fibers per graft, Carlson 1976), at the extreme periphery of the grafts Early myotubes were found at the periphery of the graft Toward the center myoblasts lined the basement membranes remaining after degeneration of the muscle fibers In some 4-day grafts a small mass of ischemic, but intact, muscle fibers persisted in the center In other 4-day grafts all of the original muscle fibers had undergone sarcolysis Five-day grafts were in the peak of the myotube stage (Fig 3) However, because of the radial gradient of differentiation, early cross striations could be seen in some of the most peripheral regenerating muscle fibers whereas in the central regions late myoblasts or early myotubes still persisted

Because of the decline in the edema reaction and the complete sarcolysis of the

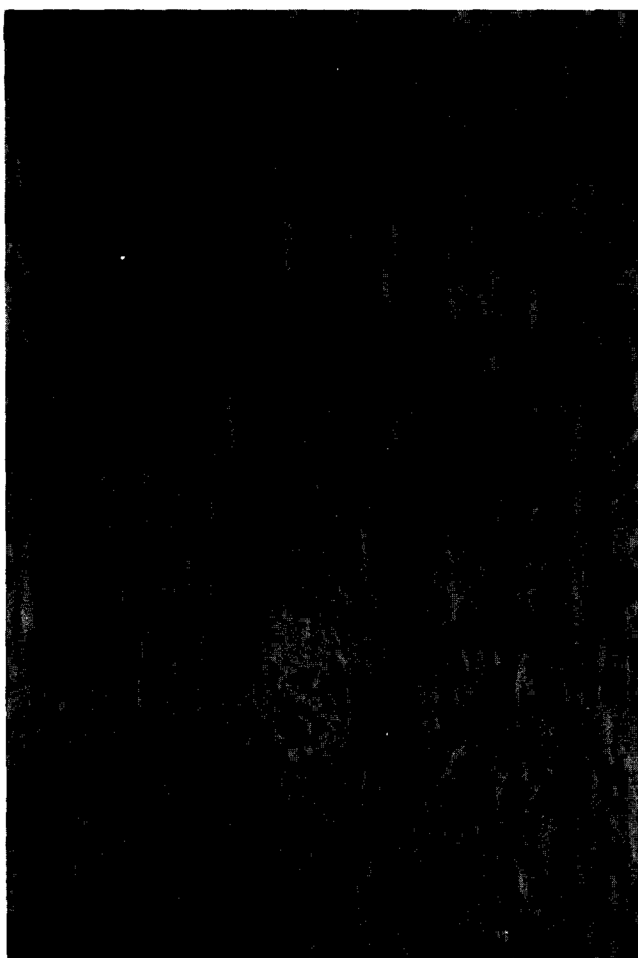


Fig 3 Longitudinal section through a 5-day muscle graft The regenerating muscle fibers are in the myotube stage A cross-section through the muscle graft revealed 3 surviving original muscle fibers H and E, × 100

original muscle fibers, the weights of the grafts declined precipitously until they were commonly about 40% of the contralateral normal muscles at the end of the first week (Fig 1) By 7–8 days the grafts were composed of a homogeneous population of young muscle fibers (Fig 4) capable of contracting (Carlson and Gutmann 1976)

As the regenerating muscle fibers matured, their cross sectional areas increased progressively (Mong 1976), and the relative weights of the grafts rose correspondingly Mature grafts (Fig 5) became stable at about 50% of the weight of control muscles (Fig 1)

Glucose-6-phosphate dehydrogenase activity began to increase on the first day after grafting, attained a maximum of 7 times the control activity by 8 days, and then gradually decreased (Fig 6a) Hexokinase activity decreased to 26% of control on the first day after grafting and subsequently increased to about 1 5 times control by

Fig 4 Cross-section through an 8-day muscle graft All of the muscle fibers in this section are re-generating and are undergoing transformation from myotubes to early cross-striated muscle fibers H and E, × 100

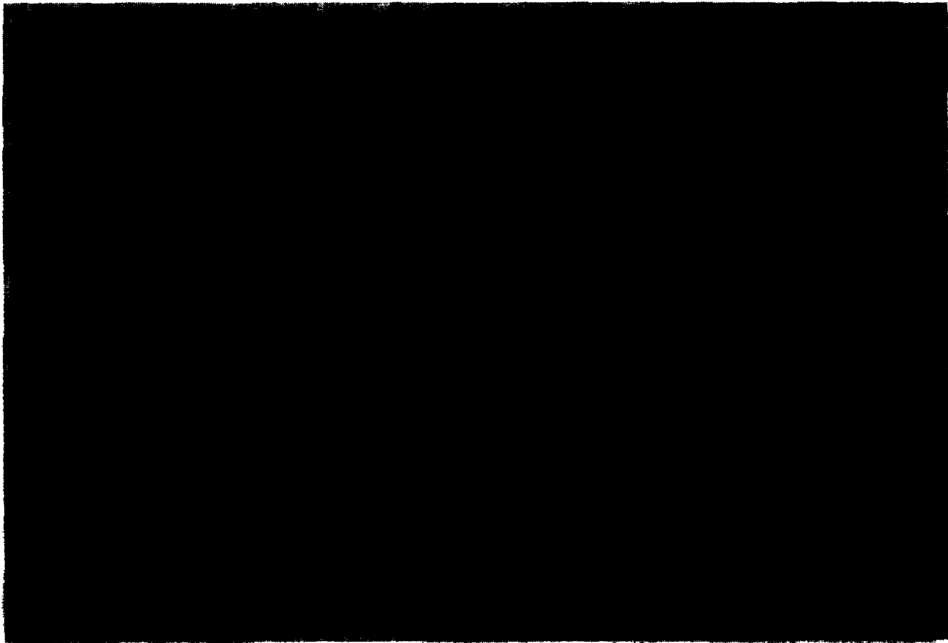
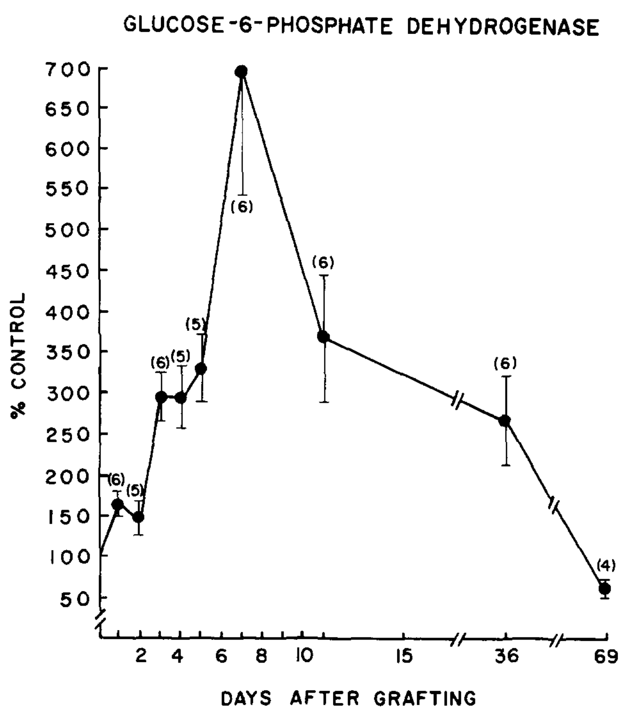
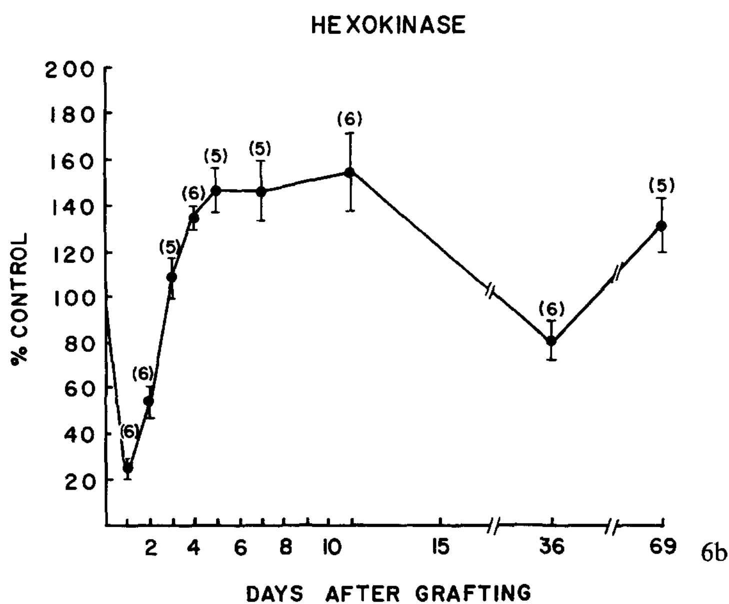


Fig 5 Cross-section through a 32-day muscle graft The muscle fibers are generally mature in appearance, but as is typical in mature regenerated muscle fibers, occasional central nuclei persist H and E, × 100

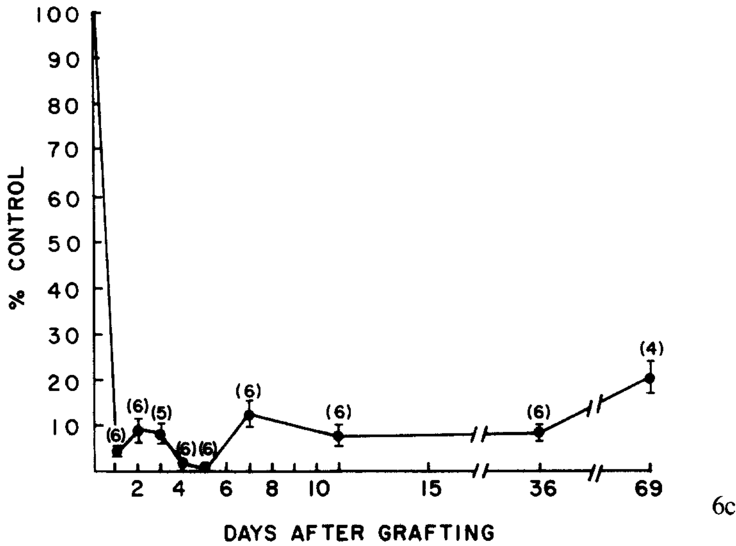


6a



6b

day 6 (Fig 6b) In contrast, phosphorylase decreased dramatically to about 5% of control on day 1 and reached only about 20% of control by day 69 (Fig 6c) Phosphofructokinase also decreased to about 5% of control on day 1, began to increase on day 11, and reached about 65% of control on day 69 (Fig. 6d) α-Glycerophosphate dehydrogenase followed a pattern similar to that of phosphofructokinase (Fig 6e),



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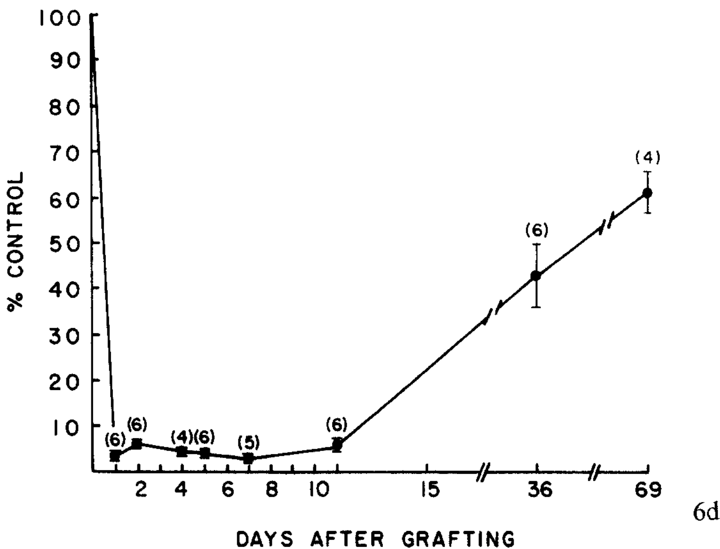
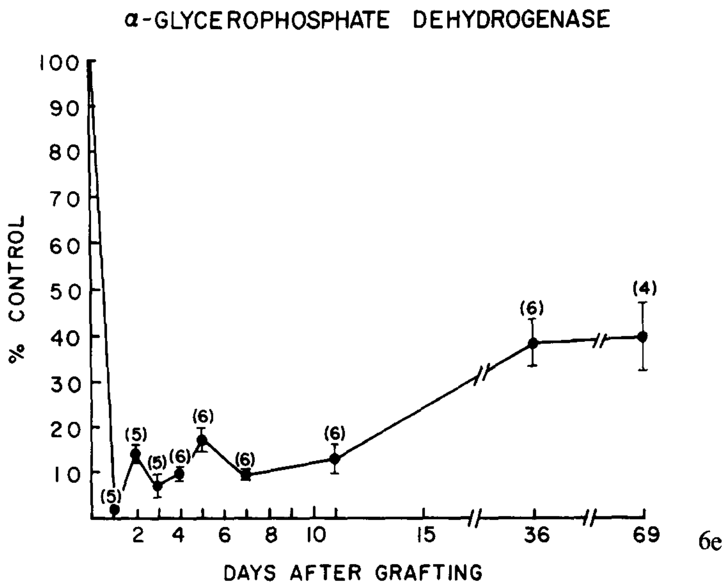


6d

in that its activity decreased strikingly on day 1 and began to rise on day 11, reaching about 40 % of control by day 69 Lactate dehydrogenase decreased to 12 % of control by day 1, began to increase after day 2, and attained about 65 % of control by day 69 (Fig 6f) Similarly, pyruvate kinase dropped to 5 % of control by day 1, began to rise immediately thereafter, and was 75 % of control on day 69 (Fig. 6g) Creatine kinase (Fig 6h) and adenylate kinase (Fig 6i) displayed similar patterns Their activities decreased to about 8 % of control on day 1, began to increase on day 4, reaching values of 130 % and 80 % of control, respectively, by day 69



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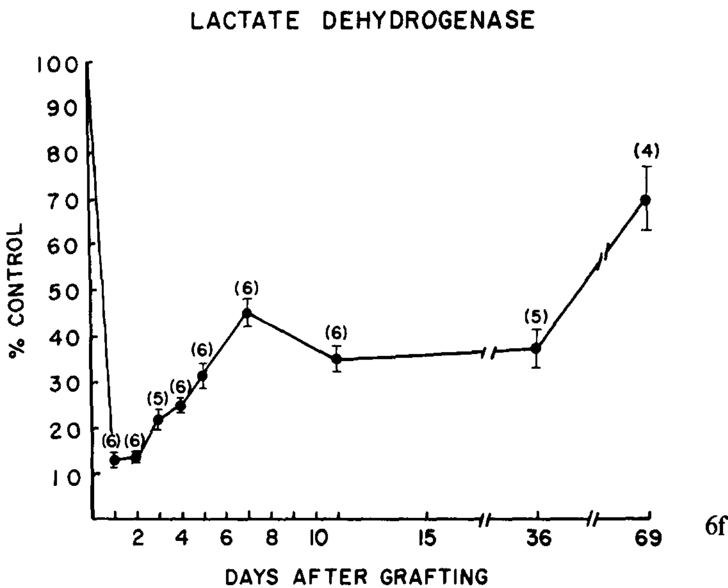
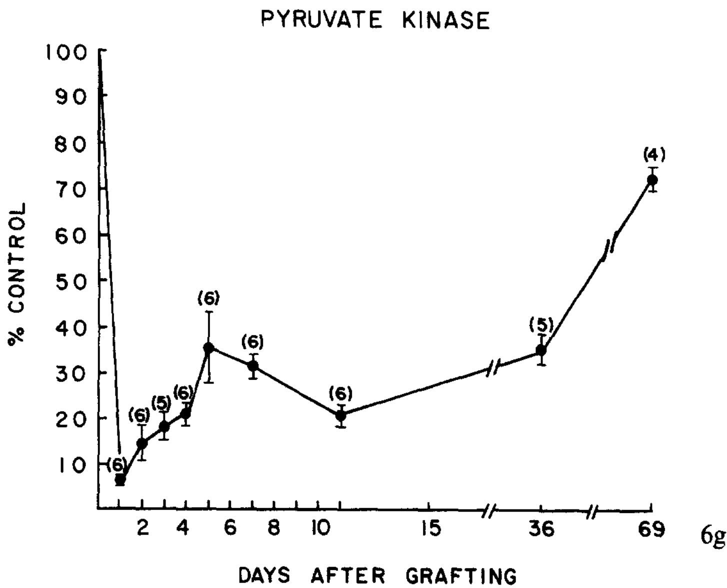


6f

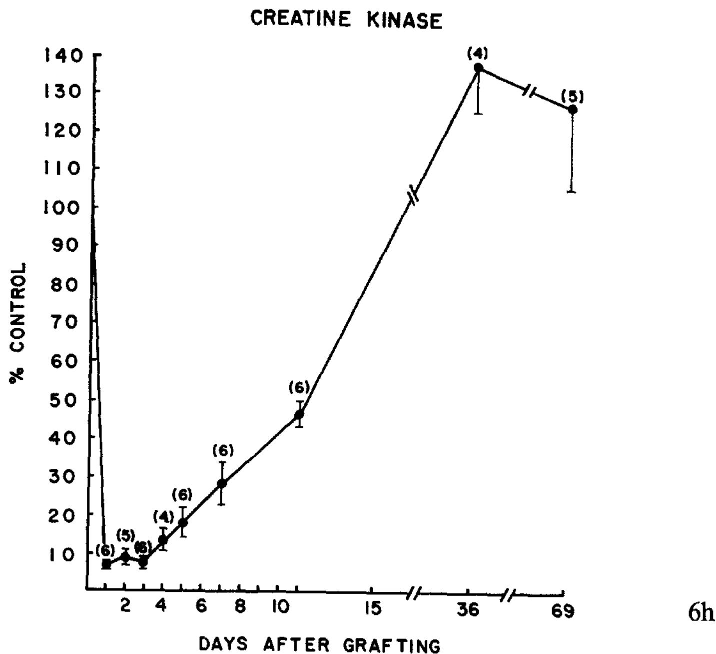
These enzyme activities can be classified into 3 groups with respect to their regenerative patterns (1) initial increase in activity hexokinase, glucose-6-phosphate dehydrogenase, (2) initial reduction with failure to recover to control values phosphorylase, phosphofructokinase, α-glycerophosphate dehydrogenase, and (3) initial decrease followed by approximate return to control levels pyruvate kinase, lactate dehydrogenase, creatine kinase and adenylate kinase

DISCUSSION

Marcaine-treated free muscle grafts are proving to be useful models for the study



6g



6h

of the physiological and biochemical properties of regenerating skeletal muscles With this model it has been possible to monitor the development of contractile properties of regenerating muscle fibers without the interference of contractile input of surviving muscle fibers (Carlson and Gutmann 1976b). In this study we employed the same model to examine the developmental patterns of glycolytic enzyme activities in regenerating muscle

There is reasonable correlation between the biochemical and morphological

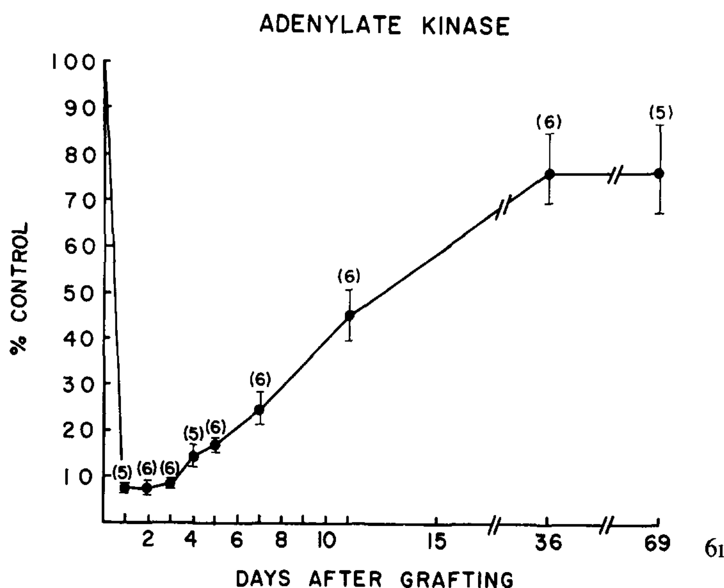


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Fig 6 Enzyme activities in rat extensor digitorum longus muscles after Marcaine treatment and orthotopic free grafting (a) glucose-6-phosphate dehydrogenase, (b) hexokinase, (c) phosphorylase, (d) phosphofructokinase, (e) α-glycerophosphate dehydrogenase, (f) lactate dehydrogenase, (g) pyruvate kinase, (h) creatine phosphokinase, (i) adenylate kinase Numbers in parentheses are numbers of rats Experimental procedures are described in the text

observations on muscle grafts Thus, on day 2, a large central zone of the graft is ischemic, while the presence of myoblastic cells between this area and the periphery is noteworthy (Fig 2, cf Carlson 1976) The dramatic decrease in most enzyme activities presumably reflects increased protein catabolism in ischemic tissue undergoing degeneration Loss of glycolytic enzymes was also observed in ischemic skeletal muscle (Archangeli, Digiesi, Masala, Serra and Congiu 1973)

The enhanced activities of glucose-6-phosphate dehydrogenase and hexokinase are associated with elevated activity of the pentose phosphate pathway (Figs 6a and b, cf Beaconsfield and Carpi 1964, Rifenberick, Koski and Max 1974, Wagner, Kauffman and Max 1977), and probably reflect accelerated glucose utilization for the production of nucleic acids (Beaconsfield and Reading 1964) and lipids in proliferating myoblastic cells In this regard, increased RNA content has been noted in a number of studies on muscle regeneration (Gallucci, Novello, Margreth and Aloisi 1966, Susheela, Hudgson and Walton 1969, Carlson 1970, Neerunjun and Dubowitz 1974b) Furthermore, Warshaw, Barrett and Coyne (1976) have provided evidence for a relationship between pentose phosphate pathway activity and growth in chick heart muscle cells

The accelerated activities of lactate dehydrogenase (Fig 6f), pyruvate kinase (Fig 6g), creatine kinase (Fig 6h) and adenylate kinase (Fig 6i) beginning at the fourth or fifth day may be a manifestation of the increased amount of regeneration noted during this period (Fig 3) Creatine kinase and adenylate kinase activities increase in association with the fusion of myoblasts in culture (Shainberg, Yagil and Yaffe 1971,

Morris and Cole 1972) Furthermore, creatine kinase accumulates rapidly after the 17th day of gestation in rat muscles (Ziter 1974)

By day 8 after grafting there are no surviving original muscle fibers and the regenerative response appears maximal (Fig 4) It is at this time that glucose-6-phosphate dehydrogenase is at its highest activity (Fig 6a) and basophilia is most prominent (Carlson 1976) The later increases in phosphofructokinase (Fig 6d) and α-glycerophosphate dehydrogenase (Fig 6e) presumably reflect maturation of regenerating muscle fibers (Fig 5)

The failure of phosphorylase (Fig 6c) to show significant recovery within the 69-day period of these experiments is interesting In a previous histochemical study of free grafts, phosphorylase appeared to be the most severely affected enzyme, although it made some recovery (Carlson and Gutmann 1975) Phosphorylase was the first enzyme to decrease after physical injury to muscle (Smith 1965) and was decreased within 24 hr after mincing and transplantation of muscle in hamsters (Neerunjun and Dubowitz, 1974b) A substantial decrease of phosphorylase was also noted following 3 injections of Marcaine plus hyaluronidase (Wagner, Max, Grollman and Koski 1976) Phosphorylase was not histochemically detectable in regenerating muscle until the fibers became histologically normal (Smith 1965) In the present study, 69 days after grafting (Fig 4) many regenerated fibers still had central nuclei, indicating that regeneration was not complete

One possible source of contamination of enzymatic activities is infiltration of the muscle by phagocytic cells However, in a recent study, glucose-6-phosphate dehydrogenase was shown histochemically to be localized primarily in skeletal muscle fibers rather than in interstitial cells following intramuscular administration of Marcaine (Wagner, Kauffman and Max 1977) Similar histochemical localization of glucose-6-phosphate dehydrogenase in regenerating muscle fibers has been observed by Snow (1973) and by Smith (1965) Thus it appears unlikely that cellular infiltration significantly influenced the enzymatic activities in muscle supernatants

Enzymatic changes associated with development of muscle both in vivo and in tissue culture have been described in a number of species Comparison of our data with these results has revealed interesting similarities and differences For example, the most striking early change observed in our study was increased activity of glucose-6-phosphate dehydrogenase and hexokinase (Fig 6a and b) Rat skeletal muscle glucose-6-phosphate dehydrogenase (Hommes and Wilmink 1968) and rabbit skeletal muscle hexokinase (Stave 1964) increased perinatally, while glucose-6-phosphate dehydrogenase activity increased about 3-fold, and hexokinase activity was unchanged in tissue culture studies of chick embryonic muscle (Schudt, Gaertner, Dolkin and Pette 1975) Similarly, hexokinase activity was unchanged during in vivo development of chick skeletal muscle (Hauschka 1968) It is likely, however, that hexokinase had already attained optimal activity in these latter two studies

Although creatine kinase and adenylate kinase (Fig 6h, i) increased more rapidly than lactate dehydrogenase and pyruvate kinase (Fig 6f and g) in regenerating muscle, in developing rabbit muscle these enzymes increased in parallel (Stave 1964) In the present study, phosphofructokinase activity increased during the second week

of regeneration (Fig 6d) In contrast, in developing rat (Hommes and Wilmink 1968) and chick (Hauschka 1968) skeletal muscle, little change in phosphofructokinase activity occurred from pre- to postnatal development On the other hand, a very early rise in activity occurred in tissue culture (Schudt, Gaertner, Dolkin and Pette 1975), as was the case in fetal muscle of the rhesus monkey in which activity was 3–5 times greater than the adult (Beatty, Young and Bocek 1976) The finding by Gallucci, Novello, Margreth and Aloisi (1968) that phosphofructokinase activity was higher in 15–21 day minced muscle implants than in 2–3 week old neonatal muscle is in contrast to our results and remains unexplained

Phosphorylase increased rapidly in chicks after hatching (Hauschka 1968, Bass, Lusch and Pette 1970), before birth in primates (Bocek, Basinger and Beatty 1969), and in developing chick and rat embryonic muscle in tissue culture (Shainberg, Yagil and Yaffe 1972, Schudt, Gaertner, Dolken and Pette 1975), but recovered poorly in the present experiments (Fig 6c) In support of our results are those of Gallucci, Novello, Margreth and Aloisi (1966) who found less total phosphorylase and glycogen synthetase in regenerating skeletal muscle than neonatal muscle

These observations suggest that regenerating muscle does not exactly recapitulate the ontogenetic pattern, although there are striking similarities between embryonic and regenerating muscle in morphological characteristics (Studitsky and Striganova 1951, Hudgson and Field 1972), in the development of contractile properties (Carlson and Gutmann 1976b), in neuromuscular relations (Mong 1975, Carlson and Gutmann 1976a), and in the establishment of histochemical fiber types (Carlson and Gutmann 1975, Mong 1975) A similar biochemical picture is seen in liver regeneration where an enzymatic profile unique to regeneration was considered to confer biological advantages upon both the regenerating cell and the host (Weber 1975) It is possible that the observed biochemical departures from the normal ontogenetic pattern represent adaptations to a harsher metabolic environment than that found in the normally developing limb This is particularly true with respect to the blood supply, which is present in abundance in and around embryonic muscles from the time their individual anlagen first become discrete entities There is no direct blood supply to the myoblastic cells during their period of activation in free muscle grafts and in minced preparations

An unanswered question concerns sources of metabolic energy which permit muscle to survive the early period of degeneration and support subsequent regeneration (Gallucci, Novello, Margreth and Aloisi 1966, Snow 1973) In a previous report we showed that glycolysis survives Marcaine plus hyaluronidase treatment (without orthotopic free grafting) and is probably sufficient to support the early stages of regeneration in the face of obliterated oxidative metabolism (Rifenberick, Koski and Max 1974, Wagner, Max, Grollman and Koski 1976) The combination of Marcaine plus free grafting is apparently so devastating that most glycolytic enzyme activities are markedly reduced In the early stages of Marcaine-induced muscle regeneration, the contraction times are very slow (Carlson and Gutmann 1976b), a disarray of contractile apparatus predominates (E C B Hall-Craggs, personal communication) and actomyosin ATPase activity is severely decreased (Wagner, Cornblath and Max, unpublished) Therefore, a regenerating muscle fiber is unlikely to utilize energy for contraction, and a low level

of glycolysis may be sufficient to provide energy for biosynthetic processes in mononuclear premyoblastic and myoblastic cells and in non-contracting muscle fibers Furthermore, since the pentose phosphate pathway can provide trioses which can enter the glycolytic scheme at triose phosphate isomerase, it seems feasible that the enhanced activity of the pentose pathway may represent a bypass around phosphofructokinase to permit glycolysis to proceed until the full recovery of glycolytic and oxidative enzymes at a later stage of regeneration

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