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The Enzymes of Adenine Nucleotide Metabolism in Developing Skeletal Muscle

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1. During late foetal and early post-natal development of rabbit skeletal muscle the total protein increased more rapidly than the non-protein nitrogen content per g. wet wt. 2. AMP-deaminase activity of rabbit leg muscles increased rapidly over the period 5–15 days after birth. In diaphragm muscle from the same animal the rapid increase to the adult enzymic activity took place at about the time of birth. 3. The rapid increase in AMP-deaminase activity of leg muscle occurred earlier in animals born relatively mature, such as the chick and guinea pig, than in animals less well developed at birth, such as the rabbit and rat. 4. The pattern of enzymic activity shown by AMP deaminase during development in diaphragm, leg and cardiac muscles in a given species was closely paralleled by those of adenylate kinase and creatine phosphokinase. 5. When young rabbits were encouraged to become active at an earlier stage than is normal, the rise in creatine-phosphokinase activity occurred at an earlier age than in the control animals. 6. The results suggest that the activity pattern of the muscle is an important factor in determining the time at which the activities of the enzymes of special significance for muscle rise sharply to the adult values. 7. Development in rabbit leg muscle also involved an increase in aldolase activity. The pattern of change was similar to that obtained with other enzymes studied.

Biochemical development in muscle involves the evolution and growth of the contractile system represented by the myofibril and the development of enzyme systems adequate to convert the metabolic fuels into ATP at the high rate required for continued contractile activity. In comparison with the investigations carried out on other tissues the study of the biochemistry of muscle development has been somewhat neglected and has been primarily concerned with the proteins of the contractile system, myosin in particular (see Trayer & Perry, 1966). The other part of the muscle cell represented by the soluble protein components of the sarcoplasm, the mitochondria and the sarcoplasmic reticulum, in which are localized the systems which produce ATP and regulate its utilization by the myofibril, have received much less attention. In contrast the sarcoplasmic enzyme, lactic dehydrogenase, has been intensively studied in recent years owing to interest in its isoenzyme complement and the changes which occur in it during development in different muscles (Cahn, Kaplan, Levine & Zwilling, 1962; Markert & Ursprung, 1962; Lindsay, 1963; Fine, Kaplan & Kuftinec, 1963). Also because of its specialized role in muscle the relationship of creatine phosphokinase to development and the onset of contractile function has received attention in recent years (Herrmann & Cox, 1951; Wollenberger & Krause, 1961; Reporter, Konigsberg & Strehler, 1963; Stave, 1964).

In all mammalian striated muscles, the contractile system is very similar both in structure and biochemical composition and differences between the fast, slow and cardiac types are reflected mainly in the non-myofibrillar fraction. For this reason the progress of specialization of muscle during development is conveniently followed by studying the biochemical properties of this fraction. The protein composition of the sarcoplasmic fraction, for example, shows marked differences between muscle types, and in earlier studies on the rabbit it was reported that although the percentage contribution of the sarcoplasmic fraction to the total nitrogen did not increase markedly, the protein composition of this fraction underwent changes, in particular in skeletal muscle, during development from foetal to adult types (Hartshorne & Perry, 1962; Perry & Hartshorne, 1963). These changes are no doubt a reflexion of the increased rate of synthesis of enzymes required for the highly developed systems for anaerobic ATP production, which occurs in response to the activity pattern

associated with the skeletal muscle of the rabbit. This tissue is composed largely of white fibres possessing a rapid contractile response.

The present study is an extension of these earlier studies with particular emphasis on the development of certain enzymes which are found in high yield in skeletal muscle, such as AMP deaminase and creatine phosphokinase, and whose presence reflects the specialization which occurs in skeletal muscle during development from foetal to adult type. Comparison of species and muscle types, together with experiments involving the increased use of muscle during development, suggests that specialized enzyme production may be a response to muscle activity during the immediate pre-natal and post-natal periods. Some aspects of this work have been briefly reported (Kendrick-Jones & Perry, 1965*a*, 1965*b*).

METHODS

Animals. Assessment of the gestational age of the New Zealand White rabbits used was based on the time after mating and the table of Chaine (1911) of foetal length and weight against age. For the period covered by these tables, the two methods gave results which agreed to within 1 day. The mean gestation period was 30 days.

Rats of the Wistar albino and guinea pigs of the Pirbright albino strains were used. Foetal age was assessed with the aid of the tables of Stotsenberg (1915) for the rat (mean gestation period 21 days) and those of Draper (1920) for the guinea pig (mean gestation period 68 days).

Fertilized eggs of the Lyditte Light Sussex strain of fowls were incubated at a constant temperature of 103°F in a moist atmosphere. The eggs were rolled three times a day, and their development was followed by the technique of 'candling' in the period of later development. The mean hatching period was 21 days.

Preparation of enzyme extracts. The majority of the enzyme extracts were prepared from rabbit muscle tissues. Modifications of the procedure for extracts from other animals are specifically mentioned where they were used.

Young and adult animals were killed by dislocation of the cervical vertebrae. The skins were then rapidly removed, the required tissue was dissected out, wrapped in thin polythene sheet and placed in ice. Foetuses were removed from the dead gravid females as quickly as possible and immersed in ice after decapitation. The whole leg muscles of rabbit foetuses older than 23 days were easily dissected out, after skinning. (The number of days after conception will be referred to throughout the text as the embryonic age in days.) For muscle samples from 18–23-day-old foetuses, the viscera were cut out and the skinned carcasses wrapped in polythene sheeting and chilled in ice.

The incubated chick eggs were 'candled' before use, and the air space was marked. The eggs were placed in special holders with this air space uppermost, a hole was cut into the top of the egg and the shell around the large air space carefully removed. The thin embryo lining was removed, and the embryo 'hooked' clear of the reserve proteins left in the egg and washed with dilute saline. After decapitation of the embryo, the pectoral and hind leg muscles were removed and stored in ice.

The chilled muscle was blotted, rapidly weighed and homogenized for 3 min. at 1000 rev./min. with a cooled Potter–Elvehjem type homogenizer in a volume of medium appropriate for the assay procedure (see below). The homogenizers had machined Teflon pestles and precision-bore glass containers, with a clearance of 0·01 in. The supernatant extract was prepared by centrifuging the muscle homogenate for 15 min. at 5000*g*, and the supernatant was filtered through glass wool to remove fat particles floating on the surface.

Enzymic assays

AMP deaminase (*EC* 3.5.4.6). The determination of AMP-deaminase activity was carried out by the method of Perry & Zydowo (1959). Preliminary experiments showed that the myofibrillar fraction contained 36%, mitochondrial 1% and the 'supernatant' fraction 61% of the total muscle AMP-deaminase activity of the whole homogenate. Assays were therefore carried out on a 2% homogenate of whole muscle in either 0·25 M-sucrose or deionized water. The incubation medium, of final volume 2 ml. containing 50 mM-sodium succinate buffer, pH 6·0, 5 mM-AMP, 20 mM-sucrose, 10 mM-KCl (incorporated for maximal activity) and a portion of the 2% muscle homogenate, was incubated at 25° for 10 min. The reaction was stopped by the addition of 1 ml. of 15% (w/v) trichloroacetic acid and portions of the protein-free filtrates were taken for ammonia estimation by using the Nessler reagent prepared by the method of Johnson (1941).

Adenosine deaminase (*EC* 3.5.4.4). The assay procedure used was essentially that described by Kaplan (1955) for the intestinal enzyme. A portion of the supernatant prepared by centrifuging a 1:1 muscle homogenate in 0·3 M-sucrose at 5000*g* for 10 min. was added to 36 μg. of adenosine in 50 mM-phosphate buffer, pH 7·6, total volume 3 ml. The decrease in extinction at 265 mμ on incubation at 25° was followed by using a Beckman DB4 spectrophotometer coupled to a chart recorder.

5′-Nucleotidase (*EC* 3.1.3.5). 5′-Nucleotidase activity was measured on extracts identical with those prepared for adenosine-deaminase estimations in a total volume of 2 ml. containing 0·1 M-tris–HCl buffer, pH 7·5, and 10 mM-AMP. The incubations were stopped after 2 hr. with 1 ml. of 15% (w/v) trichloroacetic acid, and a portion of the protein-free filtrate was taken for phosphate determination by the method of Fiske & Subbarow (1925).

Adenylate kinase (*myokinase; EC* 2.7.4.3). This was assayed by the procedure described by Colowick (1955), which is based on the estimation of AMP produced by adenylate kinase, by the addition of a purified AMP-deaminase preparation. The deaminase preparation was prepared by the method originally described by Schmidt (1932, 1933) with the modifications of Kalckar (1947). The extract, at stage 3 after $(NH_4)_2SO_4$ precipitation, was used as a convenient source of this enzyme.

The assay was performed in two distinct stages because of the different pH optima of the two enzymes. To 0·15 ml. of 0·15 mM-ADP (sodium salt), 0·75 mM-$MgCl_2$ and 12 mM-tris–HCl buffer, pH 7·6, in a 3 ml. quartz cuvette was added 0·15 ml. of the myokinase extract. After 5 min. at room temperature, 2·75 ml. of 0·05 M-citrate buffer, pH 6·0, was added to stop the reaction. A portion (0·05 ml.) of the AMP-deaminase preparation was added, mixed and the decrease in extinction at 625 mμ followed with a Beckman

DB4 spectrophotometer coupled to a chart recorder. The assay procedure was standardized by using the purified preparation of AMP deaminase and standard AMP at varying concentrations.

The muscle-enzyme extracts were prepared by the method of Colowick & Kalckar (1943). The muscle samples were extracted three times with 3 vol. of deionized water. The combined extracts were acidified with 0·05 vol. of 2N-HCl and heated at 90° for 3 min. The extract was rapidly cooled and brought to pH 7·0 with 2N-NaOH. The large protein precipitate was removed by centrifugation at 2000*g* for 10 min. The clear supernatant was used as the enzyme extract. Investigation of the intracellular localization of adenylate kinase showed that the myofibrillar fraction contained 6%, mitochondrial 2% and the supernatant fraction 92% of the total activity.

Creatine phosphokinase (*ATP–creatine phosphotransferase; EC* 2.7.3.2). Activities were determined on the back reaction by measuring the liberation of creatine with time (Chappell & Perry, 1954). The final reaction mixture, total vol. 2 ml. containing 2·5 mM-ADP (sodium salt), 2·5 mM-$MgCl_2$, 2·5 mM-creatine phosphate and 25 mM-phosphate buffer, pH 7·4, was made 5 mM with respect to cysteine–HCl (brought to pH 7·0 before addition) to prevent the oxidation of the highly labile thiol groups of the enzyme and ensure that a linear response was obtained at low enzyme concentrations.

Study of the intracellular localization of creatine-phosphokinase activity showed that the activity was localized in the 'supernatant' fraction, with a trace of activity (between 2 and 5%) associated with the myofibrillar fraction. Assays were therefore carried out on muscle supernatant extracts in 10 mM–KCl, which were diluted at least 500–1000-fold with 0·05 M-phosphate buffer, pH 7·4, before assay, and portions added to the reaction mixture which were preincubated for 5 min. at 30°. The reaction was started by the addition of 2·5 mM-creatine phosphate and after incubation for 4 min. was stopped with 0·5 ml. of 0·05 M-phenylmercuric acetate in 50% dioxan and the tubes were centrifuged to remove the insoluble residue. The creatine liberated was measured by the method of Eggleton, Elsden & Gough (1943).

Fructose 1,6-*diphosphate aldolase* (*EC* 4.1.2.7). The conditions for this assay are those described by Sibley & Lehninger (1949), with hydrazine sulphate in the assay medium. The assay medium, total volume 2·5 ml., contained 40 mM-tris–HCl buffer, pH 8·6, 5 mM-FDP and 56 mM-hydrazine sulphate (brought to pH 8·6 before addition). Assays were carried out on muscle supernatant extracts in 10 mM-KCl.

The triose phosphate produced by the aldolase action was determined by measurement of the alkali-labile phosphate. After the enzymic reaction was stopped by the addition of 2 ml. of 15% (w/v) trichloroacetic acid, the protein was removed by filtration, and 1 ml. duplicate portions were taken for free and alkali-labile phosphate determinations. Into the 25 ml. flasks for alkali-labile phosphate determination was pipetted 1 ml. of 2·3N-NaOH and, after 20 min., 2 ml. of N-HCl. Longer hydrolysis times than 20 min. did not increase the inorganic phosphate released. Into the flasks for free phosphate estimation, were pipetted 3 ml. of 2·3N-NaOH–N-HCl (1:2, v/v). Inorganic phosphate was determined by the method of Fiske & Subbarow (1925).

Nitrogen estimation. This was carried out by a micro-Kjeldahl procedure (Chibnall, Rees & Williams, 1943). Non-protein-nitrogen fractions were obtained by extracting muscle homogenates four times with 10% trichloroacetic acid. Estimations were carried out on portions of the combined washings.

RESULTS

Changes in protein content of muscle during development. One of the problems in assessing the significance of enzyme changes during development is in deciding upon the basis of reference of the changes observed. Muscle development is associated with marked increases in protein content, as illustrated in Fig. 1 for rabbit skeletal muscle. Whereas the total protein nitrogen content increased by a factor of about three during growth from a foetal age of 18 days to the adult state, the non-protein nitrogen increased somewhat less. During the late foetal and early post-natal periods the total protein nitrogen rose steadily but enzymic activities studied show marked and often much greater changes in concentration. This implies that as well as increases in the total protein content, changes in distribution of protein between enzyme fractions may also be occurring.

AMP deaminase. (*a*) Rabbit. In the 20–24-day-old rabbit foetus the leg, diaphragm and cardiac muscles were clearly distinguishable and all had low AMP-deaminase activity. As development progressed the pattern of change in specific activity of this enzyme was characteristic of the particular muscle studied. In the heart, the activity remained low in both neonatal and adult life, whereas a rapid increase occurred in the activity of the enzyme in the diaphragm during the 4 or 5 days before parturition (Fig. 2). As a result, in this muscle the maximum

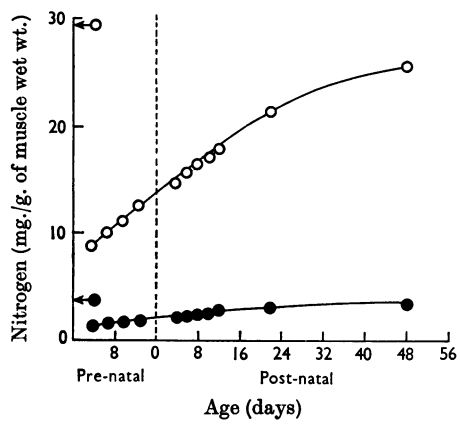

Fig. 1. Amounts of nitrogen in developing rabbit skeletal muscle: ○, protein N; ●, non-protein N. Arrows on the ordinate indicate the adult concentrations.

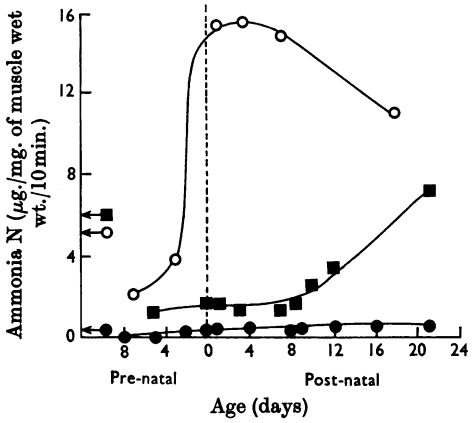

Fig. 2. AMP-deaminase activity of rabbit muscle during development. Specific activities are the means of at least three different muscle samples (S.E. within ±7%). Adult values are from rabbits at least 6 months old and are indicated by arrows on the ordinate. ■, Leg; ○, diaphragm; ●, cardiac.

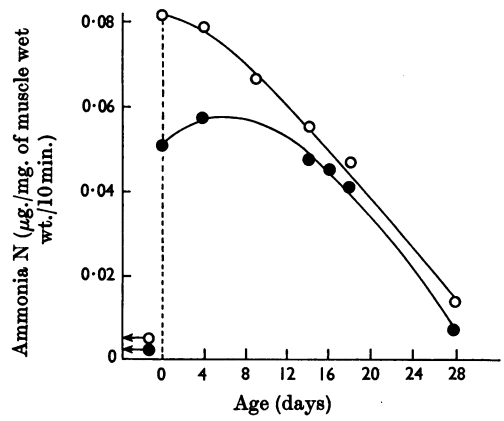

Fig. 3. Adenosine-deaminase activity of rabbit muscle sarcoplasm during development. Specific activities are the means of three different muscle samples (S.E. within ±4%). Adult values are indicated by arrows on the ordinate. ●, Leg; ○, diaphragm.

activity was obtained immediately after birth, and during the first few weeks of neonatal life slowly fell to the relatively high adult activity. In contrast the enzymic activity of the mixed leg muscle remained relatively constant until 8–9 days after birth and thence began to rise steadily until it reached a value close to the adult, seven to eight times that of the foetal muscle, within 14 days (Fig. 2). The increase in deaminase activity in leg muscle occurred during the period when the young animal began to leave the nest and move about independently.

To confirm that the AMP-deaminase activities measured were really due to this enzyme rather than to the combined activities of nucleotidase and adenosine deaminase, the activities of the latter enzymes during development were also measured. Nucleotidase activity, as measured by the liberation of inorganic phosphate from AMP, was extremely low in both foetal and adult tissue and almost undetectable. The highest activity measured was 1·5 μg. of phosphate/g. wet wt. of tissue/60 min. Likewise in leg muscle of the rabbit foetus adenosine deaminase was only about 5% of the AMP-deaminase activity when compared on the basis of ammonia produced. As the adenosine-deaminase activity fell to even lower values during the period when the AMP deaminase increased (Fig. 3) it could be concluded that the combined action of the nucleotidase and adenosine deaminase made no significant contribution to the measured rate of evolution of ammonia from AMP.

(*b*) Other species. Similar studies with the leg muscles of the rat indicated that the rapid increase in AMP deaminase also occurred after birth just at

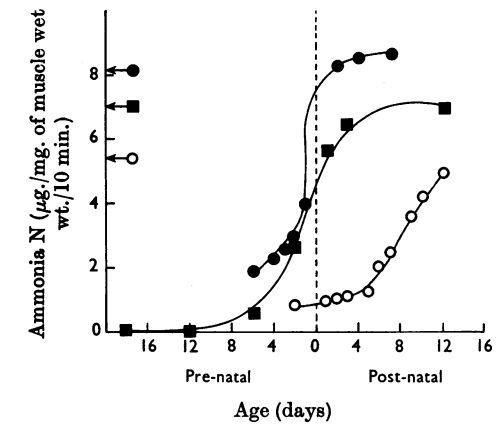

Fig. 4. AMP-deaminase activities of the leg muscles of different species during development. Specific activities are the means of at least three different muscle samples (S.E. within ±8%). Adult values are indicated by arrows on the ordinate. ■, Guinea pig; ○, rat; ●, fowl leg muscle.

the time when the young animals were beginning to move about outside the nest (Fig. 4). On the other hand AMP-deaminase activity in chick and guinea-pig leg muscle rose rapidly before birth, reaching adult activities soon afterwards. In the chick the pattern of development was similar in both pectoral and leg muscles.

Adenylate kinase. As it was possible that the increased activity of the AMP deaminase was an adaptation to greater availability of substrate it was of interest to study the activities of myokinase,

the most important enzyme for AMP production in skeletal muscle. In all the species studied, i.e. rat, rabbit, guinea pig and chick (Fig. 5), the increase in AMP deaminase was closely paralleled by an increase in adenylate kinase, i.e. in chick and guinea-pig leg muscles and rabbit diaphragm activity rose rapidly before birth whereas in rabbit (Fig. 5) and rat leg muscle the rise occurred after birth. There were suggestions in most cases that the rise in myokinase activity slightly preceded the AMP deaminase and in the rabbit and rat leg muscle the adenylate-kinase activity increased steadily after birth rather than changing rather sharply at about the time of increased activity.

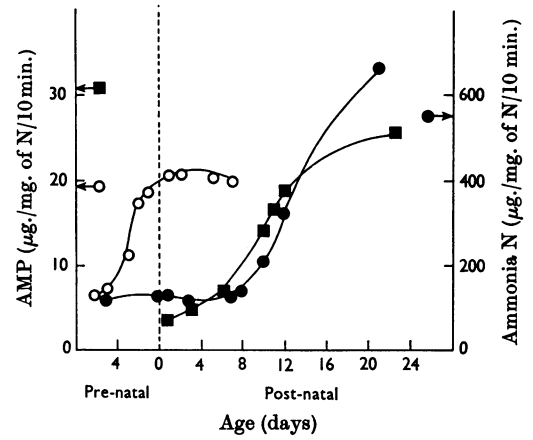

Fig. 5. Adenylate-kinase activities of leg muscles during development. Specific activities are the means of at least three different muscle samples (S.E. within ±6%). Adult values are indicated by arrows on the ordinates. ○, Fowl leg adenylate kinase; ■, rabbit leg adenylate kinase; ●, rabbit leg AMP deaminase (for comparison).

Creatine phosphokinase. Creatine phosphokinase is another example of an enzyme of special significance in muscle but in contrast with adenylate kinase this enzyme does not possess a common substrate with AMP deaminase. Also its activity would tend to maintain the adenine nucleotides in the fully phosphorylated form and hence not available for deamination. Like the deaminase and adenylate kinase its activity in foetal tissue was low and activity increased rapidly at the same stage of development, the particular age at which the activity rose depending on the species and muscle (Fig. 6), in a similar manner to that obtained with the other two enzymes.

The close correlation between the rise in activity of the three enzymes studied and the onset of activity in a skeletal muscle suggested that use of the muscle might be the stimulus for the rise in specific activities of these enzymes. To test this hypothesis an attempt was made to encourage young rabbits to move about earlier than was normal. If the female rabbit's nest was undisturbed it was unusual for the young to leave the nest before the tenth day after birth, and even for some days afterwards they spent much of the day in it. The pattern of creatine-phosphokinase development under these normal conditions is represented by the lower curve in Fig. 7. If the nest was removed from the cage on the fifth or sixth day after birth the animals moved about much more and used their limbs more than the undisturbed controls. Assays

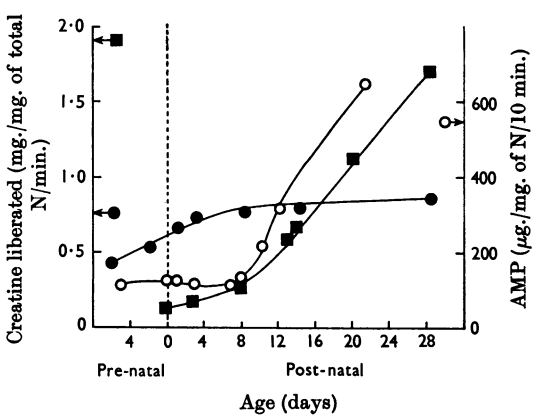

Fig. 6. Creatine-phosphokinase activities of rabbit muscle during embryonic development. Specific activities of creatine phosphokinase are the means of at least three different muscle samples (S.E. within ±5%). Adult values are indicated by arrows on the ordinate. ■, Leg creatine phosphokinase; ●, cardiac creatine phosphokinase; ○, rabbit leg AMP deaminase (for comparison).

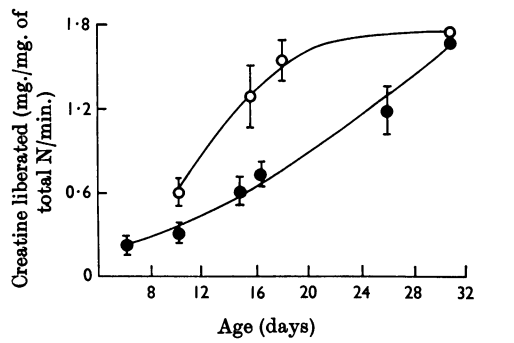

Fig. 7. Effect of activity on the creatine-phosphokinase activity of the leg muscles of young rabbits. The Figure summarizes the results obtained with six litters; ○, litters disturbed at 5 days *post partum*; ●, undisturbed control litters.

showed that in the disturbed litters the creatine-phosphokinase activity increased more rapidly and the adult values were approached at much earlier times after birth than in the control, undisturbed animals.

The exercised animals put on weight a little faster so that at 16 days they weighed $326 \pm 22{\cdot}1$ (6) g. compared with the $273 \pm 12{\cdot}7$ (6) g. of undisturbed controls. It is not possible to decide whether this increased weight was due to the increased food intake being directly stimulated by exercise or simply as a result of the animals visiting the feeding troughs more frequently as a consequence of the greater movement about the cage. The increased creatine-phosphokinase activity of the leg muscles of the exercised rabbits cannot, however, be related to the weight differences, for when they were compared with control animals of similar weight the enzymic activities of the muscle of the exercised animals were still significantly greater.

Aldolase. Foetal tissues such as the liver are in general widely believed to be more adapted to an aerobic life than their adult counter parts. Muscle, however, differs in this respect for there are indications from earlier work that development of skeletal striated muscle is associated with increased dependence on anaerobic metabolism (Hartshorne & Perry, 1962; Perry & Hartshorne, 1963).

The changes in aldolase activity of the muscle sarcoplasm during development in different striated muscles of the rabbit are somewhat similar in pattern to those obtained with the other enzymes studied, despite the fact that aldolase is not directly associated with the utilization of ATP. Activities of aldolase were generally higher in adult skeletal muscle and the stage at which the rapid increases occurred in a particular species depended on the degree of development at birth. In the rabbit, as with AMP deaminase and creatine phosphokinase, the enzyme reached adult values earlier in the diaphragm (Fig. 8).

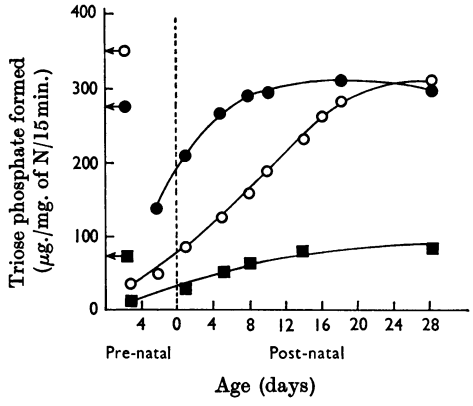

Fig. 8. Fructose 1,6-diphosphate-aldolase activity of the sarcoplasmic fraction of muscles during development. Specific activities are the means of three rabbit muscle samples (S.E. within ±5%). Adult values are indicated by arrows on the ordinate. ●, Diaphragm; ○, leg muscle; ■, cardiac muscle.

DISCUSSION

This investigation has been directed principally to the study of changes in the activities of enzymes of special significance for the metabolism of adenine nucleotides in muscle, on the hypothesis that the specialization occurring during development will be reflected in their activities. Of the three enzymes studied, creatine phosphokinase, myokinase and AMP deaminase, the last-named is probably the most specifically associated with the specialized chemistry of skeletal muscle, for in the rabbit the activity is at least 60–100 times that in any other tissue (Conway & Cooke, 1939). It is significant therefore that in all the skeletal muscles examined there was good correlation between the rapid increase in the activity of this enzyme and the time at which the contractile activity began to rise sharply. In all cases, the rapid rise in AMP-deaminase activity was paralleled by rapid increases in adenylate-kinase and creatine-phosphokinase activities, suggesting that the changes in activity of all three enzymes were in response to a common stimulus. The age at which the rapid increases in these enzymes occurred varied both with the particular skeletal muscle and the species from which it was obtained. The fact that in the rabbit, for example, the activities of the three enzymes reached adult values in the diaphragm either just before or just after birth, whereas in the leg muscle it was considerably later, strongly suggests that the change in activity is not in response to a systemic stimulus but is endogenous to the muscle itself. The change could be a response to a progress of development of the nerve system of the muscle, or possibly to the frequency of stimuli received, i.e. the activity pattern. Certainly the diaphragm is able to function effectively from birth onwards whereas the leg muscles of the newly born rabbit are little used until some days after birth. Just at the stage when the young rabbit begins to move about freely the activities of the enzymes in the leg muscles rise sharply.

This parallelism between activity pattern and enzyme activity, is also apparent when comparisons are made between a given muscle in different species. The activities of enzymes in the leg muscles of those animals born at a more advanced stage of development and capable of independent existence

(Needham, 1931), such as the chick and the guinea pig, are already at, or close to, the adult value. On the other hand, in the leg muscles of animals such as the rat and the rabbit, animals which are less mature at birth and incapable of much movement, the enzyme activities do not rise to the adult value until some time *post partum*. These differences in the stage of development of muscle at birth in different species are reflected in the histological appearance of the myofibrils (Dubowitz, 1963) and in the rates of accumulation of actomyosin (Kasavina & Tarchinsky, 1956).

There is considerable discussion in the literature about the correlation of the development of creatine phosphokinase in foetal muscle and the onset of contractile activity (Dwinnell, 1939; Read & Johnson, 1959; Herrmann & Cox, 1951; Reporter *et al.* 1963). The findings reported here throw no light on this problem but confirm the findings of Stave (1964) that the creatine-phosphokinase activities of foetal tissue are only a fraction of those of the adult muscles. The experiments with newly born animals suggest that increased use of the leg muscle stimulates the creatine-phosphokinase activity to rise earlier than in normal controls. The conclusion that the increased muscular activity is the stimulus, is confirmed by studies *in vitro* with the isolated frog sartorius reported by Kendrick-Jones & Perry (1965*b*).

The aldolase activities, which may be taken to reflect the glycolytic activity of the tissue, show a somewhat similar pattern of increase to the three enzymes discussed above although the correlation between muscle-activity pattern and enzyme activity is somewhat less striking. Insofar as the aldolase activity reflects the glycolytic activity, and hence the ability of the tissue to produce large amounts of ATP anaerobically, the results confirm the earlier preliminary findings (Hartshorne & Perry, 1962) that, unlike many other tissues (Shelley 1961), foetal skeletal tissue has a less highly developed anaerobic metabolism than its adult counterpart.

The activities measured are related to total nitrogen values. Although the extent of increase observed, ranging from five- to ten-fold, strongly suggests considerable formation of new enzymically active protein, the possibility of the activation of enzyme precursors or change in isoenzyme composition to a complement of more active enzymic components cannot be ruled out. With creatine phosphokinase there is evidence that the foetal enzyme is different from the adult in physical and enzymic properties (J. Kendrick-Jones & S. V. Perry, unpublished work). Nevertheless such changes cannot explain the large increases in enzymic activity and it seems clear that considerable increases in the amounts of myokinase, AMP deaminase, creatine phosphokinase, aldolase, and presumably many other enzymes not measured in this study, must occur. Over the period from birth to 20 days the total amount of sarcoplasmic protein only increases by 20% and therefore the much greater increases in activities of certain enzymes must imply a considerable change in the sarcoplasmic composition with possibly compensating decrease in other protein components during this period. These changes are no doubt reflected in the electrophoretic pattern of the proteins of the sarcoplasm reported by Hartshorne & Perry (1962).

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