Lactate dehydrogenase isozyme patterns in human skeletal muscle

Part II. Changes of isozyme pattern in ontogeny

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The preceding paper concerned the variation of lactate dehydrogenase (LDH) isozyme patterns in different skeletal muscles of the same human individual and on the variation in the same muscle between different individuals. In the present paper we have studied the LDH isozyme patterns in different skeletal muscles of the same human foetal individual and on the developmental changes of isozyme pattern of the same muscle during ontogeny. A preliminary communication on this topic has already appeared (Takasu and Hughes, 1966).

MATERIAL AND METHODS

TISSUE SPECIMENS A total of 47 skeletal muscle specimens were collected from four subjects—one stillborn infant and three foetuses. By careful dissection separation of individual skeletal muscles from one another could be carried out without much difficulty even in early foetuses.

In addition 13 specimens of other tissues were also examined.

The three foetuses, all male, were obtained after therapeutic abortions and their ages were estimated to be about 13, 15, and 20 weeks respectively. The stillborn infant, female, died from strangulation by the umbilical cord during labour. The foetal age was estimated at around 40 weeks.

PRESERVATION OF BODIES BEFORE COLLECTION Each foetus, immediately after discharge, was cooled in ice and kept in this way for a few hours until tissue specimens were removed.

The stillborn infant was transferred within a few hours of discharge to a refrigerator and kept for 24 hours at 4°C until dissection.

STORAGE OF SPECIMENS Specimens were stored in airtight containers at -25°C for various periods not exceeding 56 days.

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EXTRACTION OF SPECIMENS, ELECTROPHORESIS OF ISOZYMES, LOCATION OF LACTATE DEHYDROGENASE ACTIVITY, AND EVALUATION OF ISOZYME PATTERNS, ETC. These procedures were the same as described in the preceding paper, except that about 9µl. instead of 7µl. enzyme extract was placed in the slot made in the gel film for electrophoresis.

RESULTS

VISUAL SURVEY OF ISOZYME PATTERNS For comparison of isozyme patterns the classification as used in the preceding paper was followed. The patterns underlined with a continuous line in Table I were

TABLE I
CLASSIFICATION OF LDH ISOZYME PATTERNS

Group	Most prominent isozyme or isozymes
I	<u>2.3</u>
(H > M)	<u>I¹; 1.2; 1.2.3; 1.2.3.4; 1.2.4;</u> 1.3.1.4; 1.2.5; 1.2.3.5; 2
II	<u>1.2.3.4.5; 2.3.4; 3</u>
(H ≈ M)	<u>2.4; 1.5; 1.3.5; 1.2.4.5</u>
III	<u>2.3.4.5; 3.4.5; 4.5</u>
(H < M)	<u>3.4; 2.5; 1.4.5; 2.3.5; 1.3.4.5;</u> 4; 3.5; 2.4.5; 5

Isozyme combinations underlined with an unbroken line were observed in skeletal muscle specimens from one or more of the foetuses; those underlined with a dotted line were seen in specimens from the stillborn infant. The remainder are other possible combinations of isozymes which would give rise to the indicated relationships between the totals of H and M sub-units if they be present in about equal proportions, and if isozymes present in smaller amounts be ignored.

those observed in skeletal muscle specimens from one of more of the foetuses, whereas those underlined with a dotted line were seen in the stillborn infant.

In Table II all specimens which were examined are arranged according to this classification. By comparison with the adult muscle in several instances,

TABLE II
CLASSIFICATION OF TISSUE LDH ISOZYME PATTERNS

	Foetus male (13 w.)	Foetus male (15 w.)	Foetus male (20 w.)	Stillborn female (40 w.)
Group I (H > M)	Tric. sur. Rect. fem. Glut. med.	Tric. sur. Rect. fem. Pect. maj. Glut. max. Psoas Tric. br.	Erect. sp. (L) Glut. max. Thenar Bic. br. Rect. fem. Pect. maj.	(Heart)
	Glut. max. Lat. dors. Psoas ----- Pect. maj. Deltoid ----- (Heart)	Bic. br. (Liver) (Heart) Hypothen.	Erect. sp. (Th) Lat. dors. ----- (Heart) Deltoid Soleus Gastrocn. Psoas Tib. ant. Serr. lat. Flex. dig. Infraspin. Trapez. Flex. Hal. ----- (Liver)	Erect. sp. (Th) Psoas Infraspin.
Group II (H ≈ M)				
Group III (H < M)				Glut. med. Soleus ----- Deltoid Glut. max. Pect. maj. ----- Rect. fem. Diaphragm Tric. br. Thenar Bic. fem. Gastrocn.

Although belonging to the same major group, the isozyme patterns of muscles placed above the dotted lines appeared to be more anodic than those below.

it was more difficult to match observed isozyme patterns to a particular combination of isozymes, hence occasionally classification of a particular pattern into Group I, II or III is doubtful. For example, in Fig. 2, the infraspinatus muscle might have been classified as a 2.3.4.5 pattern rather than 2.3.4 which would have placed it in Group III rather than Group II. However these uncertainties do not seem sufficient to invalidate the general conclusions of this study.

VARIATION OF ISOZYME PATTERN BETWEEN INDIVIDUAL SKELETAL MUSCLES OF THE SAME SUBJECT 1. *Foetuses* Little variation in isozyme pattern was found between specimens from any of the three foetuses, indeed the uniformity of the isozyme pattern was striking. Predominant activity was in LDH 2, 3, 4, or a combination of these three, particularly 2.3, 3 or 2.3.4, and never in LDH 1 or 5, and so far as the skeletal muscle was concerned LDH 5 was invariably the least prominent (Fig. 1). The isozyme patterns all belonged to Groups I or II, and the differences

between individual specimens were minimal (Table II).

2. *40-week stillbirth* Specimens from the 40-week stillborn infant showed a much greater variety of LDH isozyme patterns (Fig. 2). With regard to isozyme patterns all skeletal muscle specimens could be placed in Groups II and III. The heart pattern was placed in Group I (Tables I and II). In comparison with adult individuals there appeared to be certain differences (see previous paper Table V).

a. None of 14 skeletal muscles examined belonged to Group I.

b. Gluteus medius muscle, Group I in two out of two adults, as well as thenar and gluteus maximus, Group I or II in five out of five and two out of two adults, were all in Group III. However, as in the adults, the gluteus medius pattern was more anodic than triceps brachii, rectus femoris, gastrocnemius, etc. (Table II).

Results for the remainder of the muscles did not differ significantly from those obtained with the adults.

FIG. 1 LDH isozyme patterns in muscles from a 20-week human foetus showing small amount of variation between muscles and, in most cases, virtual absence of LDH 5. Patterns from heart and liver are included for comparison.

FIG. 2 LDH isozyme patterns in muscles from a stillborn infant showing presence of appreciable amounts of LDH 5. Asterisk indicates grouping in doubt, see text p. 181.

COMPARISON OF THE SAME MUSCLE FROM HUMAN SUBJECTS AT VARIOUS STAGES OF DEVELOPMENT The isozyme patterns for a particular muscle were very similar in each of the three foetuses, and all the specimens could be placed in Group I or II. In the stillborn infant, of 14 different skeletal muscles 11 could be placed in Group III and three in Group II, whereas in the adults there was a spread over Groups I to III (see preceding paper Table V).

Three types of muscle could be distinguished in relation to the change of isozyme pattern during ontogeny. The first type, such as quadriceps femoris, pectoralis major, and triceps brachii, belonged to Group III both in the stillborn and in the five adults. The second type showed an increase in anodicity of the isozyme pattern, thus gluteus maximus and thenar muscles were Group III in the stillborn infant

and Groups I or II in the adults, while gluteus medius which was Group III in the stillborn infant belonged to Group I in the adults. The third type, the remaining muscles such as psoas, soleus, gastrocnemius etc., which in the stillborn infant belonged to Groups II or III, in the adult showed a variable classification and might belong to groups ranging from I to III. Examples of these types are illustrated in Fig. 3.

Despite the rather small variability of the muscles of our stillborn subject, comparison of Table II in this paper and of Table V in the previous paper shows that many of the mutual relations between different muscles with regard to the anodicity of their LDH isozyme patterns were similar in the adults and stillborn infant. In the foetuses, in which variations in isozyme pattern were in any case much smaller, these mutual relations were not observed.

FIG. 3 LDH isozyme patterns of gluteus medius, soleus and rectus femoris muscles in individuals at different stages of development.

DISCUSSION

The fact that LDH isozyme patterns of various foetal tissues differ from those of the adult, both in man and animals, has been shown by several authors. Thus Dreyfus, Demos, Schapira, and Schapira (1962) have shown in a 4-5 month foetus that the isozyme activity is predominantly LDH 2, 3 or 4 and that LDH 5 is lacking. Emery (1964) has found the same in a 400 g foetus and in a 7-month stillborn infant, as have Suzuki and Momma (1964) in a 2-day premature infant.

However, except in a report by Fine, Kaplan, and Kuftinec (1963) who stated that leg muscle was examined, previous work has not indicated the site from which the specimen was collected, and there does not as yet appear to be any information on particular foetal muscles either in man or animals. The present paper reports the LDH isozyme patterns of 21 different skeletal muscles of three human foetuses at the 13th, 15th, and 20th week of foetal life and shows that they exhibit little variation in isozyme pattern.

Despite the fact that the foetal muscle isozyme patterns are very similar they are not identical. For example as shown in Table II, some muscles are classified into Group I and others into Group II in each foetus. In addition Fine *et al.* (1963) have shown that the H sub-unit percentage of leg muscle, although it always exceeds 50% in the foetal period, gradually decreased from over 99% in the sixth week, to 85% in the third month and then to 60% in the seventh month. Consequently, the isozyme pattern of human skeletal muscle is probably changing even in early foetal life.

Reports on LDH isozymes of human muscle around birth are few. Kar and Pearson (1963) have shown the electrophoretic patterns of nine different skeletal muscles of a 2-day-old male infant. Emery (1964) has shown the electrophoretic pattern of one muscle from a newborn and from a 3-month-old

infant. The present study gives information on 14 different skeletal muscles, 10 of which do not seem to have been previously reported on.

In considering the various reports it is worth noting some disagreement as to the relative amount of LDH 5 activity. Kar and Pearson (1963) and Emery (1964) found, as in the foetuses, that LDH 5 was the least prominent isozyme in the muscles of their newborn subjects. By contrast, in our 40-week stillborn infant, LDH 5 was the most prominent isozyme in seven out of 14 muscles as it was in the muscle from the 3-month infant studied by Emery. On account of differences in methodology, caution is necessary in drawing conclusions from these conflicting findings, but the time at which LDH 5 becomes the most prominent isozyme in many muscles may vary in different subjects.

It is possible that our stillborn subject was atypical and that the isozyme patterns were all unusually 'cathodic'. Suggestive of this is the fact that certain muscles, such as the gluteus maximus and gluteus medius, which are in Group I or II in the three foetuses and also in the adults, are in Group III in the stillborn infant. However, alternatively, a general increase in the proportion of the cathodic isozymes in all muscles may occur during ontogeny but for some muscles this trend is later reversed, perhaps in relation to development of their normal functions, and consequently the anodic isozymes again predominate. The timing of these complex changes might show considerable individual variability.

In the case of our stillborn subject there appears to be less variation in isozyme pattern from muscle to muscle than occurs in adults. However, this is chiefly because certain muscles—for example, gluteus medius, gluteus maximus and thenar—in Groups I or II in the adults were all in Group III, so that for the reasons mentioned above this result may not be typical.

It is clear that in the first half of foetal life anodic isozymes predominate and muscles are largely

uniform in their isozyme pattern. At some later stage, which may perhaps be in the period just before to just after birth, there is a marked increase in LDH 5, at least in some muscles, and characteristic differences appear.

The process which takes place during ontogeny is not a general change in anodicity, but represents a complex process of differentiation. Muscles in the adult seem to fall into three categories. Firstly, there are those which have retained, or possibly have reverted to, the anodic foetal isozyme pattern and may even have become more anodic; secondly, those which have differentiated in a cathodic direction, and, finally, a third group whose isozyme pattern may show either of these changes (see preceding paper, Table V).

The factors leading to differentiation of the muscle isozyme pattern are likely to be complex. Differences in function could play a part, since in animals Blanchaer and van Wijhe (1962) have shown that tonic muscles have a more anodic isozyme pattern than phasic muscles, but at least in the case of our stillborn subject, the increase in cathodic isozymes compared with the foetuses (c.f. Figs. 1 and 2) seems to have progressed to a considerable extent before development of normal functional activity.

In other species, notably the cat, Buller, Eccles, and Eccles (1960) and later workers (Romanul and van der Meulen, 1966; Dubowitz, 1967; Prewitt and Salafsky, 1967) have demonstrated that the influence of the nervous system is very important for muscle differentiation generally, of which differentiation of the LDH isozyme pattern is one aspect, and also for its maintenance. In man our findings indicate that at a period when development of motor nerves is at an early stage, little difference exists between muscles in their isozyme patterns, a result which at least does not conflict with the suggestion that in man also, the influence of the nervous system may be an important factor in development. Moreover the atrophic muscles of patients with neurogenic disorders show a foetal isozyme pattern (Brody, 1964; Laurysens, Laurysens, and Zondag, 1964; Emery, Sherbourne, and Pusch, 1965), a fact which indicates that in man, as well as in animals, the normal function of the motor nerves is necessary for the maintenance of the differentiation which occurs during ontogeny.

Other factors such as general motor activity, the tonic function of the maintenance of posture, etc., may all play a part, but their relative importance seems obscure in the absence of much more extensive data. Neither is it clear why in primary myopathies, where there is as yet no evidence of a defect in innervation, a foetal isozyme pattern is frequently

observed (for example, Dreyfus *et al.*, 1962; Laurysens *et al.*, 1964; Nishikawa, Ueda, Matsumoto, Fushimi, and Nakata, 1964; Suzuki and Momma, 1964; Emery *et al.*, 1965).

SUMMARY

Forty-seven human skeletal muscle specimens obtained from one stillborn and three foetal subjects were examined for lactate dehydrogenase (LDH) isozyme pattern, using agar gel electrophoresis. The specimens included 14 different skeletal muscles in the stillborn and 20 in the foetal subjects respectively.

There was very little variation in LDH isozyme pattern in different skeletal muscles up to about the 20th week of foetal life.

In the 40-week stillborn infant the LDH isozyme pattern differed considerably from the foetal pattern and LDH 5 was prominent in many muscles. Between different muscles there are variations in the isozyme pattern, but to a lesser extent than in adults.

The mode of differentiation of the LDH isozyme pattern during human ontogeny and its causative factors are discussed in relation to these results and those reported in the preceding paper.

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