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Lactate dehydrogenase isozyme patterns in human skeletal muscle

Part I. Variation of isozyme pattern in the adult

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Lactate dehydrogenase (LDH) exists as five similar but not identical forms, known as isozymes. In animals (including birds) and probably in most vertebrates, the LDH isozymes are tetrameric molecules composed of four sub-units each of which may be either H (heart type) or M (muscle type) (Appella and Markert, 1961; Cahn, Kaplan, Levine, and Zwilling, 1962; Fine, Kaplan, and Kuftinec, 1963; Markert, 1963; Dawson, Goodfriend, and Kaplan, 1964). The sub-units are named after the mature tissues in which they are generally most abundant. The isozymes are numbered LDH 1-5 in order of decreasing electrophoretic mobility towards the anode and correspond to the sub-unit combinations H_4, H_3M_1, H_2M_2, H_1M_3 and M_4. Each species appears to have its own characteristic LDH isozymes (for example, Cahn *et al.*, 1962) and, with respect to LDH, individual tissues differ from one another only in the proportion of the total activity present as a particular isozyme (an exception is the isozyme which seems to be uniquely contained in sperm (Blanco and Zinkham, 1963)).

The present paper is a study of the variation of the LDH isozyme pattern in different human skeletal muscles of the same adult and of the variation in the same muscle between different adults. The following paper will present data on the changes which occur in skeletal muscle isozyme patterns during human ontogeny.

MATERIALS AND METHODS

TISSUE SPECIMENS A total of fifty-four skeletal muscle specimens were collected at necropsy from five adults, as far as possible specimens being taken from the same site in the muscle each time. None of the patients suffered from any muscle abnormality, and if the necropsy were not performed within four hours of death all bodies were left in the refrigerator at 4°C for a period not greater than 41 hours.

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STORAGE OF SPECIMENS Specimens were stored in air-tight containers at −25°C until use. The times of storage at −25°C are shown in Table I.

TABLE I

ORIGIN AND LENGTH OF STORAGE OF TISSUE SPECIMENS

Subject	*Sex age (yr)*	*Storage of tissue at −25°C (days)*
M.F.	Female 29	109-114
R.G.	Male 53	112-132
V.B.	Male 54	82-104
F.J.	Female 66	28-63
A.T.	Female 66	1-13

EXTRACTION OF SPECIMENS An aliquot (50-200 mg) of each specimen was homogenized in a cooled all-glass homogenizer with 0·5 to 2·0 ml. of cold ion-free water for 10 minutes. The homogenate was centrifuged at 10,000 *g* in a refrigerated centrifuge (4°C) for 10 minutes and the supernatant used for electrophoresis.

ELECTROPHORESIS OF EXTRACTS Electrophoresis on agar gel coated microscope slides was carried out by Wieme's method (1959a).

A known amount of tissue extract, 7 μl., was placed in a preformed slot in the gel using a capillary micro pipette. Gels were cooled by immersion in petroleum spirit (boiling range 40 to 60°C) and electrophoresis was carried out using a current stabilized power supply for 70 to 80 minutes. The potential difference across the gels varied from 24 to 28 v/cm initially to 20 to 23 v/cm at the end of the run, and the temperature of the petroleum spirit increased from 15 to 20°C at the beginning of electrophoresis to a final value of 25 to 30°C.

LOCATION OF LDH ACTIVITY After electrophoresis the slides were incubated in the dark with a reagent containing nitroblue tetrazolium, NBT, essentially as described by Van der Helm (1961), except that, following Fine *et al.* (1963), sodium cyanide was omitted. After 60 minutes the reaction was stopped by immersion in 2% acetic acid and, after thorough washing, the gels were dried in the dark at room temperature.

LDH isozyme activity appeared as purple bands of formazan which formed the isozyme pattern.

ASSESSMENT OF ISOZYME PATTERNS 1. *Transmission measurements* Gels were scanned for transmission using a Chromoscan instrument (Joyce Loebl & Co. Ltd.) with a green filter (5-040). Transmission was related to the amount of reduced formazan in the gel.

2. *Visual inspection* Although the isozyme patterns from the 54 skeletal muscle specimens and six specimens from other tissues varied greatly, in skeletal muscle all five isozymes could be demonstrated provided that sufficient tissue extract was applied to the gel.

For comparison the various patterns were placed in three groups—I, II, and III—according to which isozyme or isozymes were most prominent on visual inspection. Table II indicates the combinations of isozymes which

TABLE II

CLASSIFICATION OF LDH ISOZYME PATTERNS

Group	*Most prominent isozyme or isozymes*
I	1.2; 2; 2.3;
(H > M)	1; 1.2.3; 1.2.3.4; 1.2.4; 1.3; 1.4; 1.2.5; 1.3.4; 1.2.3.5
II	3; 2.3.4; 1.2.3.4.5;
(H ≏ M)	2.4; 1.5; 1.3.5; 1.2.4.5
III	3.4; 3.5; 2.3.4.5; 3.4.5; 4.5; 5;
(H < M)	2.5; 1.4.5; 2.3.5; 1.3.4.5; 4; 2.4.5

Underlined isozyme combinations were actually observed in skeletal muscle specimens. The rest 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 are present in about equal proportions, and if isozymes present in smaller amounts are ignored.

are possible in this classification, those underlined being actually observed. Considering only the most prominent isozymes, then combinations belonging to Groups I, II, and III are those in which, as an approximation, the total of H sub-units would be greater than, equal to, or less than the total of M sub-units respectively. Consequently the order of anodicity is Group I > Group II > Group III.

RESULTS

EVALUATION OF ASSESSMENT PROCEDURES 1. *Reproducibility* In replicate electrophoretic runs, although the absolute amount of individual isozyme activity varied by up to 25%, the proportion of each differed by less than 5%.

2. *Relation between transmission and amount of tissue extract applied* The relation between transmission and the amount of tissue extract applied was not linear, but—as reported by Wieme (1959b)—appeared to be logarithmic, so that the increment in gel formazan per unit volume of extract, although always positive, decreased as the latter increased.

As the relation between formazan produced and isozyme concentration is non-linear and, since they have different kinetic properties, the measured proportions of the various isozymes were dependent

TABLE III

EFFECT OF APPLYING VARYING AMOUNTS OF TISSUE EXTRACT TO THE GEL

Isozyme	*% Total LDH activity* (*a*)	(*b*)
LDH-1	3·6	14·5
LDH-2	19·6	30·1
LDH-3	41·1	36·3
LDH-4	34·0	16·6
LDH-5	1·8	2·5
Total LDH activity in arbitrary units	56	193

Volumes of tissue extract applied were: (*a*) 2·2 µl. (*b*) 8·9 µl. Total LDH activity was measured by scanning the gels for transmission. With the exception of LDH-5, the proportions of the more anodic isozymes were increased in (*b*) as compared with (*a*).

on the amount of extract applied. Table III illustrates a shift in favour of the more anodic isozymes with increasing enzyme concentration. The apparent exception of LDH 5 probably reflects experimental error due to the small amount present in this particular isozyme pattern.

Because of these factors, division into Groups I, II, and II is to some extent arbitrary. Thus, although the mutual relations between the isozyme patterns—that is, one more or less anodic than another—would not change, in some instances they might have been placed in different groups had a standard volume of extract, other than 7 µl., been used in preparing the electropherograms.

3. *Effect of storage* Storage of tissue specimens at room temperature (ca. 15°C) in air tight containers for up to 24 hours increased total LDH activity by about 15%, but changes in the proportions of particular isozymes did not exceed 5%. Storage at 4°C for up to six days changed the proportions of individual isozymes by only about 3%.

Finally, storage at −25°C had a negligible effect on total LDH activity and only a comparatively small effect on the relative proportions of the different isozymes (Table IV, Fig. 1).

TABLE IV

EFFECTS OF STORAGE AT −25°C FOR 72 DAYS ON THENAR MUSCLE (SUBJECT V.B.)

Isozyme	*% Total LDH activity* (*a*)	(*b*)
LDH-1	16·2	17·3
LDH-2	26·4	21·7
LDH-3	17·4	21·2
LDH-4	14·9	19·4
LDH-5	25·1	20·4
Total LDH activity in arbitrary units	235	226

Equivalent amounts of extracts prepared from aliquots of the same specimen, (*a*) before and (*b*) after storage at −25° were applied to the gels.

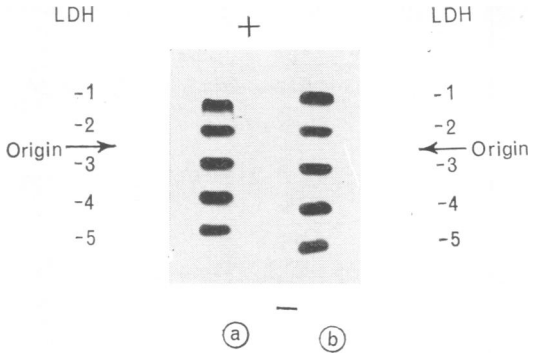

FIG. 1. *Isoenzyme pattern of thenar muscle (subject V. B.) (a) Before and (b) after storage at −25° for 72 days.*

4. *Visual assessment* Differences in the amount of formazan present in a particular isozyme band of less than 20 to 25%, as determined by transmission measurements, could not be assessed visually, and thus differences arising from unavoidable variations in other factors such as amount of extract applied, storage, etc., would not be detected by this means. Consequently, we concluded that a visually detectable difference in isozyme pattern was significant and that visual assessment was adequate for classifying the various isozyme patterns in the present study.

COMPARISON OF ISOZYME PATTERNS OF MUSCLES IN THE SAME SUBJECT AND IN DIFFERENT SUBJECTS On the whole, variations between different muscles of the same individual, in total LDH activity, were not very great but there were obvious differences between isozyme patterns of different muscles belonging to the same individual (Fig. 2, Table V).

However, when different individuals are compared there appeared to be two types of muscle: (1) those whose isozyme patterns were closely similar in all individuals examined, and (2) those whose isozyme patterns varied considerably between individuals. Instances of both types can be seen in Table V. Thus, for example, gluteus medius was in Group I in two out of two and quadriceps femoris Group III in five out of five specimens. On the other hand, of three deltoid specimens one belonged to each of the three groups.

Despite the variations in the isozyme patterns of certain muscles when different individuals are compared, on the whole their relationship to other muscles of the same individual was maintained. For example in two out of two cases the soleus pattern was less anodic than gluteus medius, but more anodic than quadriceps femoris in three out of four specimens.

Although we did not feel justified in further sub-dividing the classification of isozyme patterns, it was apparent that certain muscles which always fell in the same major group—for example, quadriceps femoris, triceps brachii (Group III)—might have more or less anodic patterns in different subjects, and it seemed likely that there was a general tendency for the muscles of one individual to have more or less anodic isozyme patterns than those of another. This point is illustrated in Figure 2.

DISCUSSION

Blanchaer and van Wijhe (1962) first showed differences in the LDH isozyme pattern of individual skeletal muscles in the rabbit, guinea-pig, and mouse, particularly between the so-called red and white muscles. The observations were subsequently confirmed and similar differences also demonstrated between human muscles (Kar and Pearson, 1963; Dawson *et al.*, 1964; Dawson and Kaplan, 1965). Although information on the LDH isozyme patterns of normal human muscles comes mainly from the observations of these authors, others have published isozyme patterns of one or two muscles which served as controls in the investigation of pathological material (Brody, 1964, 1965; Emery, Sherbourne, and Pusch, 1965). This paper presents information on 19 different adult muscles, for nine of which—namely, gluteus medius, gluteus maximus, erector spinae, semitendinosus, thenar, hypothenar, soleus, infraspinatus, and latissimus dorsi—isozyme patterns do not appear to have been published previously.

Despite differences of methodology, there is considerable agreement between the results of all these authors. Thus for the 10 muscles on which both Kar and Pearson (1963) and ourselves have obtained data, agreement is good, except that they found triceps brachii to have more anodic isozyme patterns than psoas, whereas we found the reverse in two out of two adults. Likewise our results are in general agreement with those of Dawson and Kaplan (1965) in respect of nine muscles common to both investigations.

Until recently only Dawson and Kaplan (1965) had studied the isozyme pattern of the same muscle in more than one individual. They observed differences in average H sub-unit percentages in the same muscle between men and women and between subjects over and under 65 years of age; older individuals and some women tended to have higher H sub-unit percentages. Rosalki (1967) has now investigated vastus lateralis muscle and reports similar age differences but does not comment on any variations between men and women. However, our own results do not permit any definite conclusions on isozyme pattern as related to age and sex.

TABLE V

CLASSIFICATION OF TISSUE SPECIMENS WITH RESPECT TO THEIR LDH ISOZYME PATTERNS

Subject	*M.F. female (29 yr)*	*R.G. male (53 yr)*	*V.B. male (54 yr)*	*F.J. female (66 yr)*	*A.T. female (66 yr)*
Group I (H > M)	Deltoid	Glut. med. Intercost. Soleus Thenar Psoas	Glut. med.	Infraspin. Erect. sp. Lat. dors.	(Heart) ..... Thenar ..... Gastrocn.
Group II (H ≐ M)	Glut. max. Thenar Gastrocn.		Thenar Soleus	Thenar Glut. max. Deltoid	Hypothen.
Group III (H < M)	Rect. fem. ..... Pect. maj.	Gastrocn. Rect. fem. Vast. med. ..... Pect. maj. Bic. br. Tric. br.	Deltoid Infrasp. Biceps br. ..... Gastrocn. Psoas Erect. sp. Vast. lat. Vast. med. Rect. abd. ..... Rect. fem. Sternomast. Pect. maj. Tric. br.	Bic. br. ..... Psoas Vast. med. Soleus Gastrocn. Vast. lat. Rect. fem. Rect. abd. Tric. br. ..... Pect. maj.	Rect. fem. Semitendin.

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.

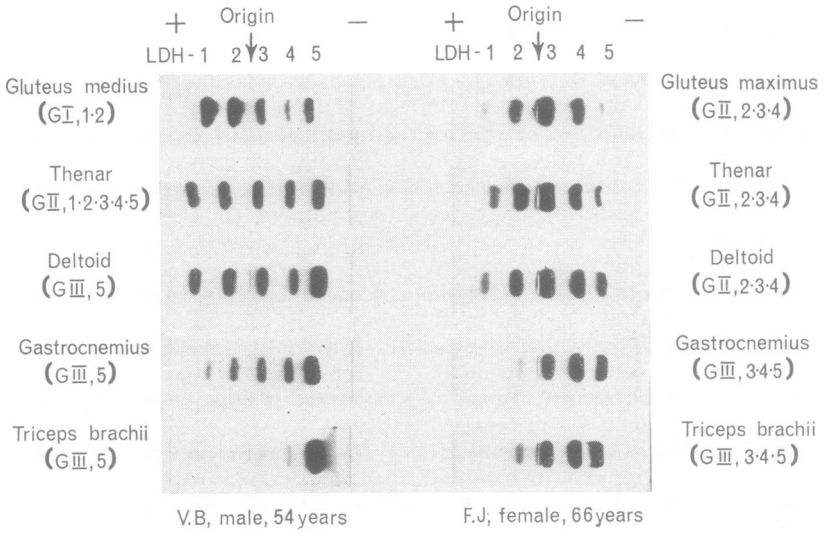

FIG. 2 *Isozyme pattern of muscles from subjects V. B. and F. J. These show marked differences between individual muscles and a tendency for muscles of subject V. B. to have less anodic isozyme patterns than those of F. J. The classification of the various patterns is indicated in parenthesis.*

It is clear that human muscles vary greatly in their isozyme content when different muscles are compared in the same individual and also to a considerable extent when the same muscle is compared in different individuals. However, Rosalki (1967) comments that, despite the wide variations in the same muscle, isozyme patterns in muscles from different sites show characteristic differences. Our results support this view, but suggest that some muscles may show considerably less variation than

others. There may thus be general relationships between certain muscles—for example, gluteus medius having a more anodic isozyme pattern than pectoralis major—which hold for most individuals, although the work of Dawson and Kaplan (1965) and Rosalki (1967) suggests that the relationship might break down for very young (< 4 years) and elderly (> 65 years) subjects.

When muscles with a more variable isozyme pattern are considered the situation appears to be somewhat different. If two subjects are compared—for example, R. G. and V. B. (Table V)—although a particular muscle, such as soleus, may be in Group I in one case and in Group II in another it generally retains its relationship to other muscles of the same subject, so that in both cases the soleus has a more anodic isozyme pattern than the pectoralis muscle. Similarly, Dawson and Kaplan (1965) found the rectus femoris muscle to contain the least LDH-H sub-units, of five muscles, in all of six subjects. Their work also implies that if one muscle of a subject has a more or less anodic isozyme pattern than the same muscle in another subject, all muscles are likely to show the same tendency. Our results, as illustrated in Fig. 2, seem to confirm this suggestion.

Any conclusions drawn from the present limited investigation must be tentative, but it may be useful in suggesting directions in which more extensive and quantitative studies might usefully be made. Moreover, it illustrates quite clearly in the case of the LDH isozyme patterns, that human skeletal muscle is not a homogeneous tissue. Consequently in any comparison of normal and diseased muscle, such as has been made by a number of authors (Brody, 1964; Lauryssens, Lauryssens, and Zondag, 1964; Emery *et al.*, 1965) it is essential that the same muscle should be used as a basis of the comparison and that due allowance is made for variations between normal individuals.

The fact that normal human muscles differ in their LDH isozyme pattern presumably implies corresponding differences in metabolism possibly for example in the relative importance of aerobic as compared with anaerobic processes of energy metabolism (Cahn *et al.*, 1962). In various pathological disorders, notably the progressive muscular dystrophies, certain muscles are often more severely affected than others. This raises the question, as yet unanswered, of whether there is a relation between the metabolic differences of particular muscles and their susceptibility to specific disease processes.

SUMMARY

Fifty-four human skeletal muscle specimens obtained at necropsy were examined for lactate dehydrogenase (LDH) isozyme patterns using agar gel electrophoresis. They included 19 different skeletal muscles.

For the purpose of the present study, visual inspection was considered adequate for delineating the major characteristics of isozyme patterns and for comparing individual muscles.

By this means, marked differences in the isozyme patterns of adult human muscle were observed ranging from those in which anodic isozymes predominated—for example, gluteus medius—to those, such as triceps brachii, in which cathodic isozymes predominated.

When different subjects were compared the isozyme patterns of some muscles were fairly constant whereas others varied considerably. However in any one individual even muscles with a variable isozyme pattern tended to preserve their mutual relationship in respect of the anodicity of their isozyme patterns.

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