Function and Metabolism of Velopharyngeal Muscle in Normal and Cleft Palate Patients

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Abstract

Activity of various enzymes and myofibrillar protein distribution were measured in biopsy material taken from velopharyngeal muscles of 30 infants with cleft palates and 20 infants with normal palates.

The results show that activity of dehydrogenases and transferases is lower in cleft palate than in normal muscle. Cathepsin D and L activities were also decreased, but calpain activity was elevated. LDH and MDH isoenzyme distribution were shifted to anaerobic metabolic pathways.

On the basis of our results cleft palate muscles seem to be genetically hypoplasic, inactive and with decreased metabolism. The large individual differences in the metabolism of certain cleft palate muscles and their deviations in the function of the already reconstructed cleft palate velum suggest that there is a cause and effect relation between them.

Key words: cleft palate, myofibrillar proteins, protease, dehydrogenase, transferase.

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During recent years a number of reviews have been published on the evaluation of functional consequences (velopharyngeal closure, speaking, hearing, etc.) of cleft palate surgery [12, 13, 14, 21]. It is well known that soft palate is of primary importance in the functioning of velopharyngeal closure. According to mentioned authors function of the reconstructed velum did not recover in 15-20% of the cases. Some of the most important reasons are: (i) decreased flexibility, (ii) shortening of the soft palate due to scars, (iii) neurovascular disturbances, (iv) insufficient postoperative logopaedical and orthodontical treatment.

Our cleft palate team observed that in case of identical clefts, using the same surgical method, following *"per primam"* healing considered anatomically ideal, the later functional results were different. Trying to find factors responsible for the above differences we measured enzyme activities, isoenzyme distributions or protein pattern in normal and cleft palate muscles. Activity of enzymes specific for various types of skeletal muscles [17] and of proteases proved to be good marker for muscle protein degradation were determined.

Objective of our studies was to find out, whether there was any difference in protein metabolism and energy transformation indicated by activity of dehydrogenases, transferases and endopeptidases in normal and cleft palate muscles.

Distribution of LDH and MDH isoenzymes as well as of the myofibrillar proteins was also studied in respect of differentiational and functional deviations in muscle of patients.

Materials and Methods

Our investigations were carried out on biopsy samples (mean weight of 50 mg) taken from cleft palate muscle of 30 infants during reconstruction from places shown in Fig. 1. Average age of the infants was 20 months. For control we used muscles obtained from normal soft palate of 20 children (average age: 20 months). Sampling was carried out within 3-5 minutes following the clinical deaths of infants from the same places as shown in Fig. 1.

The samples were homogenized with 50 volumes of 0.1% Triton-X 100 solution containing 2% KCl in an Ultra-Turrax (Janke-Kunkel, Germany) twice for 10 seconds and then centrifuged at 16000 g for 20 minutes. Soluble protein content and enzyme activity were measured in the supernatant, while the sediment was used for the separation of myofibrillar proteins. Each step of the preparation was carried out at 4°C. The activity of the following enzymes was determined: Lactate dehydrogenase EC 1.1.1.27 (LDH), Malate dehydrogenase EC 1.1.1.37 (MDH), Isocitrate dehydrogenase EC 1.1.1.42 (ICDH), Creatine kinase EC 2.7.3.2 (CPK), Glutamate-oxaloacetate transaminase EC 2.6.1.1 (GOT), Glutamate-pyruvate transaminase EC 2.6.1.2 (GPT), Cathepsin L EC 3.4.22.15 (CL), Cathepsin D EC 3.4.23.5 (CD), Ca^{++} activated neutral proteinase EC 3.4.22.17 (CANP or Calpain). The activity of LDH, MDH, ICDH, CPK, GOT, GPT enzymes was determined according to Bergmeyer [2]. Activity measurements of lysosomal cathepsin D and L were carried out with methods described by Barrett [1] and Kirschke [11], respectively, while activity of extralysosomal 1 mM Ca^{++}-activated neatral proteinase (CANP or Calpain) was determined by method of Dayton [3]. LDH and MDH isoenzymes were separated on 5.5% polyacrylamide gel electrophoresis as described by Dietz and Lubrato [4]. The different subunits were stained with tetrazolium blue and evaluated with Kipp and Zonen densitometer (The Netherlands). For measurement of protein content the microbiuret method was applied [9].

Isolation and purification of structural proteins were made with the method of Takacs [18]. The myofibrils were solubilized in 10 volumes of a mercaptoethanol and 10% sucrose solution. Myofibrillar proteins were separated on polyacrylamide gel (7.5%) containing 0.1% SDS by electrophoresis according to Weber and Osborn [20]. Myofibrillar protein patterns is illustrated on densitometric scans and numerical value of area under the curve was determined with a Kipp-Zonen densitometer-integrator.

The experimental results shown in the Figures are expressed as mathematical average of measurement values with standard deviation. Statistical evaluation of the results was made with Student' test. Mark "x" in the Figures denotes differences at a high level of significalce ($p < 0.01$) Enzyme activity values referred to 1 mg soluble protein.

Results

Enzyme activity of all the three dehydrogenase enzymes (U = μmole min^{-1}) in cleft palate muscles was significantly lower than in normal soft palate muscles (Fig. 2).

Decrease in enzyme activity was 57% at MDH and 42% at LDH or ICDH. LDH/MDH and LDH/ICDH activity ratios elevated from 0.63 (in normal) to 0.85 in cleft palate muscle. No difference in distribution of LDH isoenzymes could be found in normal and cleft palate muscles (Fig. 3). Considering H and M subunits, both cleft palate and healthy soft palate muscles proved to be fast glycolytic with similar H/M ratio (normal: 0.69, cleft palate: 0.65).

Percentage distribution of MDH isoenzymes in both muscles showed a relative excess of mitochondrial subunit, in the cleft palate muscle the M/C ratio was significantly lower (Fig. 4).

Analysing the activity values of the three transferase enzymes (CPK, GOT, GPT) it can be assumed (Fig. 5), that there was a significant decrease in the activity of transaminases in cleft palate muscles. GOT and GPT activities were about 35 and 20% lower. On the other hand, 14% decrease in activity of CPK in cleft palate muscle did not prove to be significant.

Comparing the values of endopeptidases studied in cleft palate to that of the normal, activity values measured in the later were considered 100% and the cleft palate values were given in percentage of the normal value.

Activity of lysosomal cathepsin L and D was 60% and 40% lower in cleft palate muscle than in normal, respectively, while that of extralysosomal CANP activity was about 16% higher (not significant).

Examining the densitograms of myofibrillar proteins isolated from normal (Fig. 7) and from patient with cleft palate: Sz. E. (Fig. 8), M.Z. (Fig. 8) - the similarities between the densitograms 7 and 8 can be easily noticed, but there are striking differences in the densitogram 9.

In Table 1 the percentage composition of individual myofibrillar components is illustrated in normal and cleft palate muscles. In the first two columns average values can be found, while in the other three columns the individual values of Figs. 7, 8, and 9 are shown.

In respect of average values no significant difference can be observed in the quantitative composition of myofibrillar proteins in normal or cleft palate muscles. On the other hand, there are striking individual differences in the values of cleft palate muscles not only in the activity of enzymes (Table 2), but also in the percentage distribution of myofibrillar proteins. This latter value is especially apparent in case of myosin light chains and that of troponin complex. This was why we found it necessary to analyse the obtained experimental results individually.

As not all the 50 densitograms can be shown here, we present only three examples in details: the values of normal child and two cleft palate patients, whose values were very different from each other both in their enzyme activities and in densitograms (see values of Sz.E. and M.Z.).

On the basis of the above values it can be established that values of samples taken from normal palate and from that of Sz.E. are almost identical in respect of myofibrillar pattern and percentage value. While in case of M.Z. there is a significant change even compared with the cleft palate average. Here 47% decrease in amount of myosin light chains, 34% in the troponin complex and 51% in amount of tropomyosin could be found in comparison to the cleft palate average. TN-I, U_1 and U_4 proteins were missing and between the M-α and M-β chains a peptide could be seen almost identical with the chain in quantity (indicated in Fig. 9). When these values are compared with the measured enzyme activities (Table 2.) it can be established that the activity values and the myosin light chains and the differences within the quantitative compostition of the troponin complex correlate with each other, since

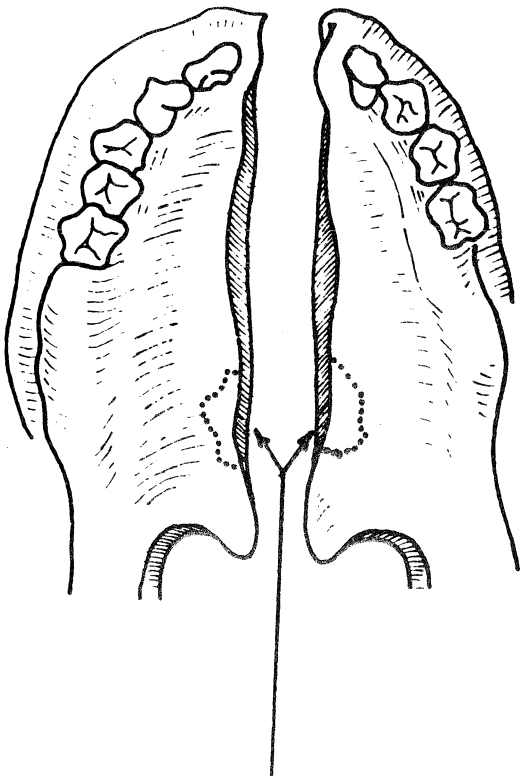

Figure 1. Location of needle biopsy.

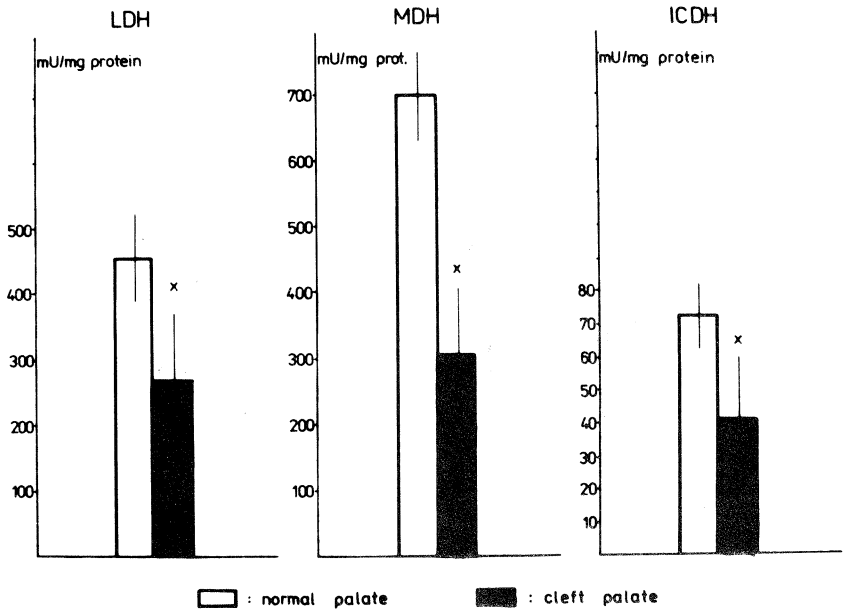

Figure 2. LDH, MDH and ICDH enzyme activity values in normal and cleft palate muscles.

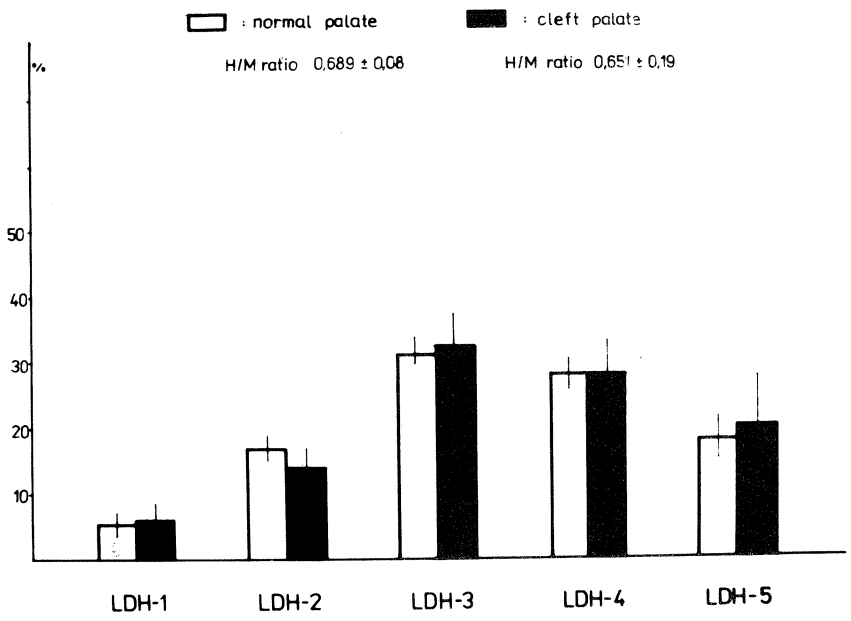

Figure 3. Percentage distribution of LDH isoenzymes in normal and cleft palate muscles.

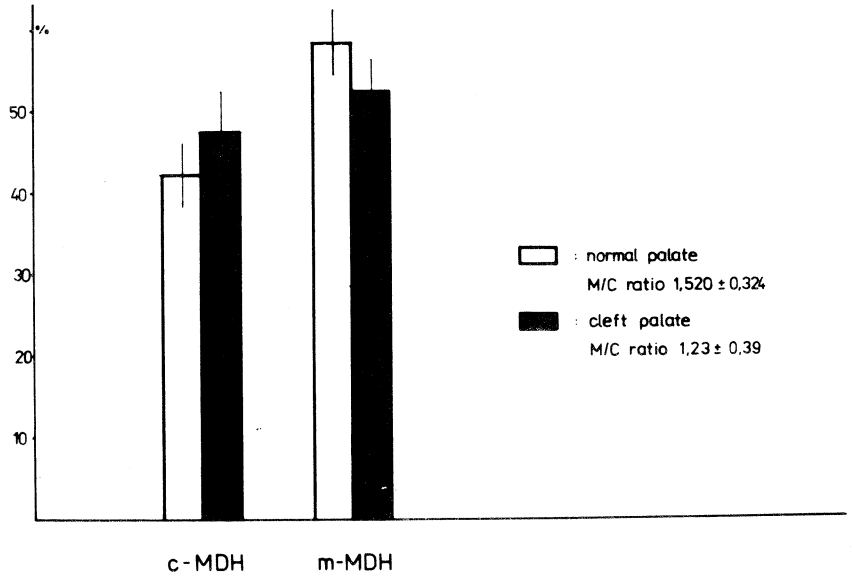

Figure 4. Percentage distribution of MDH isoenzymes.

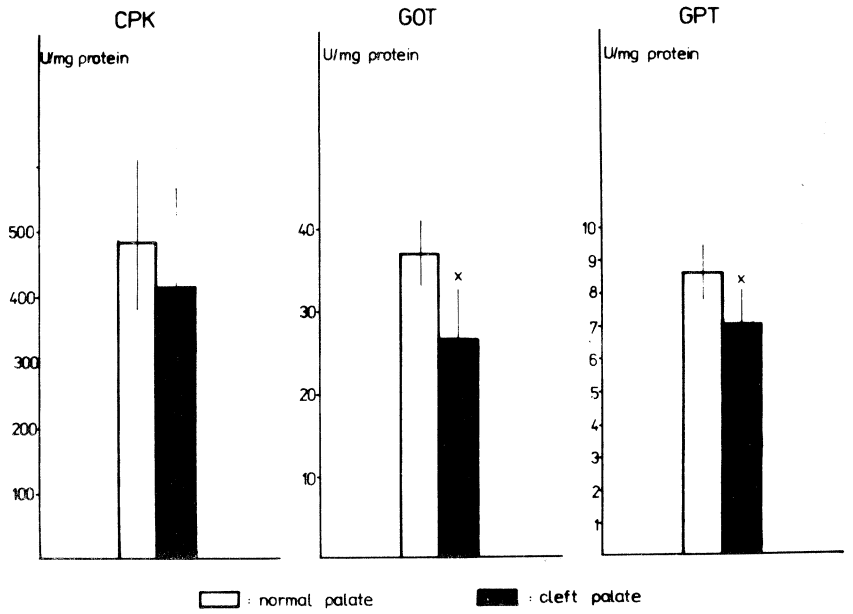

Figure 5. Activity values of three transferase enzymes in normal and cleft palate muscle.

Table 1. Percentage distribution of certain myofibrillar protein components in normal and cleft palate muscles.

Myofibrillar protein	Normal average%	Cleft palate average%	Normal KJ.See Fig. 7	Sz.E. See Fig. 8	M.Z. See Fig. 9
Myosin LC-3	3.42 ± 0.15	4.01 ± 1.12	3.33	4.06	2.10
Myosin LC-2	8.08 ± 0.75	8.95 ± 2.05	7.50	8.0	4.63
Troponin-C	5.56 ± 0.49	4.05 ± 1.21	6.13	6.84	2.81
Troponin-I	2.13 ± 0.61	1.75 ± 0.58	1.44	2.92	-
Myosin LC-1	5.22 ± 0.48	3.09 ± 1.44	5.76	5.45	1.82
Unknown-1	1.66 ± 0.04	1.27 ± 0.47	1.67	1.65	-
Unknown-2	4.13 ± 0.42	3.29 ± 0.99	3.71	3.49	0.28
Tropomyosin	3.93 ± 0.15	3.33 ± 1.01	4.09	3.93	1.54
Troponin-T	3.11 ± 0.25	3.09 ± 1.27	3.26	2.34	3.09
Actin	13.80 ± 0.34	13.80 ± 1.29	13.80	12.80	15.30
α-actinin	3.93 ± 0.61	4.11 ± 0.69	3.10	4.56	1.26
Unknown-3	2.16 ± 0.30	1.85 ± 0.71	1.82	2.92	0.70
Unknown-4	3.01 ± 0.41	2.73 ± 1.06	3.03	2.15	-
C-protein	2.85 ± 0.27	1.98 ± 0.69	2.73	4.56	0.98
M protein chain α	6.01 ± 0.61	8.57 ± 1.95	5.83	7.16	12.30
M protein chain β	9.49 ± 1.75	12.30 ± 3.05	10.40	7.67	18.80
Myosin heavy chain	21.51 ± 0.54	21.80 ± 0.99	21.70	19.51	25.00
Unknown-5	0	0	0	0	9.41

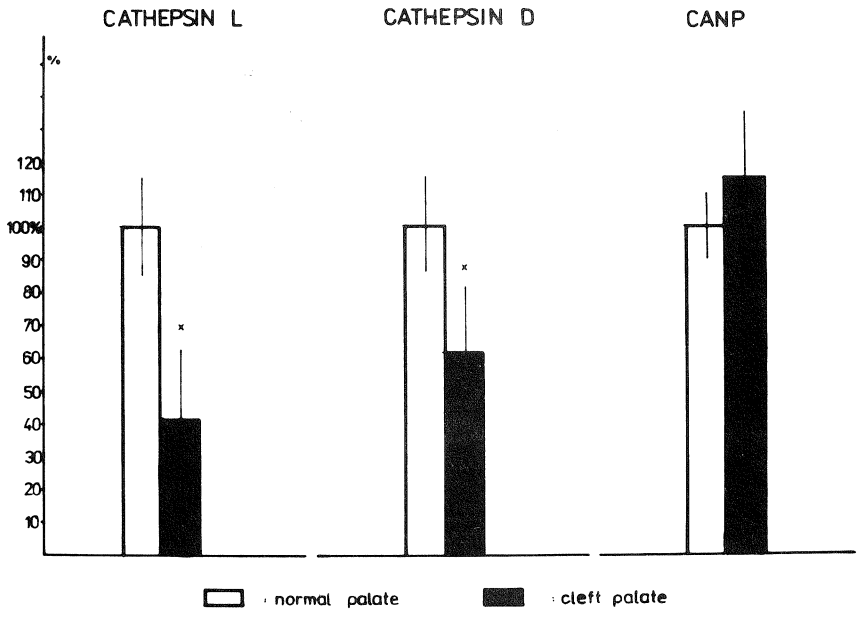

Figure 6. Endopeptidase activity values in normal and cleft palate muscles.

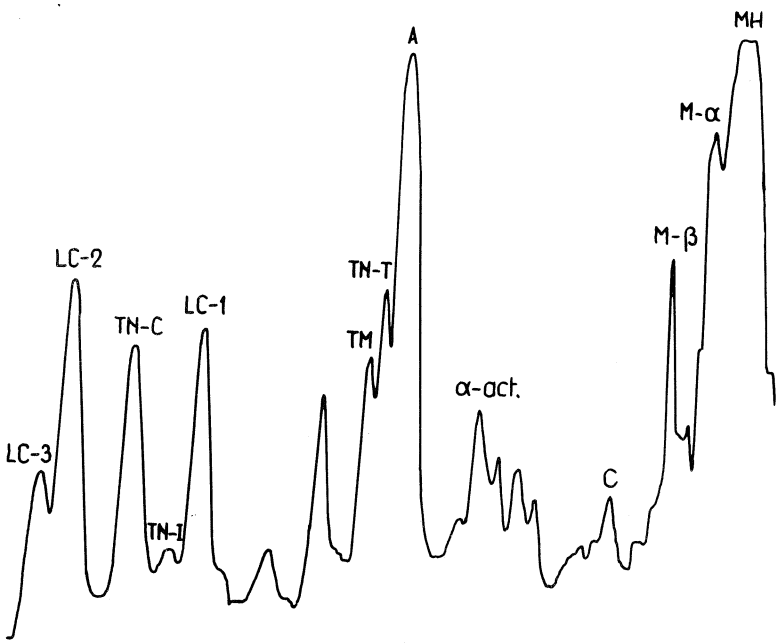

Figure 7. Densitogram of myofibrillar proteins obtained from normal muscle.

in case of M.Z. also the enzyme activity values are significantly lower than in the cleft palate average.

Among the 30 examined infants with cleft palate out of M.Z.'s values there was one more infant (V.I.) whose value was much lower than the cleft palate average.

Discussion

Summarizing our experiences it can be stated that in the cleft palate muscles, with the exception of CPK and CANP, there was a significant decrease in the examined enzyme activities compared to that of the normal. LDH/MDH, LDH/ICDH and mMDH/cMDH ratios demonstrate a decrease of aerobic processes and a relative excess of glycolytic metabolism in the cleft palate. Both dehydrogenases and transaminases changed their activity similarly to that observed in other muscle damages, e.g. immobilization [6, 17], tenotomy, denervation [8, 10, 19] and dystrophies [5, 15, 22]. The activity of these enzymes also decreased partly, due to altered enzyme synthesis and increased catabolism.

No differencies can be found, however, in cleft palate muscle compared to normal in the distribution of LDH and MDH isoenzymes, unlike immobilization and muscle dystrophies where significant change can be seen in the distribution of these isoenzymes as a sign of the undergoing dedifferentiation [17].

Decreased values of lysosomal endopeptidase activities in cleft palate were different compared with other muscle damages. Proteinase activity was elevated at the alteration period, but it turned to normal or to lower activity values at economic metabolic level. This theory was verified by lower metabolic enzyme activities.

All these facts prove that in cleft palate muscles there is a lower level of metabolism than in normal velopharyngeal muscles. Activation of proteases takes place from a reserve enzyme pool which is not supplied adequately in the state of considerable energy deficiency. Consequently such an energy-deficient state is assumed to be in cleft palate muscles the existence of which can be probed by the low activity values of LDH, MDH and ICDH. Cleft palate muscles adapt

Table 2. Enzyme activities in normal and cleft palate muscles.

Enzyme activity	Normal	Cleft palate	Normal K.J.	Sz.E.	M.Z.
LDH (mU/mg protein)	453 ± 86	266 ± 101	449.9	363.4	164.3
GOT (U/mg protein)	37 ± 4.9	22 ± 6.5	36.9	24.6	18.6
Cathepsin L (U/mg protein)	184 ± 36	122 ± 30.4	164.6	142.4	90.4

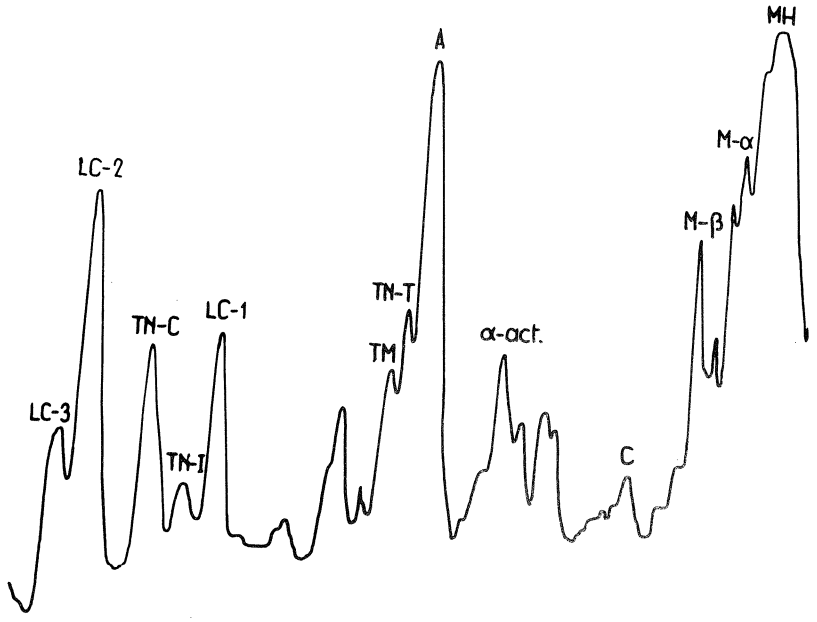

Figure 8. Densitogram of myofibrillar proteins isolated from Sz. E. cleft palate muscle.

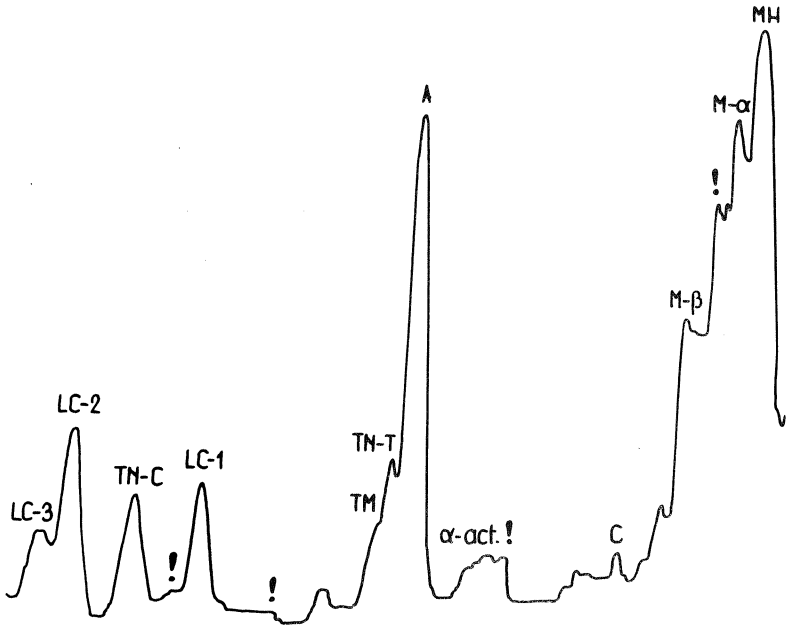

Figure 9. Densitogram of myofibrillar proteins obtained from M.Z. cleft palate muscle.

to that level of protein breakdown which correspond to the already decreased metabolic processes.

On the basis of these values the conclusion can be drawn that cleft palate muscles are genetically hypoplasic, inactive muscles, disabled in their function because of the lack of closure in middle line in aponeurosis and most probably this explains their decreased blood supply as well (Fig. 10).

The quantitative myofibrillar protein composition does not show significantly differs between normal and cleft palate muscles. From this comparison it is evident that there is no difference between the differentiation of normal and cleft palate muscles, since both muscles are of fast glycolytic type. The myosin/actin ratio is nearly identical. On the other hand, in some cases there were high individual differences in the amount of myosin light chains and troponin complex and they correlated with the different enzyme activity values of the same samples.

Thus, attention must be drawn to the high individual differences in the metabolism of cleft palate muscles in different patients. There is no doubt that after surgical reconstruction the restored function of the muscle will produce increased metabolic capacity, but its degree can not be independent from the original state which might be one of the factors explaining the differences in later functional results after cleft palate reconstruction. In the cases of the mentioned two infants (M.Z. and V.I.), where together with the extremely low activity values there was serious decrease in the quantity of certain structural proteins and the probably genetic lack of 3 proteins, it was highly probable that the later functional result was independent from the success of the chosen surgical method and the logopaedic post-care. After 5 years of follow up we can say that the children who had lower metabolic enzyme activities in cleft palate muscle at reconstruction are not able to speak as good as others having had enzyme activity near to normal.

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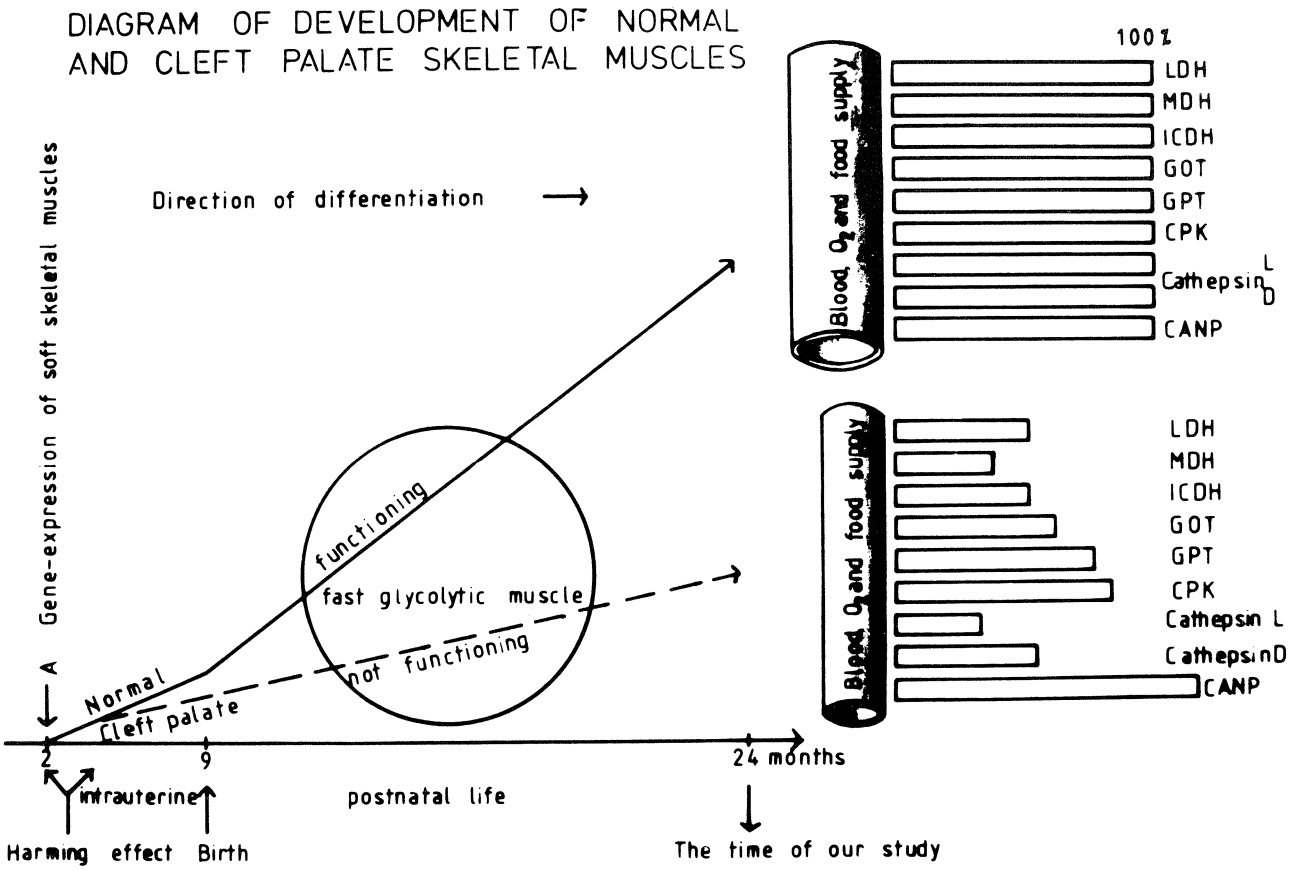

Figure 10. Diagram of development of normal and cleft palate muscles.

References

[1] Barrett AJ: Lysosomal enzymes, in Dingle JT (ed): *Lysosomes a laboratory handbook*. North Holland Pub. Comp. Amsterdam, 1972, pp. 123-126.

[2] Bergmeyer HU (ed): *Methods of Enzymatic Analysis*. Academic Press, New York, 1974.

[3] Dayton WR, Reville WJ, Goll DE, Stromer MH: A Ca^{++} activated protease possible involved in myofibrillar protein turnover. *Biochemistry* 1975; 15: 2139-2167.

[4] Dietz AA, Lubrato T: Separation and quantitation of LDH isoenzymes by disc electrophoresis. *Anal Biochem* 1967; 20: 246-251.

[5] Dolan L, Chew L, Morgan G and Kidman AD: Enzyme studies of skeletal muscle in mice with different types of neural impairment and muscular dystrophy. *Exp Neurol* 1975; 47: 105-117.

[6] Edes I, Sohár I, Mazarean H, Takacs Ö, Guba F: Changes in the aerobic and anaerobic metabolism of skeletal muscle subjected to plaster cast immobilization. *Acta Biochim et Biophys Acad Sci hung* 1980; 15: 305-311.

[7] Gaidadjiev H: Changes in thee activities of LDH, GOT and GPT in some muscular atrophies: I. Post-tenotomy changes. *Exp Med Morf* 1973; 12: 66-70.

[8] Gaidadjiev H: Changes in the activities of LDH, GOT and GPT in some muscular atrophies: II. Changes after denervation, *Exp Med Morf* 1974; 13: 223-226.

[9] Goa J: Micro biuret method for protein determination. *Scand. J Clin Lab Invest* 1953; 5: 218-222.

[10] Hogan EL, Dawson DM, Romanul, FA: Enzymatic changes in denervated muscle. *Arch Neurol* 1965; 13: 274-281.

[11] Kirschke H, Langner J, Wiederanders B, Ansorge S, Bohley P, Hanson H: Cathepsin L. A new proteinase from rat-liver lysosomes. *Eur J Biochem* 1977; 74: 293-301.

[12] Krause CJ, Tharp RF, Morris HL: A comparative study of results of the von Langenbeck and the V-Y pushback palatoplasties. *Cleft Palate J*, 1976; 13: 11-17.

[13] McEvitt WG: The incidence of persistent rhinolalia following cleft palate repair: analysis of 439 patients. *Plast Reconstr Surg* 1971; 47: 258-263.

[14] Musgrave RH, McWilliams BJ, Matthews HP: A review of the results of two different surgical procedures for the repair of the soft palate only. Cleft Palate J, 1975; 12: 281-287.

[15] Niebroj-Dobosz I: The influence of cysteine, reduced glutathione and 2-mercaptoethanol on creatine phosphokinase activity in muscular diseases. *Clin Chim Acta* 1976; 71: 327-329.

[16] Sohar I, Nagy I, Guba F: Methods for measurement of some proteolytic enzyme activities in meat. *Adv Physiol Sci* 1980; 24: 382-387.

[17] Sohar I, Takács Ö, Guba F: The influence of immobilization on soluble proteins of muscle. *Acta Biol Med* 1977; 36: 1621-1624.

[18] Takacs Ö, Szöör A, Sohar I, Kesztyüs L, Guba F: Changes in the submolecular composition of the myofibrils. *Acta Biol Acad Sci Hung* 1981; 32: 33-43.

[19] Usatenko MS: Functional interrelations between isozymes of dehydrogenases in the intact and denervated rabbit muscles. *Biochimia* 1977; 42: 311-318.

[20] Weber K, Osborn M: The reliability of molecular weight determinations by dodecyl sulphate-polyacrylamide gel electrophoresis. *J Biol Chem* 1969; 244: 4406-4410.

[21] Witzel MA, Clarke JA, Lidsay WK, Thomson HG: Comparison of results of pushback or von Langenbeck repair of the hard and soft palate. *Plast Reconstr Surg* 1979; 2: 347-352.

[22] Yasmineh WG, Ibrahim GA, Abbasnezhad M, Awad EA: Isoenzyme distribution of CK and LDH in serum and skeletal muscle in Duchenne muscular dystrophy, collagen disease, and other muscular disorders. *Clin Chem* 1978; 24: 1985-1989.