Histochemical Differentiation of Skeletal Muscle Fibres in the Bovine Foetus

Histochemical studies on foetal muscle are rare. With the available literature it appears that no work has been recorded on the histochemistry of bovine foetal muscle, while adult muscle fibres were divided into 3 types by oxidative enzyme reaction[1].

Red muscle fibres are rich in oxidative enzymes and are smaller in size, while the white fibres are rich in phosphorylase and are larger in size[1-3]. Staining for oxidative enzymes has revealed the presence of red, white and intermediate fibres in various vertebrates. Of the various oxidative enzyme activities on the muscles of many vertebrates, the difference in stainability among the 3 types of fibres was not so clearly demonstrated as that in succinic dehydrogenase[1]. Adult muscle fibres could also be differentiated by staining for myoglobin[4,5]. The red fibres are rich in myoglobin while the white fibres are poor in myoglobin.

The present work is an endeavour to differentiate the skeletal muscle fibres in the bovine foetus by the succinic dehydrogenase activity. An experiment is also designed to ascertain whether a test for myoglobin could be used for fibre differentiation in the foetus.

Materials and methods. Bovine foetuses collected from the abattoir were used. Shortly after the cows were slaughtered the foetuses were obtained and their crown rump lengths (CRL) recorded. The measurements were compared with tables of WINTERS et al.[6] to get an approximate idea of the ages. Muscle samples were taken from M. gastrocnemius, M. rectus femoris, and M. longissimus dorsi. Similar samples were collected from adult cows for comparative study. Frozen sections were cut at 20–40 µm and transferred to the incubating medium. For demonstration of succinic dehydrogenase (SDH), the method prescribed by PEARSE[7] was adopted using nitroblue tetrazolium, and for myoglobin the technique used by MORITA et al.[5] was tried. Diameters of approximately 100 muscle fibres were measured to obtain normal values.

Results. Staining for myoglobin did not reveal any distinct difference between fibre types before birth, but in the adult muscle it was possible to differentiate between fibre types (Figure 1). A positive SDH activity has been taken as the criterion for demonstrating metabolic differences between fibres.

15, 18, and 24 cm CRL foetuses (85, 100 and 110 days of gestation). Muscle samples from these age groups were mostly in the myotube stage of development. 2 distinct groups of fibres were noted by size difference. The majority of the fibres were small and they were lying around large fibres. The SDH activity was faint. A few granules were found scattered towards the central part of the fibres.

30 and 34 cm CRL foetuses (130 and 145 days gestation). The SDH activity had increased considerably and it had spread all along the fibre. No differentiation was possible between fibre types.

40, 50, 60 and 65 cm CRL foetuses (155, 175, 200, and 210 days gestation). At 40 and 50 cm CRL stages it was possible to identify light and dark fibres depending upon the staining reaction (Figure 2). In the 60 cm CRL foetus the differentiation was well advanced in all the 3 muscles examined (Figure 3).

70 and 90 cm CRL foetuses (215 and 260 days gestation). At these stages difference in staining intensity and fibre diameters made it possible to distinguish the 3 fibre types (Table and Figure 4).

Discussion. The present results show that the SDH activity, which was apparent at the 15 cm CRL foetus in the centre of the developing muscle fibres, spread steadily as the age advanced, and there was considerable increase in activity in the course of foetal development. The SDH may be taken to be the expression of tricarboxylic acid cycle activity and the presence of these enzymes in the early stages of development expresses the greater energy requirements of the muscle cells[8]. The presence of perinuclear enzymatic activity is related to the energy required for protein synthesis, while its spread throughout the sarcoplasm as the fibre matures is also related to muscle contraction.

The development of enzyme activity in the bovine foetal muscle can be divided into 3 phases: In phase I,

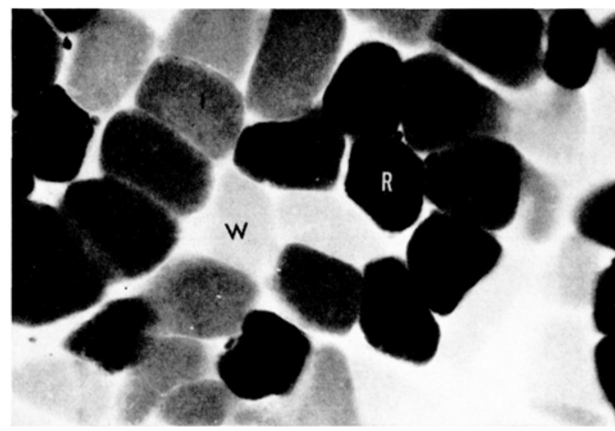

Fig. 1. Section of longissimus dorsi muscle of cow stained for myoglobin. Note red (R), white (W) and intermediate (I) fibres. ×100.

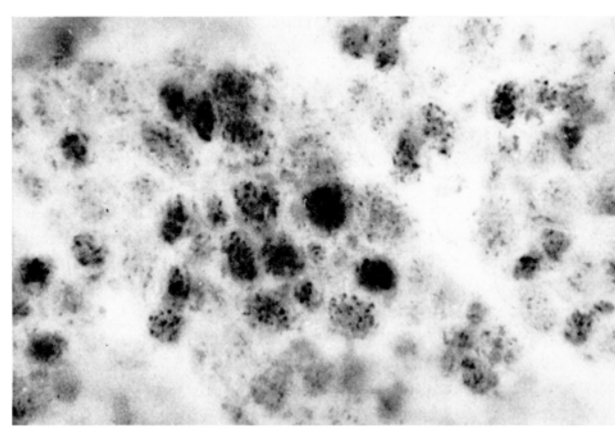

Fig. 2. 40 CRL foetus. Gastrocnemius. Note 'light' and 'dark' fibres. SDH. ×160.

[1] T. OGATA and M. MORI, J. Histochem. Cytochem. *12*, 171 (1964).
[2] V. DUBOWITZ and A. G. E. PEARSE, Nature, Lond. *185*, 701 (1960).
[3] G. JASMIN, Ann. N.Y. Acad. Sci. *138*, 186 (1966).
[4] N. T. JAMES, Nature, Lond. *219*, 1174 (1968).
[5] S. MORITA, R. G. CASSENS and E. J. BRISKEY, Stain Tech. *44*, 283 (1969).
[6] L. M. WINTERS, W. W. GREEN and R. COMSTOCK, Rep. Minn. agric. Exp. Stn. Tech. Bull. *49*, 151 (1942).
[7] A. G. E. PEARSE, *Theoretical and Applied Histochemistry*, 2nd edn (J. A. Churchill, Ltd., London 1960), p. 910.
[8] N. I. GERMINO, H. D'ALBORA and J. P. WAHRMANN, Acta Anat. *62*, 434 (1965).

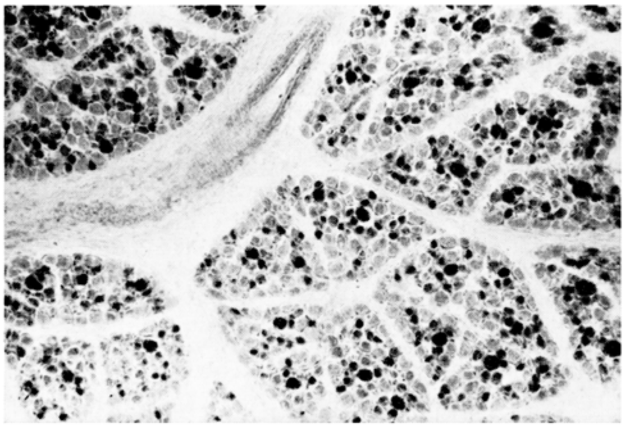

Fig. 3. 60 CRL foetus. Longissimus dorsi. Well differentiated red and white fibres are to be seen. SDH. ×40.

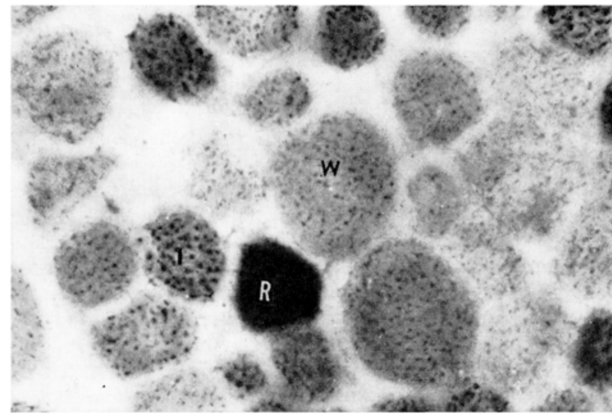

Fig. 4. 90 CRL foetus. Gastrocnemius muscle. Note red (R), white (W) and intermediate (I) fibres. SDH. ×160.

Diameters of red, white and intermediate skeletal muscle fibres at various gestational ages and in adult cows

Muscle	CRL (cm)	Gestation ages (days)	Fibre size range (μm)			Mean value (μm)		
			R	W	I	R	W	I
Gastrocnemius	50	175	3.7– 7.4	11.1– 18.5	..	5.5	14.6	..
	60	200	7.4–11.1	14.8– 18.5	..	9.6	15.9	..
	65	210	11.1–18.5	22.2– 37.0	..	15.2	28.5	..
	70	215	7.4–14.8	18.5– 22.2	14.8–18.5	12.2	19.2	12.7
	90	260	11.1–25.9	25.2– 37.0	11.1–25.9	18.1	33.7	21.8
	Cow		33.3–51.8	74.0–129.5	44.4–74.0	40.7	107.3	56.9
Rectus femoris	50	175	7.1–11.1	11.1– 14.8	..	7.8	13.7	..
	60	200	11.1–14.8	11.1– 22.2	..	12.6	17.8	..
	65	210	11.1–18.5	14.8– 18.5	..	12.6	17.0	..
	70	215	7.4–14.8	14.8– 22.2	11.1–18.5	11.3	18.6	13.3
	90	260	14.8–25.9	22.2– 44.4	14.8–25.9	18.5	32.0	21.5
	Cow		18.5–37.0	37.0– 55.5	18.5–33.3	30.3	48.4	24.8

CRL, crown rump length; R, red; W, white; I, intermediate.

approximately 155–175 days of gestation, there was some variation in the enzyme content of different fibres, but this was not consistent and there was no clear-cut subdivision into red and white fibres. In phaşe II, approximately 200–210 days of gestation, there was a clear-cut subdivision into red and white fibres. The third phase of development was noted by the appearance of intermediate fibres at 215–260 days of gestation. A similar observation almost corresponding to the above findings has been reported in the human foetal muscle[9].

BECKETT and BOURNE[10] have detected light and dark fibres at 12″ to $13^1/_2$″ goat foetus in tibialis anterior, biceps and gastrocnemius, but not in rectus femoris. They have also observed that there was no difference in size between light and dark fibres in the goat foetal muscles. On the other hand, the 3 bovine foetal muscles studied presently (viz., gastrocnemius, rectus femoris and longissimus dorsi) reacted uniformly at various gestational ages, and the histochemical differences between fibre types could be well correlated with the size of fibres.

The present results indicate that the bovine musculature begins to take on an adult appearance already at the end of the gestational period, even though the muscle fibres were much smaller than in the adult. A similar observation has been recorded in the human foetal muscle[11].

The muscle fibres were not differentiated in the longissimus dorsi muscle of the pig at any of the foetal stages[12], whereas the longissimus dorsi muscle of bovine foetuses in the present study was clearly differentiated at 200 days of gestation. The difference in maturation of fibre types may be accounted for by the gestational lengths of the animal species[11].

Zusammenfassung. Die Entwicklung der histochemischen Unterschiede in den Muskelfasern von Rinderfeten wurden mittels der Succinodehydrogenaseaktivität untersucht. Nach 155–175 Trächtigkeitstagen wurden helle und dunkle Fasern beobachtet, doch sind die Unterschiede erst nach 200 Trächtigkeitstagen deutlich. Die intermediären Fasern treten erstmals nach 215 Trächtigkeitstagen auf. Die Muskeln M. gastrocnemius, rectus femoris und longissimus dorsi zeigen eine gleichmässige Entwicklungsfolge.

P. A. OMMER[13,14]

Department of Anatomy, The Royal Veterinary and Agricultural University, Copenhagen (Denmark),
22 July 1970.

[9] V. DUBOWITZ, Nature, Lond. *211*, 884 (1966).
[10] E. B. BECKETT and G. H. BOURNE, Acta Anat. *35*, *224* (1958).
[11] V. DUBOWITZ, Nature, Lond. *197*, 1215 (1963).
[12] R. G. CASSENS, C. C. COOPER, W. G. MOODY and E. J. BRISKEY, J. Anim. Morph. Physiol. *15*, 135 (1969).
[13] Supported by the FAO Veterinary Faculty for FAO Fellows and Scholars, Copenhagen (Denmark).
[14] On deputation from the Department of Anatomy, Kerala Veterinary College, Trichur (Kerala, India).