The Nutrition of the Young Ayrshire Calf

10. Histopathology of Muscular Dystrophy and its Relation to Muscle Chemistry

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The purpose of this paper is to describe the histological appearance of tissues from the dystrophic calves reported by Blaxter, Watts & Wood (1952), to compare the dystrophic lesions with those found in naturally occurring muscular dystrophy of cattle (Hjärre & Lilleengen, 1936*a*, *b*, Vawter & Records, 1947; Slagsvold, 1925), and to relate the chemical composition of the muscle to its histological appearance.

METHODS

As described in a previous paper (Blaxter *et al.* 1952), four experimental groups of calves were involved; two receiving cod-liver oil as a source of vitamin A and D activity and two a solution of pure vitamins A and D in arachis oil. Vitamin E was given to animals in one of each of these groups, the other group receiving none. The calves are referred to by treatment and replication number as previously described. Blocks of muscle were fixed with 5 % formaldehyde or 5 % formol saline. Sections were stained by Mayer's haemalum and 5 % aqueous eosin. Gallego's method (see Langeron, 1949) was used to demonstrate connective tissue. Sections were also stained with phosphotungstic acid and haematoxylin.

RESULTS

Table 1 summarizes the results of the examination of skeletal and cardiac musculature, together with details of the macroscopic appearance of the muscle and its chemical composition. Both normal muscles from normal animals and 'normal' muscles from dystrophic animals were included in this study.

The agreement of the results was on the whole good. Muscle classified as normal macroscopically and containing the usual quantities of dry matter, total nitrogen and creatine was found to be normal when examined histologically. There were, however, several discrepancies. The flabby heart of calf no. CLO.E. 1, although containing subnormal amounts of creatine, was histologically normal, and the semimembranosus of calf no. CLO.E. 4, though of normal composition and appearance, nevertheless showed an increased number of nuclei. These discrepancies are partly due to the sampling errors involved when a small piece of muscle is excised for histo-

logical study and the remainder of the muscle macerated for chemical analysis, as well as to the subjective judgement involved in the classification of muscles into groups on the basis of their macroscopic appearance. It may be noted from the table that the severe lesion of the biceps femoris of calf no. CLO.O. 3 was bilaterally symmetrical. It may also be seen that where the lesion was localized and almost circumscribed, as in the heart of calf no. AO.O. 4, the surrounding tissue had a normal appearance.

On the basis of the experimental material presented in Table 1 a tentative account of the histogenesis of the lesion may be inferred. The earliest change appeared to be a swelling of the muscle fibre and a loss of staining power of the muscle bundles or small groups of fibres (Pl. 1, 1–3). In haematoxylin- and eosin-stained sections a deepening of the staining as the result of a closer arrangement of the anisotropic discs of the muscle fibres was found (Pl. 1, 5). The most significant change, however, was the reaction of the sarcolemmal nuclei (Pl. 1, 4, 5). These swelled in the early stages and then multiplied roughly in inverse proportion to the atrophy of the muscle cells, thus apparently maintaining the total amount of tissue (Pl. 1, 6). There was no gross fibrosis or fatty infiltration (Pl. 2, 2). The advanced lesion was remarkable for the enormous proliferation of the sarcolemma and the almost complete absence of muscle (Pl. 1, 6; Pl. 2, 1). The muscle remaining was fragmented, but retained its staining properties and its striation, though, functionally, it was clearly of no value (see Pl. 1, 6 and Pl. 2, 1). The blood vessels were normal and the nervous tissue that was present was also normal. Rarely, polymorphonuclear leucocytes were seen phagocytosing a necrotic bundle, but their rareness indicated that, essentially, the lesion was degenerative and not inflammatory.

The changes in the cardiac musculature evidently followed the same general sequence and the terminal phase is shown in Pl. 2, 1.

DISCUSSION

The lesions that we have observed in calves fed on vitamin E-deficient diets agree well with the lesions found in other species. There are, however, three discrepancies. Firstly, in the calf the muscle cells retained their striated appearance despite their considerable fragmentation, a conclusion supported by Vawter & Records (1947) on the basis of their examination of naturally occurring cases of *weisses Fleisch*. With dystrophic rats, Pappenheimer (1948) has stated that one of the earliest changes to be found was a loss of striation and in pseudo-hypertrophic dystrophy in man the striations also disappeared in the early stages of the disease (Bicknell & Prescott, 1946). Hjärre & Lilleengen (1936*a*, *b*) refer to a 'diskoider Zerfall'. This could be interpreted as a loss of striation, but such a loss is not apparent in the photographs which they have published.

The second difference between the micropathological appearance of the lesion in our calves and those in other species concerns the sarcolemmal proliferation and negligible infiltration by polymorphonuclear leucocytes. The naturally occurring cases of *weisses Fleisch* that have been described are characterized by marked variation in the interpretation of the origin of the masses of proliferating nuclei. With the exception of Nieberle (1926) all workers concur that no reactively inflammatory

Table 1. *Description of gross lesions, histopathology and chemical composition of normal and dystrophic muscles in calves*

Calf no.	Muscle	Composition of muscle: Dry matter (%)	Nitrogen (%)	Creatine (mg/100 g)	Gross appearance of muscle	Histological appearance of muscle
CLO.O. 3	Heart	22·9	2·64	227	Normal	Normal
AO.E. 4	Heart	20·6	2·79	320	Normal, dark in colour	Normal
AO.E. 1	Heart	20·0	2·38	229	Normal	Normal
AO.E. 2	Heart	20·0	2·54	235	Normal	Normal
CLO.E. 1	Heart	20·2	2·60	161	Normal, but very flabby	Apparently normal
CLO.O. 1	Heart	19·9	2·84	246	Normal	Normal
CLO.E. 2	Heart	19·9	2·68	177	Pale, otherwise normal	Scattered bundles of nuclei throughout section examined, probably representing an early stage of dystrophy
CLO.O. 4	Heart	19·5	2·51	276	Small haemorrhages in the auriculo-ventricular valves but no indication of dystrophy	Normal
AO.O. 4	Heart	20·6	2·73	235	Two areas of necrosis on outer wall of left ventricle and one large internal dystrophic lesion with smaller other lesions	(*a*) Apparently unaffected area of muscle. Normal (*b*) Severely dystrophic area. Large tracts of muscle replaced by proliferated sarcolemmal cells, each in a pocket of delicate connective tissue. Fine bands of connective tissue but no coarse replacement-fibrosis apparent. Slender wisps of muscle remained unaffected. No leucocytic infiltration
AO.E. 4	Rectus femoris	22·1	3·36	463	Normal	Some slight variation in staining power of muscle cells but normal in other respects
	Left long head of triceps	21·9	3·28	468	Normal	Normal
	Right long head of triceps	21·8	3·28	412	Normal	Normal
CLO.E. 4	Biceps femoris	21·6	3·19	418	Normal	Normal
	Semi-membranosus	21·8	3·26	512	Normal	Very slight increase in the number of nuclei present

AO.O. 3	Rectus femoris	20·6	2·91	322	Two large white areas in centre of muscle—severely affected	Several areas of increased pigmentation of muscle bundles with possibly local increases in the number of sarcolemmal nuclei. No fibrosis, and none of the muscle atrophic
AO.O. 4	Rectus femoris	22·3	3·20	417	One large pale area present	A diffuse increase in the number of sarcolemmal nuclei. Majority of muscle bundles hyalinized with loss of striations; some swollen
	Long head of triceps	20·4	2·74	261	One very large white area	A severe lesion with marked atrophy. Loss of striation and staining power throughout the whole section. Intense sarcolemmal proliferation but no evidence of leucocytic proliferation or of an increase in connective tissue
CLO.O. 3	Left biceps femoris	18·0	2·36	138	Whole muscle dead white in colour with small streaks apparently normal	A very severe lesion in which the proliferated sarcolemma was completely dominant. Normally staining muscle present only in flecks. Muscle clearly non-functional. Several of the muscles hyalinized. Multinucleate giant cells had formed from the sarcolemmal tissue. No leucocytes seen
	Right biceps femoris	—	—	—	Whole muscle dead white and the extent of dystrophy indistinguishable from that of the left biceps femoris	Lesion almost identical with that of the left biceps femoris
	Long head of triceps	18·1	2·42	205	Centre of muscle entirely white, fading to a more normal colour at the periphery	Lesion comparable to that found in the left biceps femoris
CLO.O. 4	Vastus medialis	17·8	—	208	Whole muscle white in colour	Very severe lesion comparable to that of left biceps femoris of calf CLO.O. 3
	Long head of triceps	16·8	2·21	200	Large white non-circumscribed areas throughout the muscle	Very severe lesion comparable in every respect to that of left biceps femoris of calf CLO.O. 3

changes take place in the interstitial tissues, and that only in exceptional cases are histiocytes and leucocytes observed (Ziegler, 1926; Vawter & Records, 1947; Hjärre & Lilleengen, 1936*a*, *b*). The nuclear masses have been regarded by Hjärre & Lilleengen as regenerating muscle nuclei beneath the sarcolemma, and Vawter & Records (1947) interpret them as a 'dense mass of muscle nuclei, polymorphonuclear leucocytes, histiocytes and giant cells'. Our findings are not so. Jones & Read (1948), reporting on a naturally occurring case of muscular dystrophy in a foal, emphasize that the nuclear masses are of sarcolemmal origin, but in their animal, in which the immediate cause of death was broncho-pneumonia, lymphocytes and neutrophils were also present. In the suckling rat, Olcott (1938) has shown that marked sarcolemmal proliferation occurs, and this too has been the conclusion of Follis (1948). Pappenheimer (1948), however, states that in the rat there is a 'strong inflammatory response' to the initial segmentation and hyaline degeneration of the muscle cell. In the rabbit, the multinucleate masses are thought to be fused polymorphonucleate leucocytes and histiocytes (Pappenheimer, 1940–1). It would appear, therefore, that part of the differences may perhaps be due to the difficulties of interpretation of sections in which such marked changes have occurred. From the present studies with calves, however, and published findings with rabbits, guinea-pigs and men, it appears that irrespective of species an attempt is made to maintain constant the volume of the dystrophic muscle. With the calf this is done by massive sarcolemmal proliferation, with other species by excessive fat deposition. This may reflect the quite normal differences in anabolic processes between young calves, which store negligible fat but very large amounts of protein, and mature rabbits, guinea-pigs and men, in which protein deposition is normally minimal and fat deposition predominates.

The third point of difference concerns the incidence of calcification. This aspect has already been referred to when discussing the biochemical results (Blaxter & Wood, 1952). In naturally occurring cases, Vawter & Records have commented on the extensive calcification of the muscles, and Hjärre & Lilleengen (1936*a*, *b*) have stated: '...mitunter liegen feine Ca-Körner reihenweise zwischen den Myofibrillen, was eine abnorme deutliche Längsstreifung veranlasst...'. We have not however observed such longitudinal striation in our sections.

From these results and comparisons, additional support is thus given to the contention that the naturally occurring disease of *weisses Fleisch* in calves is very closely comparable to the vitamin E-deficiency state which we have produced.

SUMMARY

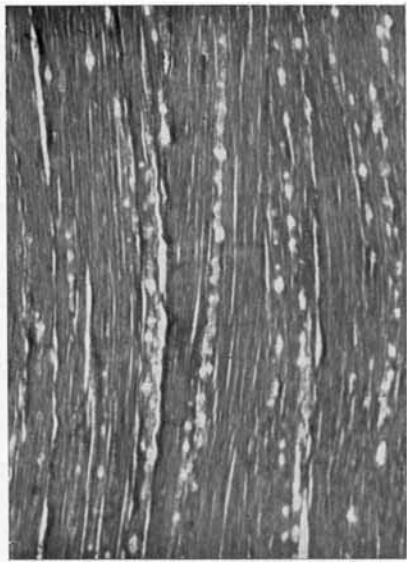
1. A description of the histology of muscular lesions observed in calves is presented.

2. It was shown that the macroscopic, biochemical and histological data were in good agreement.

3. Differences between the histology of the lesion in the dystrophic calf and in other dystrophic animals are emphasized.

We are obliged to Mr W. Mason and Mr Evett of the Children's Hospital, Glasgow, for the photographs.

Plate 1



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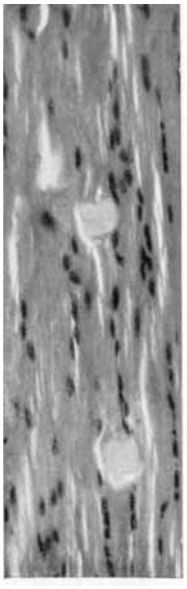

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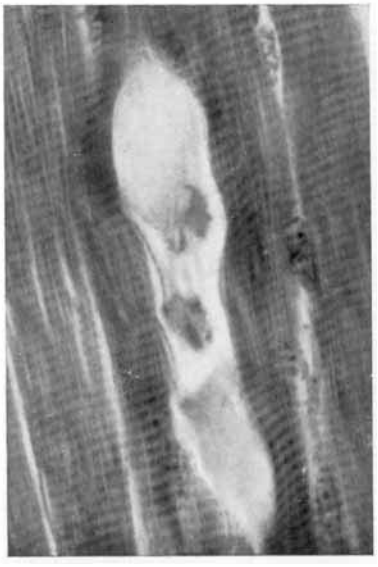

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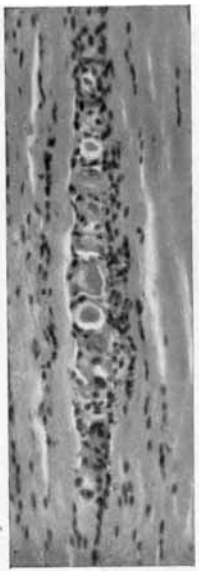

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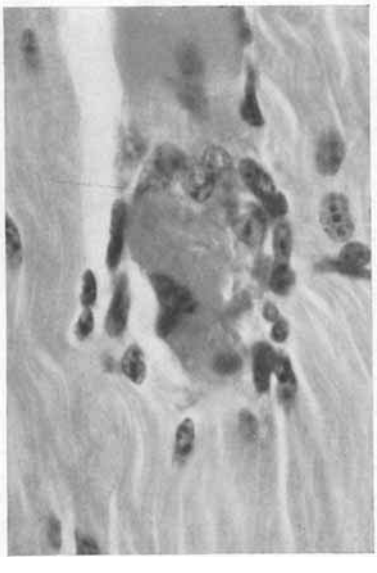

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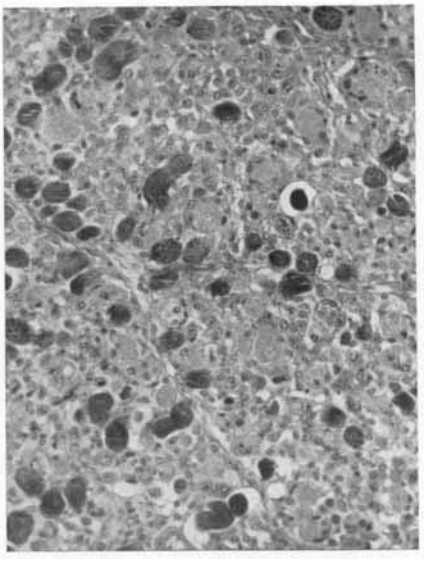

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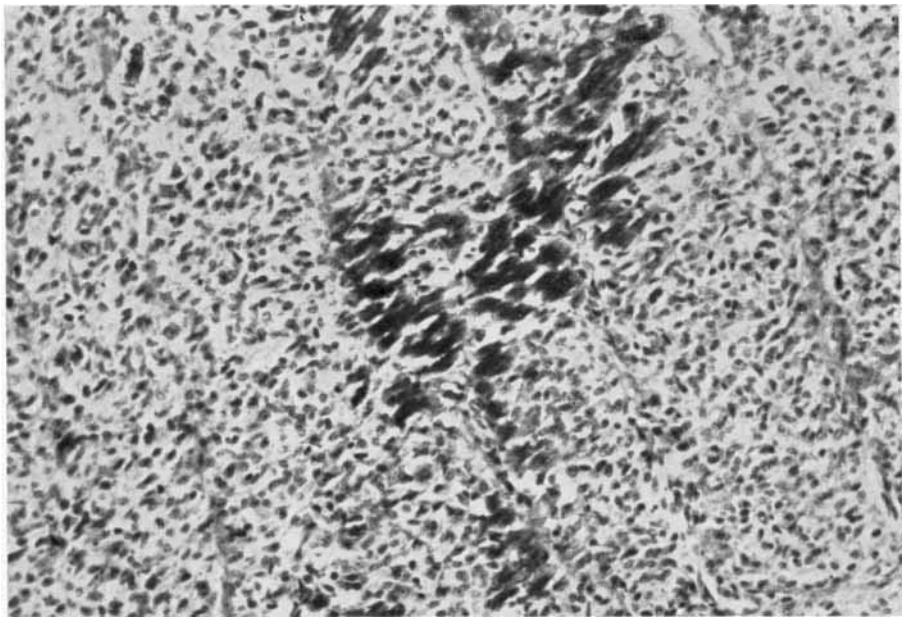
Plate 2



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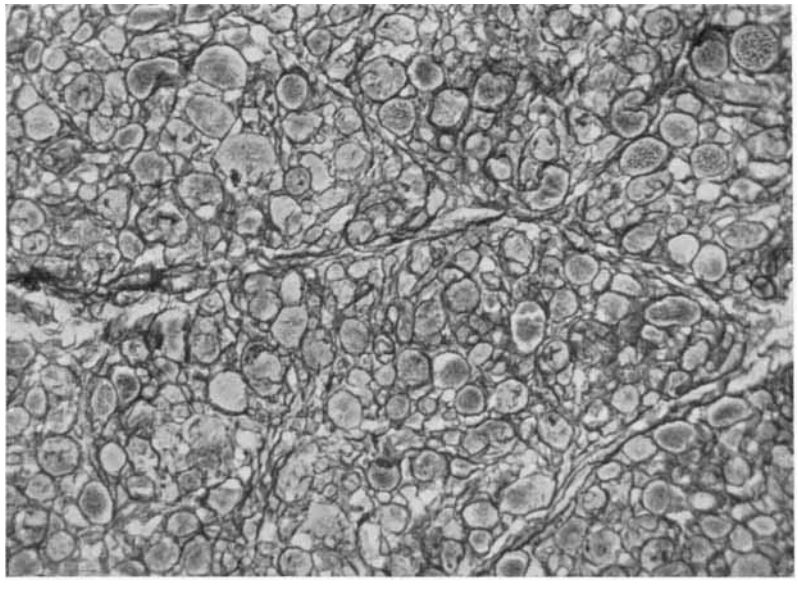

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EXPLANATION OF PLATES

Plate 1

1. Low-power magnification of skeletal muscle of calves showing affected fibres appearing as white beaded streaks in the midst of the dark normally staining muscle. The lesion is punctate and clearly defined. Phosphotungstic-acid haematoxylin stain. × 30.
2. Very early lesion of skeletal muscle of calves. Small hyaline nodes are shown in a muscle bundle or possibly fibre, surrounded by normal muscle and a normal density of sarcolemmal cells. Mayer's haemalum and eosin. × 100.
3. Lesion of skeletal muscle of calves similar to that in Pl. 1, 2. Note the isolated hyalinization surrounded by normally striated muscle. Phosphotungstic-acid haematoxylin stain. × 370.
4. Beginning of sarcolemmal proliferation around an affected muscle bundle in skeletal muscle of calves. Mayer's haemalum and eosin. × 70.
5. High-power magnification of the early sarcolemmal proliferation in skeletal muscle of calves. Irregular striation of the affected fibres is visible and the initial hyaline lesion is now necrotic. Mayer's haemalum and eosin. × 370.
6. Advanced lesion in a cross-section of skeletal muscle of calves. Great sarcolemmal proliferation; some apparently normal dark-staining muscle remains, but several muscle bundles may be seen which have completely lost their staining reactions. Phosphotungstic-acid haematoxylin stain. × 70.

Plate 2

1. Dystrophic heart muscle of calf no. AO.O. 4. A wisp of normally staining muscle remains in a massive sarcolemmal proliferation. Phosphotungstic-acid haematoxylin stain. × 70.
2. Cross-section of severely dystrophic muscle of calves stained by the Gordon-Sweets method for reticulum. × 100.