The Nutrition of the Young Ayrshire Calf

12. Factors Affecting the Tocopherol Reserves, Muscle Composition and Muscle Histology of 4-day-old Calves

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(*Received* 14 *March* 1952)

In previous experiments (Blaxter, Watts & Wood, 1951, 1952; Blaxter, Wood & MacDonald, 1953) it was observed that when each calf of a group was given the same quantity of a standard diet that produces muscular dystrophy, a very large variation occurred in the time of onset of clinical signs of the disease. With some diets, one calf was observed to show no signs, yet all its contemporaries, managed and fed under identical conditions, died. The groups of calves for these experiments were bought in the market, and in most instances little was known regarding either their nutritional history during the first few days of life, or the nutritional status of their dams. The initial reserves of the calf in respect of α-tocopherol were thought to be at least one factor responsible for the above variation. The marked seasonal variation in the incidence and severity of both the experimentally produced disease (Blaxter, Wood & MacDonald, 1953) and of that which occurs naturally (Slagsvold & Lund-Larsen, 1934; Hjärre & Lilleengen, 1936) with maximums in the late winter and early spring also suggests that variation in the tocopherol reserves of the calves is important.

It is known that the tocopherols are present in large amounts in the colostrum of rats (Mason & Bryan, 1940), women (Abderhalden, 1945*b*), and cows (Parrish, Wise & Hughes, 1947). In cows the concentration was found to be ten to twelve times that of the milk in full lactation. When calves suckle, the colostral tocopherols rapidly appear in the blood serum (Parrish, Wise, Latschar & Hughes, 1950) and the serum tocopherols of the calf increase nine- to tenfold. On this evidence, Parrish (1949) concluded that colostral transfer was far more important than placental transfer in supplying the calf with tocopherols. With rats, Mason & Bryan (1938, 1940) showed that placental transfer of vitamin E was negligible as gauged by the time of onset of testicular degeneration in the offspring. Tocopherols are, however, present in the blood and liver of newborn infants (Abderhalden, 1945*a*, *b*), lambs, kids, piglets (Whiting & Loosli, 1948) and calves (Parrish *et al.* 1950) that have never suckled their mothers, or received any food. Many studies with cows (Scherer, 1946; Parrish *et al.* 1947; Harris, Swanson & Hickman, 1947; Kachmar, Boyer, Gullickson, Liebe & Porter, 1950) have shown that an increase in the intake of tocopherols results in an increase in the concentration of the vitamin in both milk and colostrum. The same is true of sheep (Whiting, Willman & Loosli, 1949). The results of studies with sheep,

goats and pigs (Whiting & Loosli, 1948) prompted Loosli (1949) to state that since the tocopherol status of the dam is reflected in both the serum tocopherols of the newborn and in the vitamin level of the colostrum, maternal nutrition is of considerable importance in determining the vitamin E reserves of the young. Though true of the sheep, goat and pig, these results may not apply to man, since Straumfjord & Quaife (1946) found no correlation between the vitamin E status of mother and offspring as judged by tocopherol levels in the blood plasma of both the mother and the newborn child.

The present studies were designed to find whether the normal nutritional practices of farms in the west of Scotland could result in any considerable variation of the reserves of tocopherols of the calf when a few days of age. Subsidiary objects were to study the composition and histological appearance of muscles of calves from dams that had received different intakes of tocopherols. A number of bovine foetuses were examined to provide additional information.

EXPERIMENTAL

Farms

Three farms with widely differing systems of feeding and managing their Ayrshire herds co-operated in this study which was carried out during late winter and again in the early autumn of 1951. All the farms were situated within a radius of 3 miles of the laboratory. Farm 1, the Hannah Dairy Research Institute farm at Kirkhill, maintained the whole of its milking herd throughout the winter of 1950–1 on grass or grassland products supplemented from mid April till full grazing with small amounts of home-grown oats and beans. The herd was subsequently pastured on high-quality grass using an intensive system of grassland management. The calves on this farm were removed from their dams at birth and given colostrum within 6 h. Farm 2, the West of Scotland Agricultural College Farm at Auchincruive, maintained its herd during the winter on arable and grass silage, hay, mangolds and purchased concentrates. The plane of nutrition was generally high, but food shortages occurred during the latter part of the winter. The cows were subsequently pastured under an extensive grazing system. The calves on this farm were allowed to suckle their dams and remain with them for 3 days. Farm 3, Mr J. Gibb's farm at Brocklehill, maintained its herd on hay, straw, swedes or mangolds and concentrates. The cows were pastured in the summer using an extensive grazing system. Calves on this farm were removed from their dams at birth and were given colostrum within 6 h.

The farms thus represented three rather divergent types of dairy cattle nutrition. Farm 3 was very typical of sound west of Scotland farming practice. Farm 2 was similar except that emphasis was given to high-quality silage. Farm 1 represented an extreme of dependence on the conservation products of intensive grassland management.

Milking-cow studies

The farms supplied samples of the milk from two cows on one day of each of the 14 weeks beginning on 5 March. These samples were analysed for total tocopherol

content and the samples were taken to cover the period of termination of winter feeding and the inception of grass feeding in the spring and early summer. Both cows from each farm had been milking for from 2 to 3 months when the collection of samples began.

Calf studies

When a bull calf was born the following procedure was adopted. Samples of colostrum and venous (jugular) blood were taken from the dam as soon as possible after parturition. Total tocopherols were determined on these samples. Information relating to the feeding regimen of the dam, her age and previous lactational history was obtained, and her general physical well-being assessed. The calf was allowed to stay at the farm for 3 days, its management being the usual one adopted on that particular farm. It was then brought to the laboratory where it was confined in a metabolism cage, given whole milk, and its urine was collected quantitatively for 24 h. After this it was killed, and the carcass examined. Samples of blood, the diaphragm, the supraspinatus, the vastus medialis, the left wall of the ventricle, the liver and perinephric fat were then taken for estimation of creatine, dry matter and tocopherol content. One calf from farm 1 was not allowed colostrum and was killed when only a few hours old. A larger number of samples were taken from this calf to provide information on the tissues that store tocopherols during pregnancy.

Six calves were obtained from farm 1, four in the spring before the herd had access to pasture and two in the autumn at the end of the pasture season. From farm 2 two were obtained in the late winter and two in the autumn, and from farm 3, four in late winter and two in the autumn. Calves are referred to by farm, season (winter or autumn) and serial number. Thus calf no. 2 W2 is the second calf secured from farm 2 in the late winter.

Foetuses

During the summer, foetuses were obtained from nine Ayrshire cows of unknown origin. Their age was assessed from crown-rump lengths and other criteria of foetal age as listed by Hammond (1927). Samples of tissues were taken from them using the same technique as with the calves. With the smaller foetuses, however, separation of the individual muscles was impossible, and so the spinatus group, the vastus group, or both ventricles were taken for analysis, rather than the particular muscles noted above.

Chemical methods

The fat and total solids contents were determined on all samples of milk and colostrum. The urine samples were analysed quantitatively for creatine and creatinine (Folin, 1914), and qualitatively for sugar, myoglobin and other abnormal constituents. The muscles were analysed for dry matter, ash and creatine content (Blaxter & Wood, 1952). Where the tocopherol content of the tissues was determined, total lipid was also estimated.

Tocopherol estimation

Blood. The tocopherols were extracted from serum with light petroleum after precipitation of the proteins with ethanol (Quaife & Harris, 1944). The residue obtained on evaporating the petroleum layer was dissolved in benzene, and vitamin A and carotenoids were removed by passing the benzene solution through a column of Floridin Earth which had been treated with stannous chloride and concentrated hydrochloric acid just before use (Emmerie & Engel, 1939; Glavind, Kjølhede & Prange, 1942). The benzene filtrate was evaporated under reduced pressure and the residue dissolved in warm ethanol. $\alpha\alpha'$-Dipyridyl solution was added, followed by ferric-chloride solution, and after exactly 2 min the red colour was measured in a Spekker Absorptiometer, using Ilford green filters no. 604. The final colorimetric estimation was essentially that of Emmerie & Engel (1938).

Milk. The method used was the same as for blood serum, except that the residue obtained on evaporating the petroleum layer was hydrolysed with alcoholic potash in the presence of pyrogallol, exactly as described by Tošić & Moore (1945), before dissolving it in benzene for chromatography on Floridin Earth.

Tissues. The tissues were finely ground and then extracted with ethanol in a Soxhlet apparatus for 24 h (Quaife & Dju, 1949). The ethanol extract was diluted with an equal volume of water containing about 100 mg sodium sulphate, and the mixture extracted with light petroleum (Quaife & Harris, 1948). The petroleum layer was evaporated and the analysis continued in the same way as for the milk-fat residue.

RESULTS

The effect of the ration on the tocopherol content of milk

The changes in the tocopherol content of the milk of each of the six cows are summarized in Table 1. The values represent mean secretions of total tocopherol during the first 4 weeks of the studies when the cows received winter rations, during the 2 weeks immediately before the cows went out to grass and also during the last 3 weeks of observation when the cows were existing entirely on grass. Despite the rather large differences in the type of ration given, the vitamin E content of the milk fat was not grossly affected by the winter ration employed. Throughout the whole period before going to grass the milk fat of the cows of farm 1 contained the most tocopherol. Farm 2 in general gave slightly higher values than farm 3 save when feed supplies became short in the late spring. At grass, the tocopherol content of the milk of all cows increased considerably, the increase being less with farm 1 than with the others. It is not known why the tocopherol content of the milk was so high when the cows at farm 2 were at pasture.

The data also show that at any one time differences in the fat content of the milk of the two cows on any farm were not associated with any marked differences in the concentration of tocopherol in the milk fat. Large differences in the milk yield of the two cows of a pair only occurred at farm 3 where, during the last 3 weeks of the study, cow B gave only 65% of the yield of cow A. Equal opportunity to eat grass was afforded, and the fact that the tocopherol content of the milk fat was slightly

Table 1. *Mean fat content of the milk and the tocopherol content of the milk fat of the cows on the three farms*

		Period					
		Winter		Immediately before grass		Summer grazing	
Farm no.	Cow	Fat (%)	Tocopherol (μg/g fat)	Fat (%)	Tocopherol (μg/g fat)	Fat (%)	Tocopherol (μg/g fat)
1	A	4·97	29·9	6·50	32·4	2·93	45·7
	B	4·53	31·3	3·50	34·2	3·83	45·1
	Mean		30·6		33·3		45·4
2	A	4·03	31·2	3·85	29·2	4·03	53·7
	B	4·10	26·8	4·10	29·1	3·10	51·2
	Mean		29·0		29·1		52·4
3	A	4·53	23·0	4·70	31·2	3·50	48·7
	B	4·00	27·6	4·70	31·2	4·40	40·5
	Mean		25·3		31·2		44·7

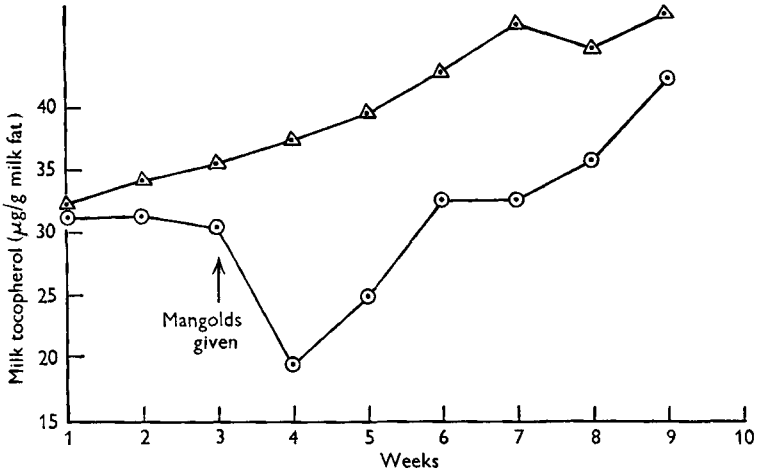

Fig. 1. Effect of the introduction of mangolds into the ration of dairy cows on the tocopherol content of the milk fat. △, farm 1 (no mangolds given); ⊙, farms 2 and 3 (mangolds given for first time between weeks 2 and 3).

less with the lower-yielding cow, suggests that any effect of the total amount of fat secreted on the tocopherol content of the milk fat is not large.

During the late winter, marked falls in the tocopherol content of the milk fat occurred on farms 2 and 3, but not on farm 1. The falls occurred 1 week earlier on farm 2 than on farm 3, and the mean values for both farms were adjusted to coincide and are shown in Fig. 1. Two changes in management and feeding had occurred immediately before the fall in tocopherol content. Firstly, the cows were allowed out for a half-hour a day on pasture which contained a very limited amount of grass, and secondly, mangolds were introduced into the ration. Cows on farm 1 were similarly let out during this mild spell of weather, but showed no depression in the tocopherol content of the fat. This suggests that mangold feeding caused the

depression. Whether trimethylamine oxide arising from the betaines of the mangolds (Davies, 1936) resulted in oxidation of the tocopherol of the milk fat was not ascertained.

The tocopherol content of the blood serum of the cows at calving

The total tocopherol content of the blood serum of the newly calved cows is given in Table 2.

The tocopherol content of the serum of the cows that calved in the late winter was highest on farm 1, intermediate on farm 2, and on farm 3 was only one-seventh of the values obtained at farm 1. In the autumn there was little difference between the serum tocopherols of newly calved animals on farms 1 and 2, but those at farm 3 were decidedly higher. During the winter period, the serum levels reflected quite closely the amount of grassland conservation-products included in the ration. Cow 3 W 3, which gave the lowest value, had been served when she was very young and was in extremely poor condition when she calved at 2 years of age. She had been given extra feed before calving with little effect on her condition. Cows and heifers on farm 3 were normally given little concentrated food before calving. Those on farm 2 received moderate quantities over the final 6-week period, but those on farm 1 were not given any.

Table 2. *Tocopherol content of the blood serum of newly calved cows on the three farms*

Time of year	Reference no.	Farm 1	Farm 2	Farm 3
		Tocopherol content (μg./100 ml. blood serum)		
Late winter	1	947	328	202
	2	1195	646	235
	3	1070	—	42
	4	1047	—	165
Mean		1065	487	161
Autumn	1	695	518	931
	2	535	484	757
Mean		615	501	844

It was expected that all the serum-tocopherol levels would be high in the autumn period, since grass, which is high in tocopherol content (Cabell & Ellis, 1942), supplied the major part of the ration. This was not so. At farm 3 no concentrated feed was given to the animals due to calve, and grass was their sole feed. At farm 2, the heifer, 2 A 1, received concentrated food, but the cow, 2 A 2, did not. At farm 1 the first animal calving in the autumn received nothing but grass, the second was brought in for several nights before she calved and given concentrated food consisting of a mixture of oats, beans, maize meal and dried beet pulp. The reason for the differences between farms in the tocopherol content of the serum of cows calving in the autumn is thus not clear. It may be partly due to the fact that the cows on farms 1 and 2 were brought in to calve, whereas those at farm 3 were left out. This would suggest that serum-tocopherol levels change rapidly in response to changes in nutrition and management as indeed has been shown by van der Kaay, Teunissen, Emmerie & van Eekelen (1949). It is quite clear, however, that under normal farm conditions, serum-tocopherol levels of individual cows can exhibit more than a tenfold variation.

The tocopherol content of the colostral fat

Table 3 shows the total tocopherol content of the colostral fat of the cows. On any one farm, there was a tendency for the tocopherol content to be higher when the sample was taken soon after parturition. This is in agreement with the results of Parrish *et al.* (1947). Further analyses of the colostral fat of a single cow, cow 1 A2, are given in Table 4, which shows that the fall in tocopherol content of the milk fat during the first 2–3 days of lactation was considerable. With this animal the maximal total yield of tocopherol occurred at the fourth milking after parturition.

Table 3. *Tocopherol content of the colostral fat of newly calved cows on the three farms*

		Farm 1		Farm 2		Farm 3	
Time of year	Reference no.	Time after calving (h)	Tocopherol content (μg/g)	Time after calving (h)	Tocopherol content (μg/g)	Time after calving (h)	Tocopherol content (μg/g)
Late winter	1	7	194	5	178	12	75
	2	7	236	—	*	10	158
	3	3	224	—	—	26	78
	4	4	368	—	—	9	155
Mean			256		178		117
Autumn	1	9	214	13	160	43	73
	2	3	345	8	166	15	219
Mean			280		163		146

* Very considerable amounts of blood were present in this sample; the analysis was suspect and therefore was excluded.

Table 4. *Yield and fat content of the colostrum and the tocopherol content of the colostral fat of an autumn-calved heifer at farm 1*

No. of milking	Yield (kg)	Fat content (%)	Tocopherol content (μg/g fat)	Daily yield of tocopherols (mg)	
1 (a.m.)*	1·1	1·4	345	5·5	38·9
2	3·2	3·4	309	33·4	
3 (a.m.)	4·8	3·7	189	33·4	87·0
4	5·4	5·0	197	53·6	
5 (a.m.)	6·4	3·4	132	28·5	67·7
6	5·4	4·8	150	39·2	
7 (a.m.)	6·4	3·8	106	25·6	38·6
8	5·9	4·5	49	13·0	
9 (a.m.)	7·5	2·9	53	11·5	

* Cow milked 3 h after parturition.

This fall in the tocopherol content of the milk fat after parturition, together with the variation in the time that elapsed between parturition and sampling in the present series of analyses, made it difficult to interpret the results of Table 3. They were therefore analysed statistically using the technique of analysis of covariance. Means, adjusted for the linear regression, are given in Table 5. The regression of the tocopherol content of the fat on the number of hours that had elapsed since calving was 5·5 μg/g/h and was statistically significant. Adjustment of the means of Table 3 using

this regression resulted in a reduction of the differences between them. The standard deviation was high (± 55 μg tocopherol/g fat) and differences between the means though large, were not statistically significant. There was a relationship, however, between the serum-tocopherol level of the cows and the amount of tocopherol present in the colostrum. The animals at farm 3, with the lowest mean serum tocopherol, also had the lowest tocopherol content of the colostral fat and the reverse applied to the animals of farm 1.

Table 5. *Mean tocopherol content of milk fat adjusted to a standard time after calving by covariance analysis*

		Mean tocopherol content (μg/g fat)		
Farm no.	Season	Before adjustment	Following adjustment to 11·4 h after calving*	Autumn increase over winter levels (%)
1	Late winter	256	221	—
	Autumn	280	249	13
2	Late winter	178	142	—
	Autumn	163	157	11
3	Late winter	117	131	—
	Autumn	146	241	84

* The regression of tocopherol content on time following parturition was statistically significant.

The tocopherol content of the blood of the calf when 4 days of age

Table 6 shows the tocopherol content of the blood serum of the calves. Together with the results of Table 2, this demonstrates that the extent of the variation in the tocopherol content of the blood serum of cattle is at least forty-eightfold, from very low levels in young calves to very high ones in older cows.

In the winter, the calves at farm 1 contained an average of 4·5 times and a maximum of 11·4 times as much tocopherol in their blood serum as did those of farm 3. In the autumn there were no distinct differences between the tocopherol contents of the serum of calves on the three farms, those at farm 3 having increased approximately fourfold.

Table 6. *Tocopherol content of the blood serum of the calves when 4 days of age*

Time of year	Reference no. of calf	Farm 1	Farm 2	Farm 3
		Tocopherol content (μg/100 ml. blood serum)		
Late winter	W1	276	209	25
	W2	*	150	81
	W3	249	—	48
	W4	284	—	87
	Mean	270	180	60
Autumn	A1	240	149	270
	A2	31†	294	202
	Mean	240	222	236

* Sample lost.

† Colostrum withheld, calf 4 h old when slaughtered.

The serum of the calf on farm 1 that was deprived of colostrum (no. 1 A2) contained approximately one-eighth of the quantity of tocopherol present in a comparable animal given colostrum. It contained, however, more tocopherol than did the serum of one calf on farm 3 which had received colostrum.

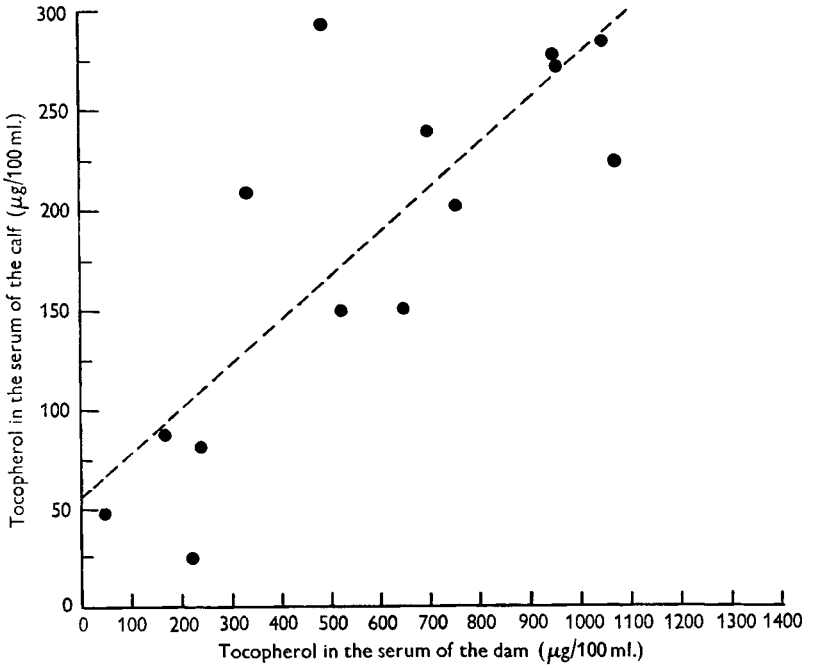

Fig. 2. Relation between the tocopherol content of the serum of the cow and that of her 4-day-old calf.

A comparison of the means of Table 6 with those of Table 5 shows that the relationship between the tocopherol content of the colostral fat and the tocopherol content of the blood serum of the calf when 4 days old was not close. Thus on farm 3 the tocopherol content of the colostral fat increased by only 86% from late winter to autumn, while the tocopherol content of blood serum of the calves increased fourfold during the same period. A much closer relationship was observed when the vitamin content of the serum of the cow was compared with that of its 4-day-old calf. These data are shown in Fig. 2. The correlation coefficient was +0·821, and was statistically highly significant.

The tocopherol content of the organs of the calves

The tocopherol content of the livers, muscles and depot (perinephric) fat of calves born in the autumn and of foetuses was determined, and the results are given in Table 7.

Comparatively large amounts of tocopherols were present in the tissues of the calves and in the tissues of calf 1 A2, which was killed at birth. A comparison of these results with those of Table 6 shows only a slight relationship between the blood level of tocopherols and tissue stores. Thus the tocopherol content of the perinephric

fat of the calves at farm 2 was double those at farm 1 yet the blood-serum tocopherol did not suggest any such differences. With liver tocopherols, however, agreement between serum and tissue level was closer.

Table 7. *Tocopherol content of the fresh liver, muscle and perinephric fat of the calves*

				Perinephric fat	
Farm no.	Calf no.	Liver (mg/kg)	Muscle* (mg/kg)	(mg/kg)	(mg/kg lipid)
1	A1	10·8	7·9	14·3	24·8
	A2†	7·5	7·8	13·2	21·2
2	A1	16·0	8·0	29·5	42·3
	A2	19·0	19·9	35·1	47·5
3	A1	15·7	16·5	26·4	39·9
	A2	11·1	7·5	19·6	36·0
Foetuses:					
(Age 3·0 months)	5	9·1	14·3	—	—
(Age 3·0 months)	10	8·6	3·6	—	—
(Age 7·0 months)	11	9·0	3·0	—	—
(Age 7·5 months)	6	15·7	6·8	—	—

* Analyses refer to the vastus medialis of the 3-day-old calves and to the spinatus group of the foetuses.

† No colostrum given.

The analyses of foetal liver and muscle show that tocopherols are present in foetal tissues even at the age of 3 months. The amounts in foetal liver tended to be smaller than those in the livers of calves given colostrum, but they were similar to the amounts found in the liver of the calf deprived of colostrum. With one exception less was found in foetal muscles than in the muscles of full-term calves. The percentage of dry material in foetal muscle, however, is considerably lower than in the muscles of the newborn. The results suggest that tocopherols pass the placenta or, less likely, are synthesized in the tissues of the foetal calf quite early in pregnancy.

The observations on the tocopherol content of the organs of the calf 1 A2 which was deprived of colostrum suggested that at farm 1 the greater part of the tocopherol reserves were acquired during gestation and not from the colostrum. The approximate total tocopherol content of this calf was computed from the analyses and weights of the organs, including muscle but excluding bone and the walls of the digestive tract, and so is a minimal estimate. It amounted to approximately 120 mg tocopherol. From its dam this calf could have received the whole of the colostrum for the first 24 h, probably 4·5 l. on the 2nd day and a further 4·5 l. on the 3rd together containing 103 mg tocopherol. Alternatively if the calf had been given the whole of the colostrum on the 1st day of life but subsequently given bulk milk from a herd—a common practice—then its intake would have been approximately 55 mg tocopherol. Both these estimates of possible intake in the first few days of life are below the estimated tocopherol content of the calf at birth. Moreover, it is highly improbable that the absorption of tocopherols by the calf is 100%. Consequently, although the serum tocopherol of the calf shows a marked increase during the first 4 days of life, the tocopherols acquired during foetal life are clearly of considerable importance.

Table 8. *Tocopherol content of tissues of calf no.* 1 A2, *deprived of colostrum*

Tissue	Tocopherol content of fresh tissue (mg/kg)	Tocopherol content of tissue lipids (mg/kg)
Skeletal muscle	7·8	558
Heart muscle	17·7	661
Lung	17·7	712
Brain	19·3	256
Liver	7·5	242
Kidney	12·8	413
Adrenals	40·9	238
Thymus	8·5	289
Perinephric fat	13·2	21

Table 7 shows also that tocopherols are not distributed in the body organs in proportion to their lipid content. Liver, containing 3–4% total lipid on a fresh basis, contained only slightly less tocopherol than did the fresh perinephric fat which in the young calf contains 60% or more of total lipid. This is further shown in Table 8, where the tocopherol content of other tissues of calf 1 A2 are given. Though it appears that the tocopherol content of tissues is not related to their total lipid content, the distribution of values in Table 8 suggests that there may be a relationship between the concentration of tocopherols and the concentration of the tissue lipids other than triglycerides. The high concentrations in muscle lipid, medium concentrations in glandular tissue lipid, and low concentrations in depot fat lipid are roughly in inverse proportion to the triglyceride content of these tissue lipids.

The creatine, dry matter and ash content of the muscles of the calves

The first few determinations of the creatine content of the muscles of the calves were compared with similar analyses of comparable muscles of older calves which had received adequate tocopherol and had been control animals in previous experiments. These comparisons all showed that the creatine content of the muscles of the 4-day-old calves was lower than that of normal calves 1–2 months of age. It was realized that the low values might have been the result of an effect of age, especially since in the rat the creatine content of muscles increases with age (Myers & Fine, 1913).

Analyses of foetal muscles are given in Table 9 together with analyses of muscles of older calves. Some of these results are also shown graphically in Fig. 3. Analyses of muscles of adult cows gave values of 430–450 mg creatine/100 g for the triceps muscle and 320–370 mg creatine/100 g for the heart ventricle. The regression of the creatine content of the dry matter of the muscles on the age of the foetus, counted in months from the date of conception, was highly significant, as shown in Table 10. These results show that the normality of the muscles of the 4-day-old calf can only be assessed by comparison with muscles of animals of the same age, known to be normal.

The creatine content of the muscles of each of the calves is given in Table 11. It will be noted that in late winter many of the muscles of the calves contained

Table 9. *Creatine and dry-matter content of certain tissues of foetal and other calves*

		Vastus group		Spinatus group		Heart	
Laboratory no.	Approximate age from conception (months)	Creatine (mg/100 g fresh muscle)	Dry matter (%)	Creatine (mg/100 g fresh muscle)	Dry matter (%)	Creatine (mg/100 g fresh muscle)	Dry matter (%)
F10	3·0	151	11·2	149	11·3	92	10·5
F5	3·0	109	9·7	95	10·0	58	14·7
F4	3·5	142	9·2	129	11·2	38	13·3
F2	6·0	169	12·8	130	10·8	50	11·9
F3	6·2	142	11·2	119	11·0	63	12·4
F8	6·5	169	12·8	156	12·9	75	13·6
F11	7·0	233	14·2	239	14·1	134	14·1
F6	7·5	239	14·9	225	15·6	80	14·9
F7	8·0	303	19·2	258	16·5	105	16·1
F9	8·5	285	18·8	280	18·8	159	16·3
F1	9·0	405	20·9	346	21·1	158	19·8
26	10·0	403	22·0	342	21·5	218	19·9
82	10·0	412	22·0	331	22·2	213	20·5
32	10·5	446	21·2	329	18·8	235	18·8
64	10·5	456	22·6	407	21·7	258	21·1
71	10·5	430	21·9	378	21·8	251	20·1
72	10·5	433	22·6	372	22·4	281	19·9
81	10·5	375	21·2	350	21·6	289	20·6
60	11·0	466	20·4	397	21·1	259	20·0
45	12·0	458	22·1	369	21·4	249	20·2
47	12·2	463	22·1	400	21·5	320	20·6

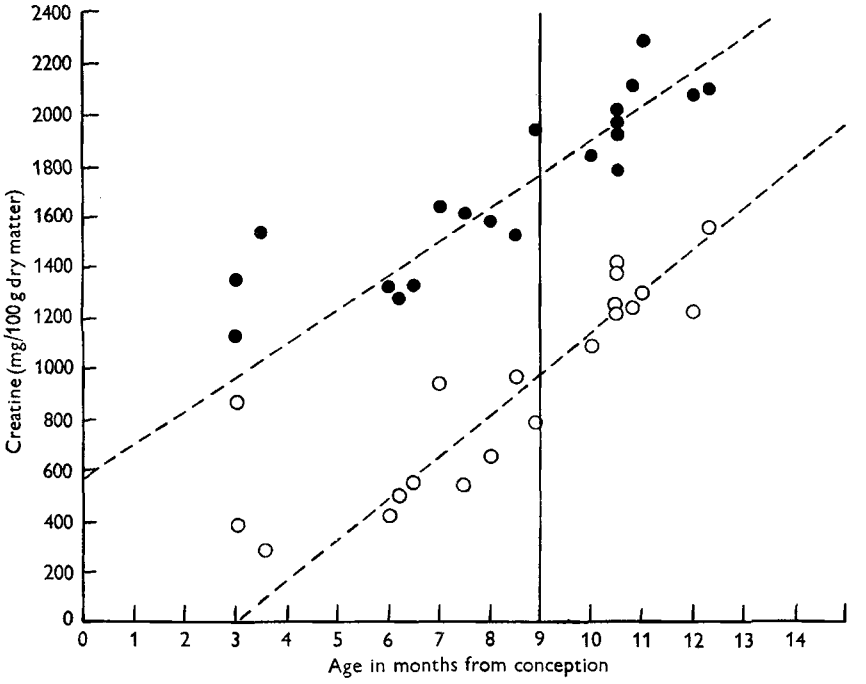

Fig. 3. Relation between the creatine content of the heart and vastus muscles of the foetus and foetal age. ○, heart; ●, vastus.

Table 10. *Significance of regression of creatine content of muscles on age of foetus or calf*

Muscle	Equation where C = creatine content of dry muscle (mg/100 g) and A = age (months from conception)	Variance ratio	Creatine content expected at birth with its standard error (mg/100 g dry muscle)
Heart	$C = 162{\cdot}6A - 496$	85·1*	968 ± 146
Vastus group	$C = 130{\cdot}8A + 573$	47·0*	1751 ± 156
Spinatus group	$C = 134{\cdot}6A + 418$	58·8*	1629 ± 144

* $P < 0{\cdot}001$.

Table 11. *Creatine content of the muscles of the calves*

Season	Farm no.	Calf no.	Creatine content of fresh muscle (mg/100 g) Infra-spinatus	Vastus medialis	Diaphragm	Heart
Late winter	1	W1	403	463	269	229
		W2	338*	377*	252*	181*
		W3	346*	405	275*	227
		W4	363	388	297	223
	2	W1	335*	381*	269*	197
		W2	351	398	288	191
	3	W1	360	382*	298	225
		W2	329*	365*	278*	211
		W3	348*	383*	264*	222
		W4	359	373*	286	173*
Autumn	1	A1	409	454	320	260
		A2	388	425	318	242
	2	A1	409	436	341	234
		A2	390	406	303	206
	3	A1	363	394	299	231
		A2	385	416	319	225

* Values judged to be abnormally low (see text, p. 118).

Table 12. *Mean creatine content of the muscles of the calves in relation to season of birth*

Season	Creatine content (mg/100 g fresh muscle) Infraspinatus	Vastus medialis	Diaphragm	Heart
Late winter	353·2	385·5	277·6	207·9
Autumn	390·7	421·8	316·7	234·0
Difference	37·5**	36·3**	39·1***	26·1*
Criterion of normality†	349	387	283	191

Significance of differences: * $0{\cdot}05 > P > 0{\cdot}01$. ** $0{\cdot}01 > P > 0{\cdot}001$. *** $P < 0{\cdot}001$. † Mean autumn value less $t \times \sigma$, taking a value of t at $P = 0{\cdot}05$.

considerably less creatine than they did in early autumn. The mean values are given in Table 12.

The differences between the mean values for calves born in the autumn and those born in the winter were statistically significant in all instances.

For the purpose of assessing the agreement of biochemical, clinical and histological findings some criterion of the normality of the muscles was essential. As an approxi-

mation, the mean creatine content of the muscles of calves slaughtered in the autumn was reduced by 2·145 × its standard deviation. This corresponds to a probability (P) of 0·05, that is odds of 1 in 40, that the values below this limit would occur by chance. Such criteria assume that the muscles of calves born in the autumn may be regarded as having an adequate creatine content. In Table 11 the analytical results for individual calves are compared with these criteria. It will be noted that on all three farms in late winter the creatine content of the musculature of some calves was judged inadequate. Calves on farm 3 tended to be slightly more affected than on farms 2 or 1. A comparison of the results for individual calves with the blood-serum tocopherols given in Table 6 did not show any correlation between vitamin E status and creatine content. Such might be expected, since the blood level of tocopherol probably reflects the recent intake of tocopherol rather than tissue reserves. Unfortunately, tissue levels of tocopherol were not available for the calves that were born in the spring, so that a comparison of tissue-tocopherol levels with muscle creatine content in the present work is impossible.

Creatine content of urine, and other abnormalities

Data relating to the freedom of each calf from apparent abnormalities of behaviour, the appearance of its muscles at post-mortem, the presence of any abnormal urinary constituents and the excretion of creatine and creatinine expressed as a ratio are summarized in Table 13. The behaviour of the calves was normal, and though several

Table 13. *Pre-mortem abnormalities of the calves and the appearance of the muscles at post-mortem*

Season	Farm no.	Calf no.	Behaviour *ante mortem*	Urine abnormalities observed	Ratio creatine : creatinine in 24 h urine specimen	Gross muscle abnormalities observed *post mortem*
te winter	1	W 1	Normal	Urine contaminated with faeces		Normal
		W 2	Dull and lethargic	Normal	1·7	Normal
		W 3	Normal	Normal	1·7	Normal
		W 4	Normal	Normal	0·8	Normal
	2	W 1	Normal	Albumin present in large amounts	2·4	Normal
		W 2	Normal	Albumin present	1·6	Normal
	3	W 1	Normal	Normal	5·3	Normal
		W 2	Weak and shivering	Normal	3·2	One area of slight pallor in left ventricle
		W 3	Weak and shivering	Albumin present	0·8	Musculature slightly pale
		W 4	Dull	Trace of a globulin present	1·2	Musculature slightly pale
ıtumn	1	A 1	Normal	Normal	0·5	Normal
		A 2	Normal	No urine passed		Slight muscle haemorrhages (birth injury?)
	2	A 1	Normal	Albumin present	1·4	Normal
		A 2	Normal	Normal	1·8	Normal
	3	A 1	Normal	Trace of a globulin present	1·4	Normal
		A 2	Normal	Albumin present	1·8	Slight haemorrhagic area in heart muscle

were dull and lethargic, these signs do not denote any serious abnormality. Protein was found in the urine of six of the calves. The ratio of creatine to creatinine in the urine was elevated in three of the calves, nos. 2 W 1, 3 W 1 and 3 W 2. These values were distinctly higher than the values observed on starvation of calves (Blaxter &

Wood, 1951) or in calves deprived of vitamin E (Blaxter, Watts & Wood, 1952). They can be regarded as serious abnormalities. The muscles of the calves were normal, with several exceptions. Pallor of the muscles is not necessarily indicative of abnormality and the small haemorrhages observed were probably due to injury at birth or to entities comparable to those described by Florence (1922). The interpretation of these mild abnormalities is given in the discussion.

Histology of the muscles

When the muscles of the calves were examined histologically several were found to have slight but definite lesions which in previous experimental work had been shown to be associated with vitamin E deficiency and cod-liver oil poisoning. These lesions have not been observed in animals that have received vitamin E without cod-liver oil. The outstanding lesions were the presence of hyaline fibrils, and typical ones are illustrated in Pl. 1, 1–3. These varied from merely the rare presence of hyaline nodes and fractures of the muscle cell, to isolated foci of hyaline fusiform swelling, with sarcolemmal reaction at the most very slight. In some muscle bundles no hyaline fibrils could be seen and yet in adjoining bundles they were present. The hyaline knots and fractures were not considered to be an agonal lesion, since they have not been observed in normal animals that had received adequate vitamin E. This mild lesion was similar to or identical with the early stage of the muscular dystrophy we have already described (MacDonald, Blaxter, Watts & Wood, 1952; Blaxter, Wood & MacDonald, 1953). No abnormalities were ever observed in the left ventricle, all the lesions being found in the skeletal musculature.

Table 14. *Criteria of normality of the calves*

	Biochemical criteria of normality				
No. of calf	Protein in urine	Ratio creatine:creatinine in the urine	Muscle creatine	Serum tocopherol*	Normality gauged by histological examination
1 W1	–	–	±	N	+
1 W2	N	±	+	–	±
1 W3	N	±	+	N	±
1 W4	N	N	N	N	±
2 W1	+	+	+	N	+
2 W2	N	N	N	N	N
3 W1	N	+	±	+	+
3 W2	N	+	+	+	+
3 W3	±	N	+	+	±
3 W4	±	N	+	+	±
1 A1	N	N	N	N	N
1 A2	–	–	N	–	N
2 A1	N	N	N	±	±
2 A2	N	±	N	N	±
3 A1	±	N	N	N	±
3 A2	N	±	N	N	N

N = normal; + = definitely abnormal; ± = equivocally abnormal. For muscle creatine these symbols indicate, respectively, adequate, definitely inadequate and equivocally inadequate.

* 150 μg/ml. serum taken as lower limit of normal range.

The relative severity of the lesions in each calf was assessed and the data are given in Table 14. It will be noted that the abnormality occurred on all farms but was more severe in calves born in late winter, in fact only very slight and indeed problematic lesions were observed in the autumn series.

Examination of the foetal muscles and hearts failed to show any sign whatever of a degeneration of the muscle cells. Only one abnormality was noted in the rectus femoris of foetus no. 11, which was slight oedema between the developing muscle bundles. These foetuses were all collected during the summer months. The significance of these findings is dealt with in the discussion.

DISCUSSION

The results of the analyses of the blood serum of the cows and calves together with the analyses of the colostrum and milk of the cows make it clear that very large differences in the tocopherol content exist as between herds even in a restricted locality. The extent of the variation in the total tissue reserves of tocopherols of the 4-day-old calf is not known, but a tenfold variation in serum tocopherols is apparent. It is probable that the serum tocopherol content bears some relation to the reserves of the vitamin in the tissues, but the results suggest that a major fraction of the serum tocopherols of the cow and calf reflects the recent intake of tocopherols rather than tocopherols present in the tissues. That tissue reserves of tocopherols are not solely determined by the ingestion of colostrum is shown by the high concentration of tocopherols present in the tissues of a newborn animal that had never received food and by the undoubted presence of large quantities in the foetal tissues. The results, therefore, suggest that calves with widely differing reserves of vitamin E may be found in normal farm practice as the result of differing levels of prenatal nutrition of the cow. It would be expected, therefore, that in late winter, calves on farm 3 would have low reserves and those at farms 2 and 1 adequate reserves, and that in the autumn calves at all three farms would have adequate reserves. The chemical analyses of urine and muscle and the histological examination of the muscles show that this was not invariably so. In winter, some calves at 4 days of age, irrespective, it appears, of the tocopherol content of their food, were found to have muscles low in creatine and to excrete abnormally high quantities of creatine in their urine. These chemical findings, together with losses of protein in the urine are abnormalities associated with muscular dystrophy. The histological examinations also showed that some calves had muscles that were in our opinion in the initial stages of a dystrophic degeneration. Table 14 makes a comparison of the chemical findings with the histological ones. It must be remembered that in making this comparison, both the biochemical and histological abnormalities represent but slight deviations from the normal. Furthermore, difficulty was experienced in arriving at criteria of biochemical abnormality. Agreement, however, was good. Thus on farm 2, in late winter, one calf was normal in behaviour, had no protein in its urine, the ratio of creatine to creatinine in its urine was classified as normal, and its muscles were judged to be normal in appearance and of adequate creatine content. Histologically, its muscles were without a trace of hyaline degeneration. Its companion, however,

excreted protein and had a high ratio of creatine to creatinine in its urine, had three muscles low in creatine, and histologically the muscle fibres were hyalinized. Biochemically a number of calves were classified as being abnormal though histologically they were classified as equivocal and vice versa. No calf was classified as abnormal by the one criterion and normal by the other. It is certain that the abnormalities observed were not artifacts, since classification of the calves by these entirely different criteria gave such good agreement.

The reason for these results is not clear. In late winter none of the calves examined from farm 3 were completely normal and in the autumn the only case classified as biochemically abnormal was also from this farm. This might suggest agreement with the tocopherol analyses. One of the most seriously affected animals, however, was born on farm 2, and calves on farm 1, which, from the study of secretion of tocopherol in milk would be expected to be superior, were by no means immune.

The histological and biochemical abnormalities, that is the hyaline foci and the lowering of the muscle creatine, were not due to immaturity of the calves as judged by their gestation times. Immaturity would probably result in a lowering of the muscle creatine, but histological examination of foetal muscles revealed no abnormality of the muscle fibres.

The only interpretation at present permissible is that an environmental factor, using the term 'environmental' in its widest sense, is involved synergistically with vitamin E in producing the abnormality. If it is a nutritional factor it is present in a high intake of fresh grass, but not in dried grass stored for 6 months, since at farm 1 dried grass was a major ingredient of the ration. Low temperature may be involved in producing the lesion since it was 10–15° F colder during the late winter period than in the autumn period, and the most severely affected calf (calf no. 2 W 1) was born when the mean outside temperature was the lowest recorded for the series. Such an hypothesis would receive support from the work of Naftalin (1951) on the relationship of environmental temperature to the incidence of liver lesions in rats given diets low in tocopherol.

The present experiments were carried out to find whether the variation in the incidence of experimental dystrophy both at different times of the year and in different individuals under like conditions was in any way related to their reserves of vitamin E. It may be concluded that though the reserves of vitamin E of calves undoubtedly vary very widely, this is not the sole reason for the variation in signs of dystrophy observed.

SUMMARY

1. The tocopherol content of various tissues, the muscle chemistry and muscle histology of calves born in late winter and early autumn on three farms were studied in an attempt to throw light upon the causes of the seasonal variation and between-calf variation in the onset of signs of nutritional muscular dystrophy under experimental conditions.

2. The tocopherol content of the full-lactation milk showed a marked rise when the cows went to grass in the spring. Mangold feeding caused a temporary depression of the tocopherol content of the milk.

3. The tocopherol content of the blood serum of newly-calved cows varied from 42 to 1195 μg/100 ml.

4. The tocopherol content of colostrum declined markedly with time after parturition.

5. The tocopherol content of the blood serum of calves when 4 days of age was correlated with the tocopherol content of the serum of their dams.

6. The tissues of 3- and 7-month foetal calves contained appreciable amounts of tocopherols. The concentrations in newborn calves were slightly greater, indicating that tissue reserves are largely acquired during gestation.

7. The creatine and dry-matter content of muscles of foetal calves increased throughout pregnancy and continued increasing during extra-uterine life up to at least the 3rd month.

8. The creatine content of the muscles and hearts of calves born in the late winter was significantly lower than that of those born in the autumn.

9. Three calves showed a profuse creatinuria on the 4th day of life which was considerably higher than that previously found on starvation of normal calves.

10. Three of the calves had hyaline foci of degeneration in their muscles, and others had doubtful lesions. These lesions were comparable with those of very early stages of nutritional muscular dystrophy of the calf. Their presence and severity were not closely related to the tocopherol content of the serum and colostrum of their dams.

11. It is postulated that the abnormality arises from the synergistic action of tocopherol with some unknown environmental factor, where the word 'environmental' is used to imply differences in nutrition, weather and management between winter and autumn.

Our thanks are due to Dr W. Holmes, Mr J. M. Thomson and Mr J. Gibb for their co-operation in placing certain animals in their herds at our disposal. We are grateful to Mr W. A. Wood for his determinations of the age of the foetal calves and for their dissection, to Miss M. K. Hutcheson and Mr D. Paterson for taking care of the animals, and to Miss G. Breckenridge for her assistance in the determination of the creatine, ash and dry-matter content of the muscles.

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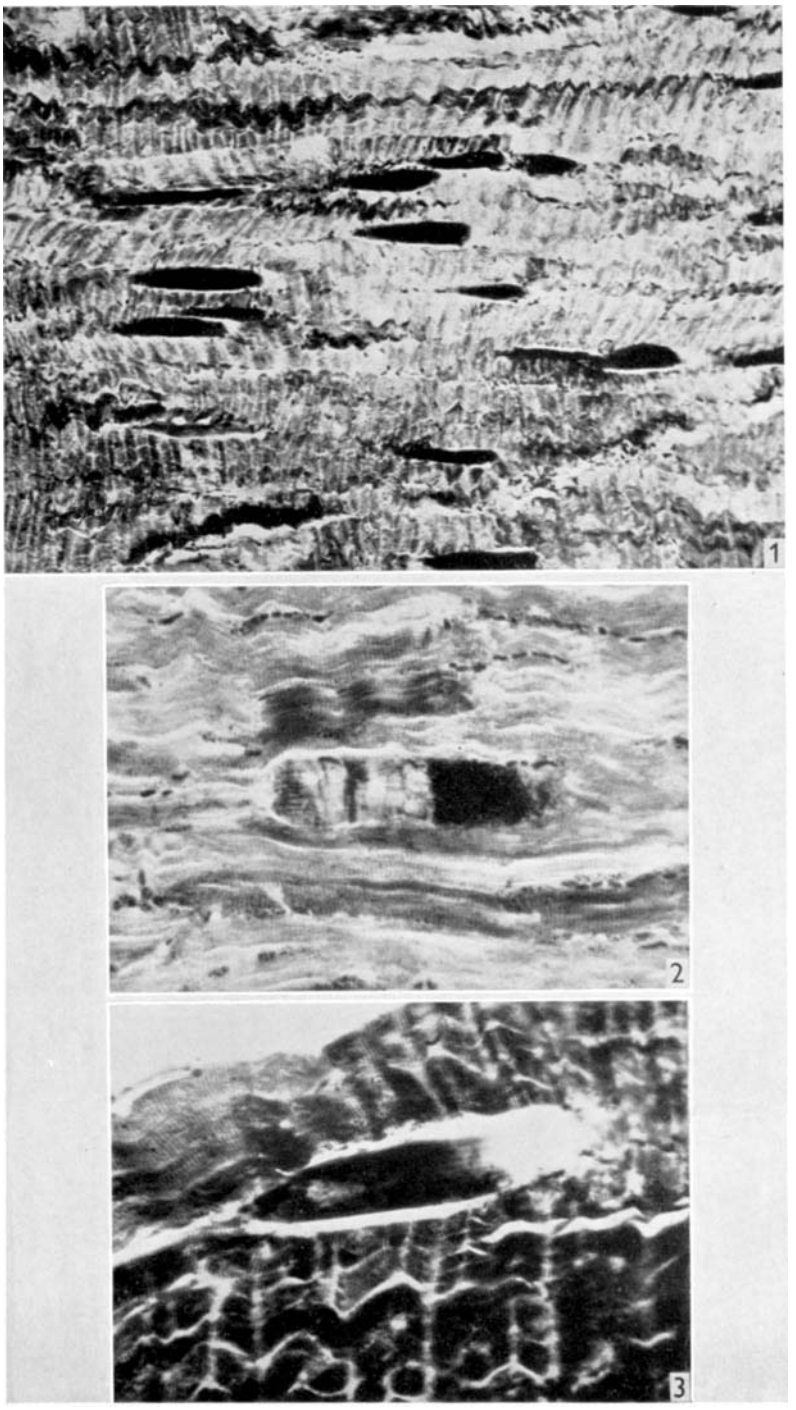

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EXPLANATION OF PLATE

Histology of affected muscles of calves

1. Low-power magnification of affected muscle. The hyaline muscle fibrils stand out clearly. There is no connective tissue section. Phosphotungstic-acid haematoxylin stain. × 50.
2. High-power magnification of affected fibril. Phosphotungstic-acid haematoxylin stain. × 175.
3. High-power magnification of affected fibril. Probably an early lesion as some striation can still be seen. Phosphotungstic-acid haematoxylin stain. × 175.