Severe undernutrition in growing and adult animals

13.* The morphology and chemistry of development and undernutrition in the sartorius muscle of the fowl

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Dickerson & McCance (1960) described the changes induced by severe undernutrition in the chemical composition of the pectoral muscles of cockerels. When growing cockerels were undernourished so that they increased in weight by only 2–3 g/week over a period of 6 months, there was an almost complete cessation of cell growth. Adult cockerels undernourished so that they lost about a third of their top body-weight lost over 90% of the cell mass in their pectoral muscles. A few observations on the changes in weight of the sartorius muscles suggested that the effect on them in the undernourished growing animal was similar to that on the pectoral muscles, but that in the undernourished adult animal the sartorius muscles did not waste so much. No observations on the morphology of the muscles were made.

Montgomery (1962*a*) reported that the morphology of the sartorius muscles of Jamaican infants dying of severe protein malnutrition was abnormal. Some fibres disappeared, the diameter of the remainder was greatly reduced, and there was a relative increase in interstitial connective tissue. The investigation now presented was planned to find out whether a calorie deficiency produced a similar change in fibre size and number in the sartorius muscle of the fowl, and to compare the results obtained from these observations with those of chemical methods which may be used to obtain similar information.

EXPERIMENTAL

Animals

The animals were pure-bred Rhode Island Red cockerels, and were cared for as described by McCance (1960). Nine birds were undernourished from about 1½ weeks of age for 26 weeks by being given very small amounts of a diet on which such cockerels grow extremely well if given enough of it. During this time their weight increased from about 100 g to about 160 g. Four undernourished birds were killed at this stage; a fifth was rehabilitated and killed at a body-weight of 2750 g when its growth curve crossed that of the undernourished adults (see below). The remaining four were fully rehabilitated. Nine well-nourished animals were killed at 1½ weeks

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of age (initial control), and three at 6 months of age (age control). Three birds well nourished to 6 months of age were killed after an average of 80 days' (75–91 days') undernutrition. During this time their weight fell from a mean value of 4000 g to 2192 g. Fig. 1 shows the mean changes in body-weight of the six groups of animals.

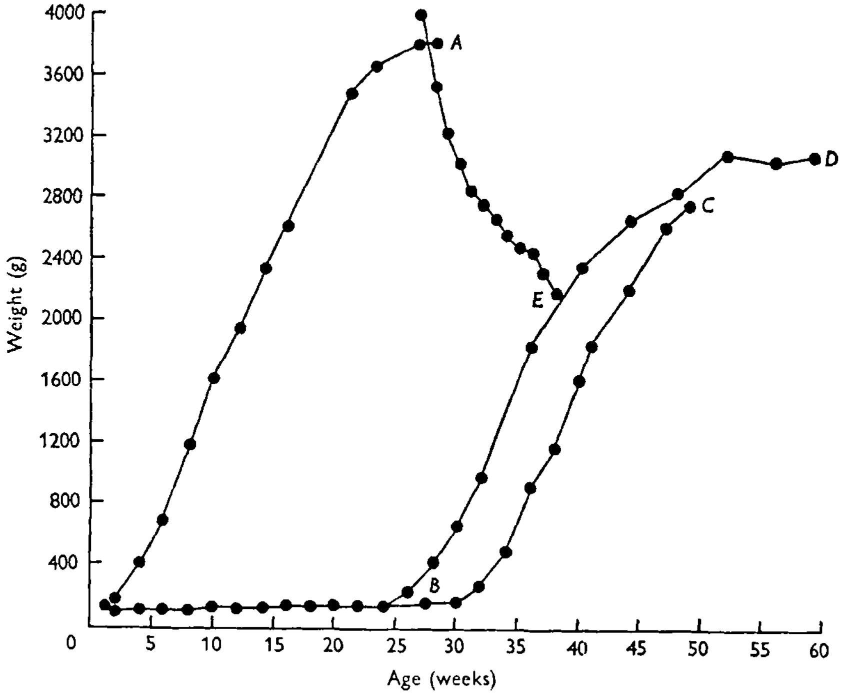

Fig. 1. Mean changes in weight of normal, undernourished and rehabilitated cockerels. *A*, growth curve of normal birds killed at 6 months of age; *B*, growth curve of undernourished birds killed at 6 months of age; *C*, growth curve of a bird rehabilitated to a weight of 2750 g; *D*, growth curve of fully rehabilitated birds; *E*, loss of weight of adult birds due to undernutrition and killed at a mean weight of 2192 g.

Methods

The birds were killed by an overdose of pentobarbitone sodium (Nembutal). Both sartorius muscles of each bird were dissected out, lightly blotted to remove adherent blood, cleared of visible fat and weighed fresh. A complete section about 5 mm in length was removed from the mid-point of the muscle and fixed in formol–saline. The remainder was frozen at $-15°$ and preserved for chemical analysis. If the cockerels were small, both sartorius muscles from two or more birds were pooled for analysis.

After fixation, the blocks were mounted in paraffin, and sections of uniform thickness were stained with haematoxylin and eosin. The whole section was projected through a photographic enlarger; the outlines were traced on paper and the area of the muscle excluding perimysial tissue was measured by planimetry. High-power images were projected through a standard microscope and counts of fibres and nuclei were made from five representative fields in each section. A section was taken from a muscle from each of three animals in a group. Standard deviations were thus calculated from fifteen estimations. The magnification of the images was obtained by projection on a haemocytometer grid.

The sartorius muscle of the fowl fans out widely towards its proximal origin, but no significant differences were found in the total number of fibres or subsarcolemmal nuclei in the proximal and distal thirds of the muscle. For the purpose of comparing total nuclear numbers in the whole muscle, the femur length was taken as an index of muscle length.

The chemical methods were those described by Dickerson (1960) with the additional separation of DNA by the method of Schmidt & Thannhauser (1945) as modified by Davidson, Frazer & Hutchison (1951). The phosphorus in the DNA was determined by King's (1932) method.

RESULTS

Morphology

Table 1 shows the effect of undernutrition and rehabilitation on the weight of the muscles, the length of the femur, the cross-sectional area, the number of fibres and the number of subsarcolemmal nuclei in the various specimens of sartorius muscle. Information is also given about the effect of normal growth over the same period of 28 weeks.

Table 1. *Effect of growth, undernutrition and rehabilitation on the length, weight, cross-sectional area, number of fibres and the number of subsarcolemmal nuclei in the fowl sartorius muscle*

	Initial control	Under-nourished	Partially rehabili-tated	Fully rehabili-tated	Age control	Under-nourished adult
No. of birds	3	3	1	3	3	3
Weight of bird (g)	102 (92–117)	163 (159–171)	2750	3035 (2620–3250)	3840 (3660–4010)	2192 (2140–2240)
Age of bird (weeks)	1·5	28	49	59	28	38
Length of femur (cm) (= approx. length of sartorius muscle)	3·3	5·0	10·4	10·4	11·2	9·7
Weight of muscle (g)	0·26	0·37	8·9	13·8	17·9	7·8
Cross-sectional area of muscle at mid-point (mm^2)	8·5 (7·5–10·5)	9·3 (7·4–11·2)	104	126 (109–143)	141 (123–163)	86 (70–105)
Total no. of fibres ($\times 10^{-3}$) ± standard deviation	25 ± 3·6	24 ± 4·5	43 ± 5·0	37 ± 4·2	46 ± 7·9	35 ± 7·8
Total no. of subsarcolemmal nuclei per cross-section ($\times 10^{-3}$) ± standard deviation	39 ± 6·0	36 ± 4·8	151 ± 10	174 ± 22	251 ± 47	138 ± 28
No. of nuclei per cross-section × length of femur ($\times 10^{-3}$) (= approx. index of number of nuclei per muscle)	130	180	1570	1810	2810	1340

A comparison of the initial control and the age control shows that during the period of observation the weight of the sartorius muscles of normal birds increased nearly twice as fast as that of the body as a whole, and the total number of fibres in the muscle increased by a factor of about two. Undernutrition from an early age grossly retarded the increase in body-weight of the animals, and their muscles remained about the same proportion of their body-weight at the end of 28 weeks as they had been at the beginning. Undernutrition prevented the normal increase in the number of fibres seen in a complete cross-section of the muscle. Rehabilitation was followed by an increase in the weight of the muscle, but the values had not reached those found in the normal adult by the time the weight of the body had ceased to increase. It is to be noted, however, that (*a*) the weights of the rehabilitated cockerels never attained those of the age controls, and (*b*) the muscle weight:body-weight ratio of both groups of birds was roughly the same. In other words the muscles were the correct weight for the body. The number of fibres increased during rehabilitation to a value which was not significantly different from that of a normal adult although the bird was already 6 months old when rehabilitation began. Undernutrition in the adult halved the weight and the cross-sectional area of the muscle, but did not significantly reduce the number of fibres.

The number of subsarcolemmal nuclei did not increase in the normal way during prolonged undernutrition. The number of nuclei found in the undernourished adult bird appeared to be less than that in the normal adult although the difference was not statistically significant.

Composition

Table 2 shows the effect of growth, undernutrition and rehabilitation on the chemical composition of the sartorius muscle. The increase in the amounts of water, extracellular protein, sodium and chloride brought about by undernutrition at each age were characteristic (Dickerson & McCance, 1960; Widdowson, Dickerson & McCance, 1960). In keeping with this there was less fibrillar and sarcoplasmic protein nitrogen per kg in the undernourished animals. When the undernourished animals were rehabilitated the mineral and protein components of their sartorius muscles became similar to those of the normal bird.

The concentration and absolute amounts of DNA-P in the muscle of the chick undernourished for 6 months were not significantly different from those in the muscle of the initial control. The concentration of DNA-P in the muscle was a little higher in the undernourished adult than in the normal bird. Since the total weight of the muscle was somewhat less than half after the period of undernutrition (see Table 1) the total amount of DNA-P present was lower.

The values for Cl and Na show that undernutrition both in the young and adult birds increased the proportion of extracellular fluid. Undernutrition also reduced, and rehabilitation increased, the amount of intracellular protein (sarcoplasmic + fibrillar protein). The amount of intracellular protein N per unit of DNA-P, which is a measure of the size of the 'cell unit', was the same in the undernourished animal as it was in the initial control. Undernourishing the adult animal led to a reduction in this ratio; rehabilitating the small undernourished animal led to an increase up to the value for the normal adult.

Table 2. *Effect of growth, undernutrition and rehabilitation on the composition of the fowl sartorius muscle*

(Values are means for the number of animals shown and are expressed per kg of fat-free muscle unless otherwise stated)

	Initial control	Under-nourished	Partially rehabili-tated	Fully rehabili-tated	Age control	Under-nourished adult
No. of birds	9	4	1	4	3	3
Fat (g/kg fresh muscle)	33	Nil	50	20*	22	Nil
Water (g)	772	799	764	766*	767	788
Total N (g)	34·6	32·2	34·2	36·4	34·6	34·2
Non-protein N (g)	4·4	3·5	3·7	3·4	3·8	3·9
Sarcoplasmic protein N (g)	6·2	3·9	7·8	5·4	6·1	6·0
Fibrillar protein N (g)	21·9	15·7	21·2	22·7	20·8	17·6
Extracellular protein N (g)	2·0	9·5	3·5	4·8	3·7	6·4
Cl (m-equiv.)	27·3	36·8	20·3	20·0*	21·8	30·4
Na (m-equiv.)	33·2	63·6	32·8	38·4*	32·9	40·6
K (m-equiv.)	80·6	80·5	91·1	95·5*	91·6	83·4
DNA-P (m-moles)	3·5	3·3	2·4	1·3	1·3	1·7
Total intracellular protein N (g)	28·1	19·6	29·0	28·1	26·6	23·6
Intracellular protein N/DNA-P (g/m-mole)	8·5	6·0	12·2	21·3	21·0	14·2
Range	(5·5–10·4)	(5·5–6·7)		(17·0–26·0)	(18·6–23·7)	(11·8–16·1)
Absolute amount DNA-P per muscle (μmoles)	0·9	1·2	21·2	18·0	23·0	13·0
Range	(0·7–1·3)	(0·8–1·5)		(13·9–22·7)	(15·7–29·7)	(11·0–15·7)

* Values for two birds only.

DISCUSSION

Morphology

It is generally considered that there is little increase in the number of fibres in the skeletal muscle of a mammal after birth (McMeekan, 1940; Joubert, 1955, 1956; Montgomery, 1962*b*); the present results suggest that there may be a considerable increase in the number of fibres in the sartorius muscle of a bird after it has hatched. The results, however, do not exclude the possibility that the increase in number may only be an apparent one due to an increase in the length of pre-existing fibres.

In the sartorius muscles of human infants with severe protein malnutrition (kwashiorkor) Montgomery (1962*a*) found that the fibres were fewer and smaller than in normal children of the same age and that some fibres had undergone visible degeneration. There was also a reduction in the ratio of the intracellular to the extracellular cross-sectional area of the muscles.

The histological appearance of the muscle of the undernourished chick was indistinguishable from that of its initial control as was that of the fully rehabilitated bird from that of the normal adult. The muscle of the partially rehabilitated animal differed from that of the undernourished adult of similar body-weight only in the slightly larger intermysial connective tissue spaces in the latter.

Comparison of the chemical results with those of the morphological study shows good agreement between the total number of subsarcolemmal nuclei and the total amount of DNA-P per muscle. Such close agreement was somewhat unexpected since the DNA of nuclei other than the subsarcolemmal ones is included in the chemical determination. The morphological studies, moreover, indicated that the diameter of the fibre in the birds undernourished from an early age was the same as that in their initial control and the ratio intracellular protein N:DNA-P was in agreement with this. In the birds undernourished after reaching maturity both morphology and chemistry also agreed in suggesting a considerable reduction in 'cell' size. In the undernourished adult birds both methods demonstrated that the amount of extracellular tissue (extracellular water + extracellular protein) was increased.

Undernutrition of the type to which these birds were subjected clearly does not produce the same changes in the structure of skeletal muscle as those found in 'protein malnutrition' in infants. Thus in the birds undernourished from an early age there was no shrinkage in the diameter of the fibres, no degeneration or loss of fibres and no loss of subsarcolemmal nuclei. The relative increase in the amount of interstitial connective tissue is a feature common both to severe undernutrition in the growing bird and to severe protein malnutrition in the human infant. Chemically, however, the changes may not be so very different and any chemical approach should clearly be supported by morphological study.

SUMMARY

1. Nine cockerels were severely undernourished for the first 6 months of their lives and five of them rehabilitated. Three adult birds were also severely undernourished.

2. Undernutrition early in life did not prevent some increase taking place in the weight of their muscles, but it did prevent all the normal increase in cross-sectional area of the muscle and in the total numbers of fibres and subsarcolemmal nuclei seen in cross-section.

3. When the undernourished birds were rehabilitated the weights of their muscles increased but at maximum body-weight had not reached those found in the normal adult. The number of fibres rose during rehabilitation to the normal adult value.

4. Undernutrition in the adult halved the weight and cross-sectional area of the muscles, but did not change the number of fibres.

5. The changes in chemical structure of the muscles corroborated the morphological findings.

6. The changes did not resemble those found in severe human kwashiorkor.

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