Severe undernutrition in growing and adult animals

3.* Avian skeletal muscle

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Apart from its economic value, the domestic fowl is in many respects a convenient experimental animal. Studies of the effect of undernutrition on growing animals have, however, been mostly made on mammals and there is ample evidence that underfeeding results in a retardation of growth (Jackson, 1925). Drummond (1916) obtained similar results in chickens. As part of an investigation of the effects of prolonged undernutrition (McCance, 1960) its effect on the composition of both avian and mammalian tissues was studied. The results of the study on mammalian tissues will be presented in a separate paper (Widdowson, Dickerson & McCance, 1960).

Of all the soft tissues, skeletal muscle contributes more than any other to the body-weight of an adult animal, but little is known about the changes that take place in it during undernutrition. Changes in its composition may result from the differential growth or resorption of the component parts. They have been investigated and an attempt has also been made to find if the composition of the cells themselves was changed by underfeeding.

Several histological observations have been reported which indicate that the cells of skeletal muscle may become smaller in times of nutritional stress (Waters, 1909; Meyer, 1917; Speidel, 1938; Waterlow, 1948). Few attempts have been made, however, to find out what cell proteins are lost (Moulton, 1920; Hagan & Scow, 1957). The work now being reported on the muscle of adult cockerels has provided an opportunity for doing so.

The cockerels, which had been held at weights below 200 g for 6 months, rapidly gained weight when allowed free access to food (McCance, 1960). The nitrogenous constituents in the muscle of rehabilitated cockerels have therefore been investigated to find if they were permanently affected by underfeeding early in the period of growth.

The effect of growth on the composition of avian pectoral muscle has been described elsewhere (Dickerson, 1960). Some of the results were obtained on animals used as controls for the present investigation. Three kinds of controls were necessary: (1) normal birds of the same age as the undernourished ones at the beginning of underfeeding, (2) normal birds of the same size as the undernourished ones at the time of their death (weight controls) and (3) normal birds of the same age as the undernourished ones at the time of their death (age controls).

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EXPERIMENTAL

Animals

The animals were all pure-bred Rhode-Island Red cockerels. Nine birds which had been undernourished for 25 weeks from $2\frac{1}{2}$ weeks of age, three birds undernourished for 15 weeks from 27 weeks of age and three birds which were rehabilitated for 15 weeks after being undernourished for 25 weeks from $2\frac{1}{2}$ weeks of age, were used. The feeding and care of all the birds has already been described (McCance, 1960).

Methods

The nine birds, undernourished from an early age and killed at 27 weeks, were divided into three groups, each of three animals. The pectoral and sartorius muscles from the individuals in a group were pooled for weighing and analysis. The muscles from each of the adult birds were weighed and analysed separately. The killing of the animals, their dissection and all the chemical methods were the same as those described by Dickerson (1960) and the absolute figures for the composition of the control animals are also given in that paper.

RESULTS

Table 1 shows the mean body-weight and the mean weight of the pectoral and sartorius muscles of the undernourished cockerels at the termination of the experiment, in absolute terms and also expressed as a percentage of those of normal animals at the beginning of underfeeding (Dickerson, 1960). The relative weights show that

Table 1. *Mean live-weight and mean weights of the pectoral and sartorius muscles of (a) nine cockerels undernourished from $2\frac{1}{2}$ to 27 weeks of age, and (b) three undernourished from 27 to 42 weeks of age*

Animal or tissue	Absolute weight (g)		Relative weight: Amount in normal birds at $2\frac{1}{2}$ weeks taken as 100	Relative weight: Amount in normal birds at 27 weeks taken as 100
	(a)	(b)	(a)	(b)
Live bird	181	2274	195	67·5
Pectoral muscle	5·6	29	137	8·9
Sartorius muscle	0·39	6·7	139	48·3

the birds undernourished from $2\frac{1}{2}$ weeks of age did not quite double their weight in 25 weeks. Healthy control birds gained thirty times their initial weight during this period. The birds undernourished from 27 weeks of age lost a third of their body-weight in 15 weeks. The 'weight controls' for the birds undernourished till they were 27 weeks old reached their killing weight in $1\frac{1}{2}$ weeks when they were 4 weeks old. During this time the pectoral muscles almost trebled their weight but the weight of the sartorius muscles was barely doubled (Dickerson, 1960). In the birds undernourished from $2\frac{1}{2}$ to 27 weeks, both pairs of muscles gained about the same amount reckoned as a percentage of their initial weight, and the growth of the pectoral muscles was therefore affected more than that of the sartorius. This difference between the two pairs of muscles was more pronounced in the birds undernourished from 27 to 42 weeks, for in them the pectoral muscles were grossly atrophied and had lost about 90 % of their initial weight, whereas the sartorius muscles sustained only a 50 % loss.

Effect of undernutrition on the composition of pectoral muscle

The results of the analyses of pectoral muscle from the two groups of undernourished birds will be considered relative to their controls in two ways. First, as changes in the amounts of the various constituents per unit weight of muscle and, secondly, as changes in the total amounts of the constituents per muscle.

Table 2 shows the effect of underfeeding cockerels (*a*) from 2½ to 27 weeks of age, and (*b*) from 27 to 42 weeks of age on the composition of pectoral muscle. The analytical results for the experimental animals and their controls are expressed on a fresh-weight basis. Fat was estimated in a few samples of muscle from birds of the same weight and of the same age, and did not account for more than 2 % of the muscle at either age. This small percentage of fat would not affect the conclusions to be drawn from the results.

Table 2. *Effect of underfeeding (a) nine cockerels from 2½ to 27 weeks of age, and (b) three from 27 to 42 weeks of age on the mean composition of pectoral muscle*

(Amounts expressed per kg fresh muscle. Well-nourished birds at 2½ weeks of age represent birds in group (*a*) when underfeeding began. Well-nourished birds at 4 weeks were 'weight controls' for group (*a*). Well-nourished birds at 27 weeks were 'age controls' for group (*a*) and also represent birds in group (*b*) when underfeeding began)

	Undernourished		Well-nourished controls		
Constituent	(*a*)	(*b*)	2½ weeks	4 weeks	27 weeks
Water (g)	796	763	771	756	737
Total N (g)	27·5	33·7	29·5	34·1	37·0
Non-protein N (g)	3·8	5·9	4·2	4·5	5·5
Sarcoplasmic protein N (g)	5·5	6·2	6·6	10·8	11·1
Fibrillar protein N (g)	15·1	15·4	16·9	17·9	19·4
Extracellular protein N (g)	4·0	6·7	1·6	1·1	1·2
Collagen N (g)	2·4	6·6	1·4	0·8	1·1
Cl (m-equiv.)	30·7	28·6	20·4	19·6	17·1
Na (m-equiv.)	39·5	35·1	19·3	20·1	19·9
K (m-equiv.)	109	87·0	124	116	104
P (m-moles)	70·1	66·5	87·8	82·3	80·0

In both lots of undernourished birds, underfeeding greatly increased the amounts per unit weight of the extracellular electrolytes, Cl^- and Na^+, and of total extracellular protein nitrogen and collagen N. There was a small rise in the amount of water and a fall in that of total N. In the birds undernourished from an early age, the amount of non-protein N was reduced, but it was increased in the birds undernourished during later life. In both lots of undernourished animals, the intracellular proteins, belonging to the sarcoplasm and fibrils, were reduced more than the total N. When the results for these two types of protein in the birds undernourished from 2½ weeks of age are considered in relation to the normal developmental changes which take place over the same range of weight increase, the sarcoplasmic proteins were clearly more affected by underfeeding than the fibrillar proteins. A similar difference was also found in the muscles of the animals undernourished from 27 to 42 weeks of age. The fall in the amounts of the intracellular proteins per unit weight was accompanied by a fall in the amounts of the intracellular minerals K and P.

Effect of undernutrition on the distribution of water and on the composition of the muscle cell

From the analytical results presented in Table 2, the distribution of the water in the muscle has been calculated on the assumption that the chloride 'space' is equal to the volume of extracellular fluid. The composition of the muscle cells has then been deduced with reference to intracellular water and intracellular protein N. These calculations were described in detail by Dickerson & Widdowson (1960). The results of the calculations on the undernourished animals have been expressed as a percentage of the initial values, i.e. of the values for the muscle of normal birds at $2\frac{1}{2}$ weeks of age and of the muscle of normal birds at 27 weeks of age. Table 3 shows the effect of undernutrition on the distribution of water and on the composition of the muscle cell.

Table 3. *Effect of underfeeding (a) nine cockerels from $2\frac{1}{2}$ to 27 weeks of age, and (b) three from 27 to 42 weeks of age on the distribution of water and the composition of the muscle cell*

(Mean values expressed as percentages of initial values)

Variable	(*a*)	(*b*)
Chloride 'space'	148	178
Intracellular water	92	90
Ratio, intracellular protein N: intracellular water	92	78
Ratio, intracellular K + intracellular Na: intracellular protein N	102	111

During underfeeding, the chloride 'space' and hence the volume of extracellular fluid per unit weight of muscle was greatly increased. Since there was only a small rise in the amount of total water per unit weight of muscle, the volume of intracellular water was reduced. Relatively more protein than water was, however, lost from the cells, for the ratio of protein to water in the cells was reduced and the ratio of intracellular Na + K to protein was increased. The changes in cell composition were larger in the birds undernourished from 27 weeks than in those undernourished from $2\frac{1}{2}$ weeks, but the evidence in both suggests that the muscle cells were overhydrated.

Effect of undernutrition on the pectoral muscles as a whole

Table 4 shows the total amounts of some of the intracellular and extracellular constituents in the pectoral muscles of the undernourished birds expressed as a percentage of the total amount present when undernutrition began. In spite of the undernutrition, there was an increase in all the constituents in the birds that were $2\frac{1}{2}$ weeks old at the time the restrictions began. Some, if very little, true growth must have taken place. There was, however, a much greater increase in the extracellular constituents than in the intracellular ones. Underfeeding the birds that had been reared normally till they were 27 weeks old resulted in a loss of all the muscular constituents but a greater loss of the cellular than of the extracellular components. In these birds, the values for Cl, Na and extracellular protein N suggest that more extracellular fluid than protein disappeared from the extracellular phase.

Table 4. *Effect of underfeeding (a) nine cockerels from 2½ to 27 weeks of age, and (b) three from 27 to 42 weeks of age on the total amounts of cellular and extracellular constituents in pectoral muscles*

(Mean values expressed as percentages of initial values)

Constituent	(a)	(b)
Intracellular:		
Sarcoplasmic protein N	113	5·0
Fibrillar protein N	122	7·1
K	120	7·4
P	108	7·4
Extracellular:		
Extracellular protein N	340	49·5
Cl	212	14·9
Na	314	15·7

Effect of rehabilitating birds undernourished from 2½ to 27 weeks of age

Table 5 shows the amounts of N per kg in the pectoral muscle of birds undernourished from 2½ to 27 weeks of age and then allowed free access to food for 15 weeks.

During rehabilitation, the amount of N per unit weight in the non-protein fraction and in each of the intracellular protein fractions increased, whilst that in the extracellular fraction decreased. After 15 weeks' rehabilitation the amounts of the various nitrogenous components per unit weight of muscle were similar to, but not yet quite the same as, those in the muscle of normal birds at 27 weeks of age. It seems unlikely that the gross retardation of growth resulted in any permanent change in skeletal muscle.

Table 5. *Effect of rehabilitating cockerels undernourished from 2½ to 27 weeks of age on the nitrogenous constituents of pectoral muscle*

(Mean values expressed as g/kg fresh muscle)

Constituent	Undernourished from 2½ to 27 weeks of age	Rehabilitated from 27 to 42 weeks of age	Normal birds, 27 weeks of age
Non-protein N	3·8	5·2	5·5
Sarcoplasmic protein N	5·5	9·5	11·1
Fibrillar protein N	15·1	19·2	19·4
Extracellular protein N	4·0	1·6	1·2

DISCUSSION

Jackson (1915) found that when rats were subjected to chronic underfeeding the muscles tended to lose a greater proportion of their initial weight than the body as a whole. It is evident from the results presented in Table 1 that some muscles may be affected more than others. It has been suggested that the susceptibility of different muscles to atrophy is related to their rate of growth (Babinski & Onanoff, 1888), and the finding that the faster-growing pectoral muscles were more seriously affected than the sartorius is in agreement with this suggestion. It is hoped to investigate the turnover rates of various constituents in the two muscles.

The effect of underfeeding on the different muscles is yet another manifestation of the well-established fact that underfeeding affects the various organs of the body to a different degree. There is ample evidence that the skeleton continues to grow, though at a slower rate, when growing animals are undernourished (Jackson, 1925). It has been found that the femurs of the cockerels undernourished from an early age in this investigation grew, albeit slowly, and were larger than femurs from normal immature animals of the same body-weight (Dickerson & McCance, unpublished). The percentage of collagen in the skin hardly changed during underfeeding (Dickerson & McCance, unpublished) but, since the undernourished animals increased in size, collagen must have been deposited in the skin. In skeletal muscle, the amount of extracellular protein, the greater part of which is collagen, continued to increase whilst that of the cell protein remained almost unchanged throughout the period of underfeeding, which resulted in the muscle becoming much more fibrous and similar in this respect to that of the chick at hatching (Dickerson, 1960). A sustained synthesis of collagen was also found by Mendes & Waterlow (1958) in the muscle of rats fed on a protein-deficient 'Jamaican diet'. Neuberger, Perrone & Slack (1951) found that the initial activity of [^{14}C]glycine isolated from the tendon collagen of young rats was almost as high as of that isolated from the muscle proteins. In old rats, on the other hand, the initial activity of tendon collagen was negligible compared with that of the muscle proteins. These workers considered that their results were compatible with the assumption that collagen, once it is deposited in the intercellular spaces, becomes metabolically inert and is only slowly, if at all, degraded in the normal animal. Our findings with undernourished animals are in agreement with this assumption and they support the conclusion of Mendes & Waterlow (1958) that changes in total N provide only an uncertain guide to the loss of cell protein as the result of nutritional stress.

Underfeeding cockerels at the two stages of growth induced, on the whole, similar changes in the composition of their pectoral muscles. The results for the muscle as a whole show that these changes were brought about in the young cockerels by the greater retardation of cell growth relative to that of connective tissue, and in the older birds by the greater atrophy of the cells relative to that of the connective tissue. A relative increase in the volume of extracellular fluid in the body is a characteristic sign of undernutrition (Widdowson & McCance, 1951), but Fourman & McConkey (1958) and McConkey (1959) found that the absolute volume of extracellular fluid was not increased in their human subjects during wasting. They pointed out that a relative increase was a necessity if the shrunken muscle cells with their relatively large ratio of surface area to volume were to retain a surrounding film of interstitial fluid. If the wasting is severe, as it was in the pectoral muscles of the birds undernourished from 27 weeks of age, cells not only shrink but have been said to disappear (Frankl & Freund, 1884). The absolute volume of extracellular fluid was certainly reduced in the muscles of these animals.

The results presented in Table 3 provide some evidence of intracellular hydration in the muscle of undernourished chickens, particularly in the adults. These changes would not have been apparent had values for total nitrogen been used in calculating the ratios. It is, therefore, possible that the failure of Widdowson & McCance (1957) and Stanier (1957) to find any evidence of intracellular hydration in their protein-deficient rats may have been due to an increased proportion of collagen masking a decrease in the amount of protein in the cells. This matter is being further investigated.

There have been suggestions that the protein lost from tissues, particularly liver and muscle, during starvation or protein deficiency may be some particular 'storage' protein. Though this view is now largely discounted for the liver (Kosterlitz & Campbell, 1945–6), little evidence has been up till now obtained on skeletal muscle. The work on the muscle of the undernourished adult cockerels has shown that there was a partial loss of all the intracellular proteins. Although possibly the sarcoplasmic proteins were affected to a greater degree than those of the fibrils, the results for both types of protein and also for the non-protein N are, in the main, in agreement with those of Hagan & Scow (1957) on the muscle of starved rats. These workers did not separate denatured fibrillar from extracellular proteins and the present work suggests that, though Hagan & Scow found no change in the sum of these two groups, there may, in fact, have been a change in their proportion.

SUMMARY

1. Investigations have been made of the muscles of Rhode-Island Red cockerels: (*a*) nine undernourished from $2\frac{1}{2}$ to 27 weeks of age (weight gain 100 g) and (*b*) three undernourished from 27 to 42 weeks of age (weight loss 1100 g).

2. Both pectoral and sartorius muscles gained weight in the animals undernourished from the age of $2\frac{1}{2}$ weeks and lost weight in the animals undernourished from the age of 27 weeks. The pectoral muscles were the more seriously affected, and in the animals undernourished from 27 to 42 weeks of age lost over 90% of their initial weight.

3. The pectoral muscles were analysed for water, chloride, sodium, potassium and phosphorus, and the total nitrogen was divided into non-protein, sarcoplasmic, fibrillar and extracellular protein N.

4. Underfeeding in both groups of animals increased the amounts of water, Cl, Na and the extracellular proteins per unit weight of muscle and decreased those of K, P and the intracellular proteins. The non-protein N fell when undernutrition began at $2\frac{1}{2}$ weeks, and rose when it began at 27 weeks.

5. When undernutrition began early in life, the growth of the cells was retarded to a greater extent than that of the connective tissue, and in the adult animals the loss of cell substance was greater than that of connective tissue.

6. Underfeeding probably resulted in overhydration of the cells.

7. Rehabilitation for 15 weeks almost restored the pectoral muscles to normal.

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