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The Effect of Growth on the Composition of Avian Muscle

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Muscular growth in birds and mammals is known to involve a fall in the percentage of water and extracellular electrolytes, and a rise in the percentage of protein, potassium and phosphorus (Leslie & Davidson, 1951; Robinson, 1952*a*; Barlow & Manery, 1954). McCance & Widdowson (1956) found that during the development of human and pig muscle there was an increase in the nitrogen/potassium ratio. This was interpreted to mean that development might be associated with a change in the gross composition of the muscle cell. The work on these two mammals has been extended and the results are reported elsewhere (Dickerson & Widdowson, 1960). The present paper describes observations of a similar nature on avian muscle.

EXPERIMENTAL

Birds used in this investigation were pure-bred Rhode-Island Red cockerels. Twenty-four birds were taken as soon as possible after they had hatched. A further 12 birds aged 2–3 weeks (wt. approx. 100 g.), nine aged 4 weeks (wt. approx. 200 g.) and four aged 27 weeks (wt. approx. 3·5 kg.) were used. The 27-week-old birds were assumed to be adult for the present purpose.

Methods

Newly hatched chicks. These were divided into three groups, each of eight animals, in such a way that the average weight of the birds in each group was approximately the same. The pectoral muscles of all the birds in each group were pooled for analysis. The birds aged 2–3 weeks and 4 weeks were also each divided into three groups and treated in a similar way. The muscle from each adult bird was analysed separately.

Young birds. These were killed by a blow on the head and about 1 ml. of blood was taken by cardiac puncture from each, and this clotted satisfactorily under liquid paraffin. The samples taken from the individual birds in each group were pooled so that three pooled samples of serum were obtained at each of these ages. The pectoral and sartorius muscles of each bird were dissected as completely as possible and placed in tared specimen tubes which had been previously cooled on solid CO_2. The frozen muscle was weighed.

Adult birds. These were killed by the injection of 180 mg. of pentobarbitone sodium (Nembutal). About 5 ml. of blood was taken by heart puncture before the animal died and allowed to clot under paraffin. Further haemorrhage was avoided (Widdowson & Southgate, 1959). The pectoral muscles of one side and the sartorius muscle of both sides were dissected as completely as possible, weighed at once and representative samples frozen on solid CO_2. The weight of the pectoral muscles of these animals was obtained by multiplying the weight taken from one side by two. A small piece of muscle from one animal of each age and kind was fixed in formalin–0·9% NaCl for histological examination. In preparation for analysis, the pectoral muscle was thawed, freed of visible fat and tendon, and thoroughly cut up with scissors.

Analyses

Proteins. These were separated by the method described by Robinson (1952*a*). Analyses were made on duplicate samples where possible. Each sample of 0·5–2·0 g. was weighed in a screw-capped Universal container (Baird and Tatlock Ltd.) which fitted the micro-attachment of a MSE homogenizer. The muscle samples were extracted in the homogenizer three times with 'dilute salt' solution (0·1 M-KCl, 0·066 M-NaH_2PO_4–K_2HPO_4, pH 7·1, *I* 0·2). The suspension was centrifuged between each extraction and the supernatant liquid removed. These extractions with salt solution were followed by two further extractions with 0·1 N-NaOH, after which the muscle samples were left to stand in 0·1 N-NaOH overnight at 4°. The 'dilute salt' and the NaOH extracts and the residue were retained. Actomyosin in the 'dilute salt' extract was precipitated by the addition of an equal volume of 30% (v/v) ethanol and separated in the centrifuge. The N in both the supernatant liquid and the precipitate was estimated. The former consisted of sarcoplasmic-protein N and non-protein N, and the sarcoplasmic-protein N was obtained by deducting the non-protein N obtained by separate estimation. The N in the extract with NaOH was estimated and this, together with the N in the actomyosin precipitate, constituted the fibrillar-protein N. The result for total fibrillar protein obtained in this way was in good agreement with that obtained by extraction of further samples with 'strong salt' solution (1·25 M-KCl, 0·066 M-K_2HPO_4, pH 8·5, *I* 1·45) followed by 0·1 N-NaOH as described by Robinson, and in some cases this further extraction was omitted. In all samples the 'fibrillar proteins' described in this and other work (Dickerson & McCance, 1960; Dickerson & Widdowson, 1960; Widdowson, Dickerson & McCance, 1960) correspond to the sum of Robinson's fibrillar and denatured fibrillar fractions. The residue after extraction with salt solution and NaOH has been assumed to be extracellular proteins (Lowry, Gilligan & Katersky, 1941). The sum of the fractions agreed with the total N within the limits ±5%.

Total protein nitrogen. This (actually protein + nucleic acid) was precipitated by the addition of 10 ml. of 10% (w/v) trichloroacetic acid to 0·5–1·0 g. of muscle. The muscle was snipped as finely as possible with scissors in the trichloroacetic acid and the suspension allowed to stand for at least 1 hr. After centrifuging, the supernatant liquid was removed and the precipitated protein extracted twice more with trichloroacetic acid solution. The supernatants were combined, the volume was measured and a sample was pipetted into a Kjeldahl flask for estimation of the non-protein N. The precipitated protein was dissolved in conc. H_2SO_4, made up to a known volume and a sample taken for estimation of N. All samples for estimation of N were digested with 2 ml. of conc. H_2SO_4 in the presence of copper selenite as catalyst. The N was subsequently estimated by micro-Kjeldahl distillation. The total N was either estimated independently or obtained by adding together the protein and non-protein N.

Collagen. This was estimated by direct determination of hydroxyproline in the hydrolysate of the mixed proteins of the tissue by the method of Neuman & Logan (1950). Skeletal muscle was dried to constant weight at 100°. The dry tissue was powdered and duplicate samples of 100–300 mg. were used for the estimation of collagen. The samples were hydrolysed with 6 N-HCl in sealed tubes in a boiling-water bath for 48 hr., 1 ml. of HCl/50 mg. of dry tissue being used. The subsequent procedure was the same as that described by Neuman & Logan (1950), except that 0·05 M-$CuSO_4$ solution was used as advocated by Baker, Lampitt & Brown (1953), and the extinction was read at a wavelength of 560 mμ. It was assumed that the collagen in fowl muscle contained 14·0% of hydroxyproline (Leach, 1957). No allowance was made for interference by tyrosine.

Water, chloride, sodium, potassium, phosphorus and magnesium. These were estimated by the same methods as those described elsewhere (Dickerson & Widdowson, 1960). The serum electrolytes were estimated as described by Widdowson & McCance (1956).

Chloride 'space'. This was calculated in the conventional way from the concentration of Cl in the muscle and that in the serum water. The Na 'space' has been calculated in a similar way. The Cl 'space' has been used as a measure of the proportion of extracellular fluid (Barlow & Manery, 1954). The concentrations of electrolytes found in the serum were similar to those reported by Barlow & Manery (1954) and these have been converted into concentrations in the extracellular fluid by applying factors of 1·05 for Cl and 0·94 for Na and K to make allowance for the Gibbs–Donnan equilibrium.

Changes in cell composition have been followed as described by Dickerson & Widdowson (1960), by using intracellular water and intracellular-protein N as reference standards for the cellular constituents.

RESULTS

Table 1 shows the effect of growth on the body weight and on the weight of the pectoral and sartorius muscles. The body weight of the chicks was almost doubled during the first 2½ weeks after hatching, but there was a tenfold increase in the weight of the pectoral muscles during this time. The sartorius muscles grew at a slower rate than the pectoral muscles and were always lighter. Only the pectoral muscles were chemically analysed.

Table 2 shows the effect of growth on the composition of skeletal muscle. Most of the fall in the amounts of water, Na and Cl/kg. of muscle took place during the first 2½ weeks after hatching. The large concentration of Na and the small concentration of K in the muscle of the chick at hatching agrees with the findings of Barlow & Manery (1954). During the first 2½ weeks the large fall in the concentration of Na was accompanied by an increase in the concentration of K. Potassium did not, however, continue to increase during further growth as

Table 1. *Effect of growth on the body weight and on the weight of the pectoral and sartorius muscles*

Average weights (g.) per bird are given.

Age (weeks) ...	0	2½	4	27
Body wt.	52·4	93	195	3365
Pectoral muscles	0·39	4·12	12·1	326
Sartorius muscles	0·13	0·28	0·53	13·5

Table 2. *Effect of growth on the composition of fowl pectoral muscle*

Amounts/kg. of fresh muscle are given as average and range.

Age (weeks)	0	2½	4	27
Number of analyses	3	3	3	4
Water (g.)	854 (847–864)	771 (768–774)	756 (753–760)	737 (724–740)
Total N (g.)	17·3 (16·5–18·1)	29·5 (28·4–30·5)	34·1 (33·1–35·5)	37·0 (33·8–38·9)
Non-protein N (g.)	1·7 (1·6–1·8)	4·2 (4·0–4·7)	4·5* (4·2, 4·9)	5·5 (5·0–6·1)
Total-protein N (g.)	15·6 (14·7–16·4)	25·3 (24·5–26·5)	29·5* (28·9, 30·1)	31·6 (28·8–33·5)
Sarcoplasmic-protein N (g.)	4·0 (3·4–4·6)	6·6 (6·5–7·4)	10·8* (10·6, 11·0)	11·1 (10·0–12·1)
Fibrillar-protein N (g.)	8·8 (8·4–9·0)	16·9 (16·7–17·1)	17·9* (17·3, 18·4)	19·4 (18·2–20·6)
Extracellular-protein N (g.)	2·8 (2·7–2·9)	1·6 (1·4–1·9)	1·1* (1·0, 1·2)	1·2 (1·0–1·4)
Collagen N (g.)	2·3 (2·0–2·5)	1·4 (all 1·4)	0·8 (all 0·8)	1·1 (0·8–1·4)
Cl (m-equiv.)	74·7 (71·0–80·1)	20·4 (18·8–21·8)	19·6 (19·4–19·7)	17·1 (13·6–19·0)
Na (m-equiv.)	137·3 (122·1–149·8)	19·3 (18·8–19·7)	20·1 (18·0–24·4)	19·9 (17·4–22·3)
K (m-equiv.)	46·0 (44·1–49·4)	124 (124–125)	116 (all 116)	104 (91–116)
P (m-moles)	53·9 (45·5–66·5)	87·8 (85·2–89·3)	82·3 (81·3–83·2)	80·0 (77·4–82·6)
Mg (m-equiv.)	23·2* (21·3, 25·0)	28·0 (26·9–29·5)	29·2* (27·7, 30·8)	28·8 (27·0–30·6)

* Two results only.

Table 3. *Effect of growth on the distribution of the total nitrogen*

Results are the amounts of N in the various fractions expressed as a percentage of the total N, and are the averages of the individual values.

Age (weeks)	0	2½	4	27
Non-protein N	9·9	14·2	13·4	14·8
Sarcoplasmic-protein N	22·8	22·9	31·7	30·0
Fibrillar-protein N	50·9	58·2	52·4	53·2
Extracellular-protein N	16·3	5·2	3·2	3·3
Collagen N	12·7	4·8	2·3	3·0

it does in human muscle (Dickerson & Widdowson, 1960), but steadily decreased to a somewhat lower value in the muscle of the adult. The concentration of total P changed in parallel with that of K.

The fall in the amount of water/kg. with development was accompanied by an increase in the amount of total N and, as with the water, most of the change took place in the first month of life. Parallel increases also took place in the non-protein N and in the total-protein N. The non-protein N was the nitrogen extracted from the muscle with trichloroacetic acid, and for this reason will include amino acids and polypeptides besides urea, creatine and purines.

The two groups of intracellular proteins, those of the sarcoplasm and fibrils, increased with development. The great increase in the fibrillar proteins took place in the first 2½ weeks. Between 2½ and 4 weeks they increased only a little more, whereas the sarcoplasmic proteins went on doing so. The amount of extracellular-protein N/kg. decreased rapidly after hatching and by 4 weeks had reached its adult amount. The changes in the amount of collagen N paralleled those of the total extracellular proteins, but, as collagen forms only a part of the latter, it was at all ages lower.

Table 3 shows the distribution of the N as a percentage of the total N. The non-protein N had almost reached its adult proportion of the total N by 2½ weeks, and by 4 weeks the composition of the pectoral muscle resembled that of an adult. The distribution of N amongst the various protein fractions is very similar to the pattern obtained by Robinson (1952*a*) at corresponding ages. The small differences may be due to the analysis of a different breed of bird.

Table 4 shows the distribution of the water and the relationship of intracellular-protein N, K, P and Mg to the intracellular water and of K, P and Mg to intracellular-protein N.

Table 4. *Effect of growth on the composition of avian skeletal muscle*

Data derived from average results shown in Table 2 for the distribution of water and the composition of the cells relative to cellular water and cellular-protein N.

Age (weeks)	0	2½	4	27
	Per kg. of muscle			
Chloride 'space' (g.)	550	161	144	124
Sodium 'space' (g.)	967	126	135	128
Intracellular water (g.)	304	610	612	613
Intracellular-protein N (g.)	12·8	23·7	28·4	30·4
Intracellular K (m-equiv.)	40·8	123	115	103
Intracellular Na (m-equiv.)	59·3	0	0	1·4
	Per kg. of intracellular water			
Intracellular-protein N (g.)	42·1	38·9	46·4	49·6
Intracellular potassium (m-equiv.)	134	202	188	168
Intracellular potassium plus intracellular sodium (m-equiv.)	330	202	188	168
P (m-moles)	177	144	135	130
Mg (m-equiv.)	26·6	38	40·1	39·5
	Per g. of intracellular-protein N			
Intracellular potassium (m-equiv.)	3·19	5·19	4·1	3·39
Intracellular potassium plus intracellular sodium (m-equiv.)	7·83	5·19	4·1	3·59
P (m-moles)	4·2	3·7	2·9	2·63
Mg (m-equiv.)	0·63	0·98	0·87	0·80

The changes in the Cl 'space' and intracellular water during growth were similar to those described by Barlow & Manery (1954) in fowl muscle and by a number of investigators (Yannet & Darrow, 1938; Hines & Knowlton, 1939; McCance & Widdowson, 1956; Dickerson & Widdowson, 1960) in mammalian muscle. The proportion of protein to liquid in the muscle cells decreased slightly during the first 2½ weeks of life but thereafter steadily increased. A similar sequence of events has been met with in the skeletal muscle of the pig and man (Dickerson & Widdowson, 1960). The concentration of K in the intracellular water moved in the opposite direction. The quantity of P steadily decreased whereas that of Mg decreased during the first 2½ weeks and then probably did not change further. The ratio of intracellular K to intracellular-protein N was lower at hatching than at 2½ weeks but from this time onwards decreased. The ratio of Mg to intracellular-protein N was higher at hatching than at 2½ weeks but changed little after this age. The ratio of P to intracellular-protein N decreased over the whole of the growth period. The large amount of Na in excess of that in the extracellular fluid was thought by Barlow & Manery (1954) possibly to be bound to solid in the extracellular phase, but if it was, in fact, inside the cells, then the ratios of intracellular Na plus intracellular K to intracellular water and to intracellular-protein N show a decrease over the whole of the growth period.

DISCUSSION

The pectoral muscles increased in weight some tenfold during the first 2½ weeks of life and this was associated with a decrease in the quantity of extracellular proteins in each kilogram of muscle. Similar changes have been found to take place in mammalian muscle (Dickerson & Widdowson, 1960) and have been attributed to an increase in the diameter of the muscle fibres. The rapid change in fowl muscle could hardly be attributed to this, however, for there was only a small increase in the diameter of the muscle fibres during the first 2½ weeks, though there was an increase during later life. Another reason which, however, may be put forward for these changes in the pectoral muscles is a change in shape of the muscle. At hatching, the pectoral muscle of the chick is a thin sheet of tissue with a relatively large surface area/volume ratio. Consequently, the thickest of its connective-tissue components, the epimysium, accounted for a large part of its extracellular proteins. During the first 2½ weeks, the muscle probably grew by increasing the number of fibres, and this growth took place at a faster rate than that of the bone underlying it. The result of this was that the muscle increased in thickness and the surface area/volume ratio fell.

It has been assumed in making some of the calculations shown in Table 4 that the intracellular water was evenly distributed throughout the cell.

This may not be so (Huxley & Hanson, 1957). Furthermore, skeletal-muscle cells contain intracellular structures which are common to all true cells. Of these, the nucleus is the most prominent and accounts for a larger proportion of the protein in the muscle cells of young animals (Robinson, 1952*b*), and it has been suggested that this might account for some of the differences in the composition of the muscle cell at different ages (Dickerson & Widdowson, 1960). It might, for example, partly account for the large amounts of sodium in the muscles of 'day-old' chicks and to a lesser extent in those of foetal mammals (Dickerson & Widdowson, 1960), for Itoh & Schwartz (1956) showed that the nuclei of liver and thymus contain more sodium than the cytoplasm and the same may be true of muscle nuclei.

The cells of chick-heart muscle at hatching also contain apparently more sodium than they do at later stages of growth (Dickerson, 1959). Oxygen is essential if the mechanism by which sodium is extruded from muscle cells is to work efficiently (Hercus, McDowall & Mendel, 1955) and it may be that the real explanation of the high sodium content of chick-muscle cells at hatching is to be found in the oxygen and energy supply of the muscles.

SUMMARY

1. Pectoral muscle from Rhode-Island Red cockerels at 0, 2½, 4 and 27 weeks of age were analysed for water, chloride, sodium, potassium, phosphorus and magnesium, and the total nitrogen was divided into non-protein, sarcoplasmic-, fibrillar- and extracellular-protein nitrogen.

2. The amounts of water, chloride, sodium and extracellular-protein nitrogen/kg. decreased and those of potassium, phosphorus and magnesium increased during the first 2½ weeks. Potassium and phosphorus fell from this age to a lower level in the adult. The fibrillar proteins increased mainly during the first 2½ weeks whereas the main increase in the sarcoplasmic proteins was between 2½ and 4 weeks. At 4 weeks the muscle had an almost adult composition.

3. Calculations showed that the increase in the proportion of the cellular phase was accompanied by an increase in the amount of protein nitrogen/l. of cell water, whereas the amount of sodium plus potassium/l. of cell water decreased.

4. The possible influence on cell composition of the uneven distribution of cellular constituents is briefly discussed.

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