Calomel electrode for use with the glass electrode

A convenient type of calomel electrode assembly consisted of a short test tube (10 × 2·5 cm. diameter) carrying in a two-hole rubber bung a calomel electrode (Cambridge Instrument Company) and an inverted U-tube filled with agar-KCl. The test tube contained saturated KCl plus excess of solid KCl and the end of the U-tube outside the test tube was drawn out to a capillary (2 mm. external diameter). The assembly was clamped so that the tip of its capillary was a few mm. above the upper end of the electrode capillary (Fig. 2*d*).

Testing the electrode

The leads from the calomel and Ag-AgCl electrodes were connected to a Cambridge pH meter (Cambridge Instrument Company), and the latter was adjusted after rinsing the glass electrode with standard phthalate buffer (pH 4·00) and filling with the same buffer. The pH's of a range of buffers were then determined and compared with the results obtained using the glass electrode assembly supplied by the makers of the meter. The capillary electrode was considered to be satisfactory if the results agreed within 0·02 pH units within the range 2–9. On introducing 0·3 ml. liquid at the top and closing the stopcock as soon as the liquid reached the lower end of the capillary, ample liquid remained above the capillary to ensure a junction with the agar bridge. If the tip of this bridge is < 2 mm. diameter it is impossible for it to reach and possibly to damage the narrow part of the electrode capillary.

SUMMARY

The making of a simple and robust glass electrode for pH measurements on 0·3 ml. liquid is described.

REFERENCES

Britton, H. T. S. (1942). *Hydrogen Ions.* London: Chapman and Hall.
Chinoy, J. J. (1947). *Curr. Sci.* **16**, 25.
Dietz, V. H. (1948). *Science*, **108**, 338.
Hanes, C. S. (1951). *Nature, Lond.*, **168**, 566.
Michaelis, L. (1936). *Science*, **83**, 213.
Morton, C. (1930). *J. Sci. Instrum.* **7**, 187.
Païc, M. (1938). *J. chim. phys.* **35**, 327.
Pickford, G. E. (1937). *Proc. Soc. exp. Biol., N.Y.*, **36**, 154.

Changes in the Protein Composition of Chick Muscle During Development

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Although the earliest systematic investigations of the proteins of muscular tissue date from the work of Fürth (1895) we still know very little concerning the composition of muscles other than those of the normal adult animal. At the present time there exists a formidable array of facts and theories relating to the composition, detailed structure and functioning of adult skeletal muscle and yet, apart from a few isolated papers in the literature, little biochemical work has been carried out upon the developing tissue. Thus, the information which is available is due largely to the work of the embryologist, cytologist and histologist and is therefore mainly descriptive in character.

The changes occurring in the appearance of the muscle cell from the myoblast to the adult myofibril are reasonably well known. In the chick, with which this study is concerned, fibres can be observed in the premuscular mass of the limb buds on the 6th day of incubation which, by the 8th day, have become striated and contractile (Hunt, 1932). In the adult, the muscle fibres are composed of numerous myofibrils of diameter about 1 μ. embedded in a viscous medium, the sarcoplasm. Connective tissue fibres of collagen and elastin ramify throughout the muscle. The cell nuclei lie under the sarcolemma, having moved there during development, from a central position in the fibre.

The intracellular proteins of the adult tissue may be conveniently divided into the proteins of the sarcoplasm, such as myogen, myoalbumin, and globulin X, which together contain all the enzymes of the glycolytic cycle; and the proteins of the myofibril, such as actin and myosin. The former are extracted from minced muscle by dilute salt solutions at a neutral pH and are characterized by their distinctive physicochemical properties. The myofibrillar protein, actomyosin, is extracted only by strong salt solutions and precipitates on dilution. If extraction is prolonged at an alkaline pH then the actin content of the extract rises and precipitation on dilution yields an actin-rich actomyosin complex.

The protein tropomyosin (Bailey, 1948), while also probably located within the myofibril, can only be extracted by special methods.

Remaining after strong salt extraction is a residue which is partly soluble in dilute acid. Bate Smith (1937) thought that the acid-soluble part consisted largely of denatured myosin and globulin X and belonged to the intracellular fraction, while the acid-insoluble residue consisted of collagen, elastin and reticulin which together formed the extracellular fraction.

Such studies on the solubilities and distribution of the muscle proteins of the adult have led to the development of schemes for their fractionation such as those used by Weber & Meyer (1933) and by Bate Smith (1934, 1935, 1937). In this paper a similar scheme has been applied to the developing tissue and the quantitative relations between certain groups of muscle proteins have been determined. Such a survey seems an essential preliminary to any more detailed work on the biochemistry of muscle development. Subsequent papers describe the nucleoprotein and adenosinetriphosphatase distribution in the developing tissue (Robinson, 1952*a*, *b*).

EXPERIMENTAL

The solubility characteristics of the proteins of adult striated muscle

Bate Smith (1934, 1935) has demonstrated that the extraction of minced adult rabbit muscle with strong salt solutions at neutral pH brings into solution the major part of the myofibrillar and sarcoplasmic proteins, though the discrepancy between the amount extracted by this procedure and that using 0·1 N-HCl may amount to 15% of the total intracellular protein. It is probable that some of the actin is not extracted by neutral salts under Bate Smith's conditions, but is soluble in 0·1 N-HCl.

The myofibrillar proteins are precipitated from such a salt extract at pH 7 by dilution to an ionic strength (I) of 0·05; globulin X and the other sarcoplasmic components remain in solution. According to Bate Smith, however, the completeness of the precipitation depends upon the nature of the ions present. He was able to estimate the amounts remaining in solution under different conditions and found, for example, that by 20-fold dilution of a 7% (w/v) LiCl extract only 88% of the myofibrillar proteins were precipitated. Similarly, it was found by the author that dilution of an actomyosin preparation in 1·25 M-KCl-phosphate buffer to an ionic strength of 0·1 did not bring about complete precipitation of the protein. Dilution to an ionic strength of 0·05 would probably have further reduced the amount remaining in solution, but the volume of liquid to be handled would have become too large for the routine fractionations which were contemplated.

Work in a related field (Cohn *et al.* 1946) on the fractionation of plasma proteins, using ethanol at low temperatures as the precipitating agent, has indicated that fibrinogen, a protein similar in its solubility to actomyosin, is precipitated from plasma at pH 7 by an ethanol concentration of 7% (v/v) at −3°. It seemed possible that a similar system could be used for the precipitation of actomyosin. Experiments carried out on a pure preparation of rabbit actomyosin in strong KCl-phosphate, showed that, on dilution to an ionic strength of 0·12 and addition of 70% (v/v) ethanol from a fine capillary to the stirred preparation at 0°, all the protein was precipitated at an ethanol concentration of 7% (v/v). Using artificial mixtures of an actomyosin preparation and a sarcoplasmic extract of adult rabbit muscle, actomyosin could be quantitatively recovered under the same conditions with an error of ±5%. With a sarcoplasmic extract in dilute KCl-phosphate, addition of ethanol to 20% (v/v) gave no precipitation of any protein component.

The effect of different salt solutions upon the extraction of muscle proteins

Bate Smith (1934) concluded from a survey of various salts that 7% (w/v) LiCl solution at pH 7 extracted the largest percentage of the intracellular proteins from minced muscle. A similar study was carried out using the newer extraction techniques described in detail below, but at pH 8·5 rather than at pH 7 to facilitate the solution of the protein, actin. The results of three such experiments are given in Table 1.

Table 1. *The effect of different salts upon the extraction of the muscle proteins*

Extractant	Protein N extracted (g./100 g. fresh muscle)
0·5 M-KCl in 0·066 M-phosphate buffer pH 8·5	2·07
1·25 M-KCl in 0·066 M-phosphate buffer pH 8·5	2·38
7% LiCl in 0·05 M-borate buffer pH 8·5	2·45

The efficiency of the 1·25 M-KCl-phosphate solution at pH 8·5 seems to be very little below that of the 7% (w/v) LiCl-borate solution and because of the rapid destruction of double refraction of flow of myosin which occurs in the presence of the latter, according to Edsall & Mehl (1940), the strong KCl-phosphate solution has been preferred in this study.

The residue remaining after extraction with a strong KCl*-phosphate solution*

This fraction contains approximately 20–25% of the total protein of adult rabbit muscle. When extracted with 0·1 N-NaOH much more protein material goes into solution leaving a residue forming about 5% of the total protein. This is believed to consist only of collagen and elastin, since it has been shown (Lowry, Gilligan & Katersky, 1941; Bowes & Kenten, 1948) that while other tissue proteins are soluble these two are insoluble in 0·1 N-NaOH solution.

The state of division of the tissue

Many workers have stressed the importance of the state of dispersion of the tissue in the extraction of the intracellular proteins (Bate Smith, 1937; Guba & Straub, 1943; Dubuisson, 1950). Experiments were made with a Latapie mincer followed by a glass homogenizer, a Waring Blendor, a treatment which involved grinding with sand after mincing with

scissors, and with a stainless-steel homogenizer, described by Marsh & Snow (1951). The last was found to be the most satisfactory for adult muscle, and in less than a minute reduced it to disintegrated and broken myofibrils.

There appears to be no denaturation of the sarcoplasmic proteins in the homogenizer when running at top speed in the presence of either the strong KCl-phosphate solution or the dilute KCl-phosphate solution. The small amount which occurs when actomyosin solutions are similarly treated at 0° does not amount to more than 4% of the total protein.

Fractionation procedures

The studies already described suggest certain factors which should be considered in any proposed fractionation. It seems clear that the state of division of the tissue, the pretreatment of the muscle, the time and conditions of the extraction (pH, temperature, etc.) and the nature of the salts used are important in obtaining a quantitative recovery of any particular fraction.

The procedures we have used are based on extraction with three different solutions which the preliminary studies suggest will yield four distinct fractions of muscle proteins. The details are given below.

Extraction with a dilute salt solution. Weighed samples of fresh muscle (from 1 to 3·5 g.) were finely minced with scissors in the cooled extractant solution. This had the composition, 0·1 M-KCl, 0·066 M-NaH_2PO_4/K_2HPO_4 pH 7·1, $I=0·2$, and will be referred to as the dilute KCl-phosphate solution. The whole was transferred to the homogenizer which was never more than half full of solution (capacity 50 ml.) and was surrounded by an ice bath. A drop of 'capryl alcohol' was added to reduce frothing. After homogenization for 5 min. at top speed, the homogenizer, designed to fit into a 50 ml. centrifuge cup, was centrifuged and the supernatant solution poured off. Second and third extractions for 10 min. at half speed were carried out in a similar fashion. The three extracts were combined and samples diluted at pH 7 with 30% ethanol (v/v) to $I=0·1$ at an ethanol concentration of 15% (v/v). The ethanol was added from a fine capillary to the stirred extract at 0°. The small precipitate of actomyosin which formed was removed on the centrifuge and the protein N content of the supernatant solution determined.

The residue remaining after extraction with dilute KCl-phosphate was extracted twice with 0·1 N-NaOH for periods of 10 min. in the homogenizer at half speed and then stood overnight in 0·1 N-NaOH at 0°. The extract was discarded and the residue dissolved in concentrated H_2SO_4 and samples taken for protein N determination. The scheme is summarized in Fig. 1.

Protein N was estimated by the micro-Kjeldahl method (Chibnall, Rees & Williams, 1943), but 15 ml. lipped Pyrex test tubes were used instead of the customary 50 ml. digestion flasks. Thus the precipitation of the protein, the washing of the precipitate and the digestion with H_2SO_4 could be carried out in the same vessel. All micro-Kjeldahl estimations were made in triplicate: at the earliest developmental stages where the amount of protein N/sample was less than 9·2 mg. a Markham apparatus was used instead of the usual distillation system and the subsequent titration carried out with a 1 ml. horizontal burette.

Extraction with strong salt solution. Weighed samples of fresh muscle were treated as above, except that a stronger salt solution of composition 1·25 M-KCl, 0·066 M-K_2HPO_4 pH 8·5, $I=1·45$ was used. This will be called the strong KCl-phosphate solution. The combined salt extracts were

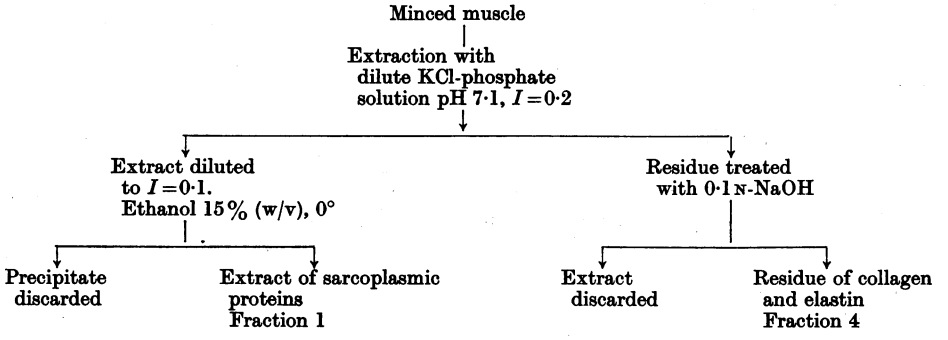

Fig. 1. Scheme showing the fractionation achieved with dilute KCl-phosphate and 0·1 N-NaOH solutions.

then diluted with 0·066 M-NaH_2PO_4 and water to a final KCl concentration of 0·5 M and an ionic strength of 0·58 at pH 7·1. Samples of this extract were further diluted in the Pyrex tubes with 19% ethanol (v/v) to an ionic strength of 0·12 and a final ethanol concentration of 15% (v/v). The temperature was kept at 0° throughout the addition. The precipitated protein was centrifuged and washed twice with 5% trichloroacetic acid (w/v) before determination of the protein N content.

The residue after extraction with the strong KCl-phosphate solution was treated with 0·1 N-NaOH as previously described, and both the NaOH extract and residue retained for nitrogen determination. The scheme is summarized in Fig. 2.

In addition, it was usual, as a check upon the analyses of the fractions, to determine the total protein N directly. Weighed samples of muscle were minced with 10% trichloroacetic acid (w/v) and, after centrifuging and washing, the residue was dissolved in concentrated H_2SO_4 before samples were taken for final digestion.

A summary of the fractions estimated. The protein fractions determined are, therefore:

Fraction 1. Soluble in dilute KCl-phosphate at pH 7·1 and not precipitated on dilution to an ionic strength of 0·1 at an ethanol concentration of 15% (v/v) at 0° (Fig. 4*A*).

Fraction 2. Soluble in strong KCl-phosphate at pH 8·5, but precipitated on dilution to an ionic strength of 0·12 and

a final ethanol concentration of 15% (v/v) at pH 7·1 at 0° (Fig. 4*B*).

Fraction 3. Insoluble in strong KCl-phosphate at pH 8·5, but soluble in 0·1 N-NaOH (Fig. 4*C*).

Fraction 4. The residue after extraction with 0·1 N-NaOH (Fig. 5*A*).

Fraction 5. Total protein N (Fig. 5*B*).

In the later stages of development after hatching, when all the muscle was not required, samples as representative as possible were taken. Analyses carried out on three such samples, taken from the breast muscle of a single month-old chick, agreed to within 5%. Consequently, the larger variations when muscle was taken from different birds of the same weight must be attributed to general environmental

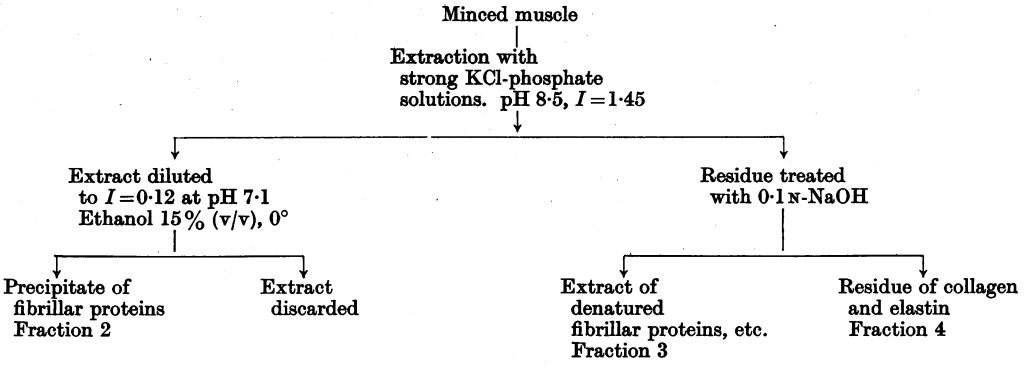

Fig. 2. Scheme showing the fractionation achieved with strong KCl-phosphate and 0·1 N-NaOH solutions.

Materials

Incubation. The eggs, incubated in a standard incubator or in a hot room at 37°, were from a single strain of White Leghorns. The description of an embryo's stage of development in terms of the length of time of incubation is far from satisfactory, and the weight of the embryo or chicken has been used as a better, and easily available, measure of the development state. The relation between age and weight of the chick embryo is shown in Fig. 3 (data from Romanoff, Smith & Sullivan, 1938).

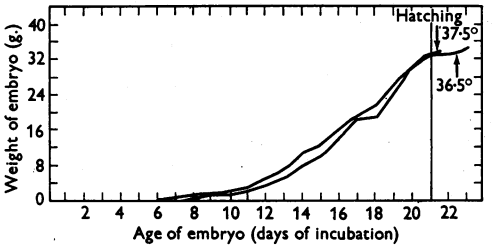

Fig. 3. The relation between age and weight of the chick embryo at incubation temperatures of 37·5 and 36·5° (average values for 700 White Leghorn embryos, Romanoff *et al.* 1938).

Dissection techniques. The analyses covered a range from the chick embryo of 13 days' incubation, through the hatching period around 21 days' incubation, to the adult bird. A routine method of dissection was devised and adhered to throughout. The egg was broken and the embryo removed, dried lightly on filter paper, weighed rapidly and immediately dissected. The muscle from each breast was removed as completely as possible and transferred to the appropriate extracting solution contained in a weighed vessel at 0°.

factors and a comparatively large number of analyses had to be carried out, therefore, on the older birds. In the earlier stages where at least 1 g. of muscle was required for a complete fractionation and, consequently, several embryos had to be harvested, the variations were less. The facilities and help given by Dr D. B. Cater are gratefully acknowledged.

RESULTS

In Figs. 4*A–C* and 5*A*, *B* the protein nitrogen content of each fraction has been expressed as g./100 g. fresh weight of muscle. In Fig. 6 the individual curves are shown together to facilitate comparison (the individual determinations are not given). In Fig. 7 the protein nitrogen of the different fractions is expressed as a percentage of the total protein nitrogen. The breaks which occur in all the curves at hatching are most probably due to changes in the water content of the tissue and have not been studied in detail.

Fraction 1 consists of sarcoplasmic proteins, including many of the enzymes of the glycolytic cycle, and the particulate components of the cell cytoplasm. As a percentage of the fresh weight it steadily increases during growth though it forms a rapidly decreasing proportion of the total protein throughout the embryonic period. It reaches a steady value about a fortnight after hatching.

Fraction 2 consists largely of the proteins of the muscle fibril. However, the deoxyribonucleoproteins of the cell nuclei will form a considerable proportion of the whole in the embryonic stages since, like the myofibrillar proteins, they are extracted by strong salt solutions and precipitated on dilution. Their contribution is assessed in a subsequent paper

(Robinson, 1952*a*). The fraction appears to increase throughout the period over which the analyses have been carried out, both as a percentage of the fresh weight and of the total protein, though the rate of increase declines about a fortnight after hatching.

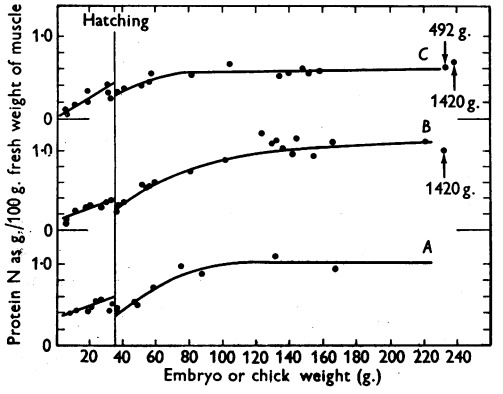

Fig. 4. Changes in the protein nitrogen content of certain muscle fractions during embryonic development; expressed as g./100 g. fresh weight of muscle. *A*, protein nitrogen soluble in dilute KCl-phosphate at pH 7 and not precipitated on dilution to an ionic strength of 0·1 at an ethanol concentration of 15% (v/v) (Fraction 1). *B*, protein nitrogen soluble in strong KCl-phosphate at pH 8·5, but precipitated on dilution to an ionic strength of 0·12 and a final ethanol concentration of 15% (v/v) at pH 7 (Fraction 2). *C*, protein nitrogen insoluble in strong KCl-phosphate at pH 8·5, but soluble in 0·1 N-NaOH (Fraction 3).

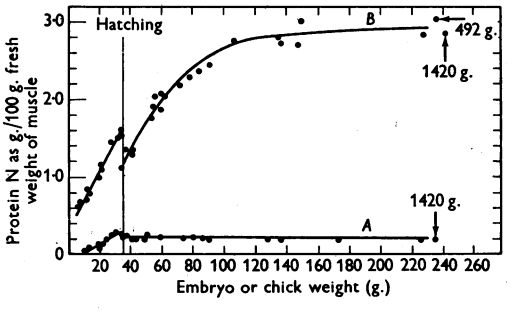

Fig. 5. Changes in the protein nitrogen content of certain muscle fractions during embryonic development; expressed as g./100 g. fresh weight of muscle. *A*, protein nitrogen insoluble in 0·1 N-NaOH (Fraction 4). *B*, total protein nitrogen (Fraction 5).

Fraction 3 is complex and almost certainly contains unextracted actomyosin, either in the native or denatured form. In addition, however, the ill-defined 'ground substance' is probably a component in the embryonic stages where it forms, together with the extracellular proteins, a structural basis upon which the cell architecture is built. Certainly its importance in growing and differentiating tissues should not be minimized and it is interesting to note the large proportion of the total protein of the muscle which the fraction forms during the earlier periods of development. At hatching it represents as much as 25% of the total protein and, therefore, at this stage the residual proteins after extraction with strong salt (Fractions 3 and 4) form almost 50% of the total protein of a muscle which is apparently fully functional. As a percentage of the fresh weight, the fraction appears to increase more

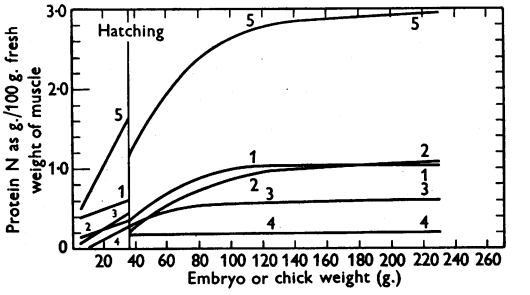

Fig. 6. The protein nitrogen of Fractions 1–5, expressed as g./100 g. fresh weight of muscle at different developmental stages.

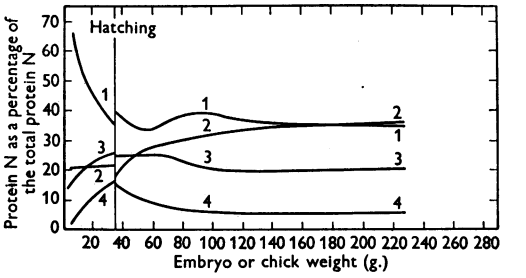

Fig. 7. The protein nitrogen of Fractions 1–4, expressed as percentage of the total protein nitrogen (Fraction 5) at different developmental stages.

rapidly than the other fractions during the embryonic stages; after hatching its increase is less marked, and as a percentage of the total protein it decreases to a constant, yet still high, level about a fortnight after hatching. Here it probably gives a better measure of the unextracted actomyosin of the muscle.

The mucopolysaccharides extracted from muscle by Dische & Osnos (1950) would probably be located in this fraction, which from a brief histochemical study does appear to contain such material. Part of the fraction is soluble in urea and its solutions in 0·1 N-sodium hydroxide have a high viscosity which, however, falls on standing, possibly due to depolymerization. The fraction will shrink in the presence of adenosinetriphosphate suggesting that some actomyosin is present, although strong salt

solutions will not dissolve the precipitate either before or after syneresis.

Fraction 4 increases as a percentage of the fresh weight until hatching and then forms a relatively constant percentage throughout post-embryonic growth. It contributes 15% of the total protein nitrogen of the muscle at hatching and appears to be the first component to be fully differentiated. As development proceeds it forms less and less of the total protein, falling to a constant level, about 5% of the whole, a month after hatching.

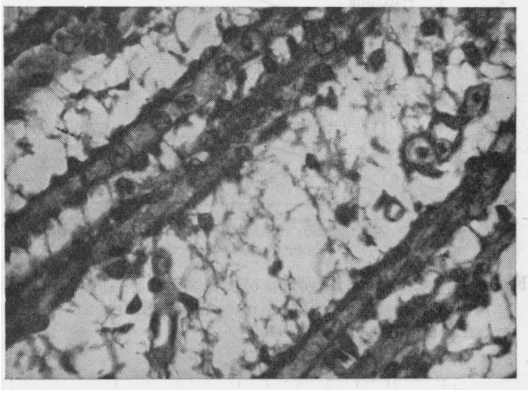

Fig. 8. A section of 13-days'-incubated embryonic chick muscle stained with haematoxylin and eosin. Magnification ×660. Note the developing muscle fibrils with centrally placed nuclei and the 'reticulin' network.

The fraction is made up of the extracellular proteins, collagen, elastin and reticulin. Histologically, the young embryonic muscle shows a vast interlacing network of fibrils which from their staining properties appear to consist largely of reticulin. They stand out very clearly in sections (Fig. 8) and may well form a structural framework for myofibril development, thus accounting for the relatively early differentiation of the fraction. In the adult the histologists suppose it probable that reticulin becomes converted to collagen, and certainly a stained section of the adult muscle shows no such obvious ramifying network as exists in the embryo (Fig. 9). The author is indebted to Mr D. Canwell of the Physiology Department, Cambridge, for these preparations.

In Fraction 5 the total protein nitrogen as a percentage of the fresh weight increases steadily throughout development, reaching its maximum value when the chick is about a month old. It is merely the resultant curve of the changes observed in the other fractions analysed and cannot yield very much information concerning the structural differentiation of the tissue.

It is interesting to contrast the analyses which have been presented with those of Bate Smith (1937)

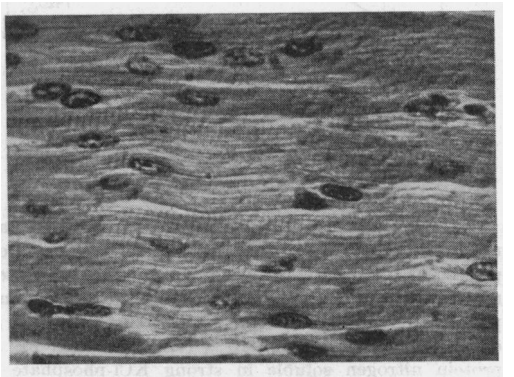

Fig. 9. A section of adult fowl muscle, stained with haematoxylin and eosin. Magnification ×880. Note the striated myofibrils and elongated nuclei. The 'reticulin' network has disappeared.

and Hasselbach & Schneider (1951) on adult rabbit muscle. In Table 2, protein analyses of the muscles of month-old and newly hatched chicks are presented together with those of adult rabbit muscle. In the month-old chick the muscle has acquired its adult form and the partition of nitrogen between the fractions has reached a steady level.

The true contribution of the extracellular proteins appears to be less than Bate Smith (1937) and Hasselbach & Schneider (1951) found in the rabbit. However, it must be emphasized that the extracellular fractions are not directly comparable in these analyses. Thus, in the analyses of Bate Smith they have been calculated from extractions with

Table 2. *Analyses of chicken breast muscle and adult rabbit striated muscle expressed as percentages of the total protein*

Description of fraction	Chicken breast muscle (immediately after hatching)	Chicken breast muscle (1 month after hatching)	Adult rabbit striated muscle	
			Bate Smith	Hasselbach & Schneider
Intracellular:				
Myofibrillar protein	17·5	40·0	53·6	
Residual protein	25·0 } 42·5	22·0 } 62	15·0 } 68·6	53–55
Sarcoplasmic protein	40·0	33·0	16·4	28
Extracellular:				
Collagen, elastin, reticulin	17·5	5·0	15·0	15–17

dilute acid and in those of Hasselbach & Schneider (1951) from extractions with urea, whereas in our fractionation dilute sodium hydroxide has been used. Certainly neither 0·1N-hydrochloric acid nor urea solutions extract from chick muscle as large a percentage of the total protein as 0·1N-sodium hydroxide and it seems that this last leaves a residue which consists solely of collagen, elastin and reticulin. These factors as well as species differences may be, in part, responsible for the differences in the analyses.

The high extracellular fraction in the young chick has already been commented upon. Except for this extracellular fraction the analytical values during development appear to be tending towards those of the adult rabbit muscle.

DISCUSSION

The results have shown marked differences in the rates of increase of the protein fractions, and, moreover, that the rates vary in themselves during development. From the form of the curves presented it is difficult to decide whether there is any interchange between the fractions. For example, expressed as a percentage of the total protein, the sarcoplasmic proteins (Fraction 1) decrease during the embryonic stages while the myofibrillar fraction (Fraction 2) is increasing. It may be that protein is being laid down in the developing fibril, having been preformed in the sarcoplasm. This concept will receive added point in a subsequent paper when the adenosinetriphosphatase activities of these two fractions are considered (Robinson, 1952*b*).

Similar work to that described has been carried out by Hermann & Nicholas (1948) on the muscle proteins of growing rats. Their analyses are similar to those given here, although they did not attempt to estimate the true collagen, elastin and reticulin content of the residue after strong salt extraction, though they appreciated its probable complexity. The time relations of their curves differ from ours somewhat, but that is understandable since the rat at birth is at an earlier stage of general development than is the newly hatched chick. In the rat the residue left after extraction with strong salt solutions, when expressed as a percentage of the fresh weight, reaches a high value about 15 days after birth and then falls to a lower value in the adult. This has no counterpart in the chick where the comparable fraction (Fractions 3+4) rises to a high value at an earlier stage about 10 days after hatching, and thereafter forms a constant percentage of the fresh weight of the muscle. During the development of the rat the sarcoplasmic fraction (Fraction 1) forms a lower percentage of the total protein than it does in the chick, while the rate of increase of the myofibrillar fraction (Fraction 2) after birth is much greater than it is after hatching.

Throughout this work one of the primary aims has been to obtain a quantitative recovery of each fraction and no attempt has been made to isolate any protein component of the muscle in the pure state. A single estimation of the tropomyosin content of embryonic chick muscle has, however, been made in view of Bailey's (1948) suggestion that tropomyosin might function as a prototype of the myosin molecule. If this were so it might occur in large amounts in embryonic tissues where the elaboration of the fibril is taking place. However, in embryos incubated for 16 days, it formed only about 1% of the total protein of the muscle, an amount comparable to that found in the adult.

So far as any individual protein is concerned, analyses of the type reported can throw little light on its behaviour. All the fractions which have been estimated are complex and doubtless contain several distinct proteins. However, the fractionation can form the basis for more detailed work on the interchange between the protein components. In addition, it is an essential to any further work on such individual components and it is perhaps in that direction that it will be of most value. In particular those proteins which may be characterized easily by their physicochemical properties could be studied in relation to the fractionation scheme as a whole. One such example, tropomyosin, has already been mentioned.

A further examination of the residue remaining after strong salt extraction would be extremely interesting. Study of the poorly defined 'ground substance' is urgently needed since a detailed knowledge of its nature, both as a matrix in which particular cell structures are elaborated, and possibly as a source for their elaboration, would be extremely valuable at the embryonic level where differentiation is proceeding.

SUMMARY

1. A study has been made of the quantitative changes occurring in the protein composition of the chick breast muscle as the tissue develops from the embryonic to the adult state.

2. A standard method for the extraction and estimation of several protein fractions has been developed. The sarcoplasmic proteins extracted from the homogenized muscle tissue by dilute salt solutions, and the fibrillar proteins extracted in strong salt solutions but precipitated on dilution, form two such fractions. That protein fraction insoluble in strong salt solutions but soluble in dilute alkali forms a third complex fraction and the final extracellular residue, a fourth.

3. The changes found in the protein nitrogen content of each fraction during the development of

the chick have been presented with reference both to the fresh weight of the tissue and the total protein. Protein analyses of the muscles of month-old and newly hatched chicks are contrasted with those of adult muscle from the rabbit.

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REFERENCES

Bailey, K. (1948). *Biochem. J.* **43**, 271.
Bate Smith, E. C. (1934). *J. Soc. chem. Ind., Lond.*, **53**, 351.
Bate Smith, E. C. (1935). *J. Soc. chem. Ind., Lond.*. **54**, 152.
Bate Smith, E. C. (1937). *Proc. Roy. Soc.* B, **124**, 136.
Bowes, J. H. & Kenten, R. H. (1948). *Biochem. J.* **43**, 365.
Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 372.
Cohn, E. J., Strong, L. E., Hughes, W. L., Mulford, D. J., Ashworth, J. N., Melin, M. & Taylor, H. L. (1946). *J. Amer. chem. Soc.* **68**, 459.
Dische, Z. & Osnos, M. (1950). *Fed. Proc.* **9**, 165.
Dubuisson, M. (1950). *Biol. Rev.* **25**, 46.
Edsall, J. T. & Mehl, J. W. (1940). *J. biol. Chem.* **133**, 409.
Fürth, O. von (1895). *Arch. exp. Path. Pharmak.* **36**, 231.
Guba, F. & Straub, F. B. (1943). *Stud. Inst. med. Chem. Univ. Szeged*, **3**, 46.
Hasselbach, W. & Schneider, G. (1951). *Biochem. Z.* **321**, 462.
Hermann, H. & Nicholas, J. S. (1948). *J. exp. Zool.* **107**, 165.
Hunt, E. A. (1932). *J. exp. Zool.* **62**, 57.
Lowry, O. H., Gilligan, D. R. & Katersky, E. M. (1941). *J. biol. Chem.* **139**, 795.
Marsh, B. B. & Snow, A. (1952). *J. agric. Sci.* **1**, 190.
Robinson, D. S. (1952*a*). *Biochem. J.* **52**, 628.
Robinson, D. S. (1952*b*). *Biochem. J.* **52**, 633.
Romanoff, A. L., Smith, L. L. & Sullivan, R. A. (1938). *Mem. Cornell agric. Exp. Sta.* **216**, 1.
Weber, H. H. & Meyer, K. (1933). *Biochem. Z.* **266**, 137.

Changes in the Nucleoprotein Content of Chick Muscle during Development

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Whilst adult striated muscle is particularly well adapted to perform its specific function in the body, it nevertheless possesses certain elements, such as the nuclei and the particulate components of the cytoplasm, which are common to all cells. The quantitative contribution of these latter to the whole varies during development; it is large in the earlier embryonic stages, but falls, as the myofibrils differentiate, to a lower level in the adult muscle (Graff & Barth, 1938; Caspersson, 1941, 1947, 1950; Brachet, 1945, 1947).

In a previous paper a protein fractionation procedure has been described and applied to chick muscle at various developmental stages (Robinson, 1952). Since the nucleoproteins of the cell nucleus have solubility properties similar to those of the myofibrillar proteins (Brachet & Jeener, 1947; Mirsky & Pollister, 1942, 1943), they have been estimated with them in this scheme. Thus, whilst none of the deoxyribonucleic acid of the cell nucleus was found in dilute KCl-phosphate extracts of muscle (Fraction 1) over 80% was extracted in strong KCl-phosphate solutions and was precipitated, with the myofibrillar proteins, by 15% (v/v) ethanol on dilution to an ionic strength of 0·12 at 0° (Fraction 2). The remaining 20% of the deoxyribonucleic acid was not extracted and was estimated in the residual fractions (Fractions 3 and 4). The numerical designations of the fractions refer throughout this paper to Robinson (1952).

In this study the contribution made by deoxyribonucleoproteins of the cell nucleus has been assessed as follows. Nuclei have been isolated from chick muscle at different developmental stages and the deoxyribonucleic acid/protein ratio has been determined. Then, knowing the deoxyribonucleic acid content of the muscle residue after extraction with dilute KCl-phosphate solution (Fraction 2 + 3 + 4), the amount of deoxyribonucleoprotein in this fraction has been calculated. Furthermore, a better assessment of the myofibrillar protein content of muscle has been made by correcting the previous analyses for the newly determined nuclear protein contribution.