THE METABOLISM OF MYOSIN, THE MEROMYOSINS, ACTIN AND TROPOMYOSIN IN THE RABBIT*

by

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Isotopic experiments establish that proteins are continually being lost and regenerated in mammalian tissues at rates characteristic for each tissue[1,2]. More detailed interpretations of such experiments based upon observation of the whole protein complex of the tissue are in many respects ambiguous. A clearer definition of the problems may result when individual proteins are examined. Although the protein metabolism of muscle is slow, muscle contains an array of accessible and uniquely defined proteins for metabolic study. Three of these proteins, aldolase, glyceraldehyde-3-phosphate dehydrogenase, and phosphorylase, have been the subjects of previous metabolic investigations[3,4]. The results established certain relations of the proteins with each other and with their common pool of amino acid precursors. An initial approach is described here to the metabolism of another group of muscle proteins of a quite different type. The proteins are myosin, actin, and tropomyosin. As before the interpretations are based upon the specific activities of labelled amino acids in several individual proteins of the same animal. To provide a basis of reference two of the glycolytic enzymes and two individual blood proteins were also examined.

METHODS

Labelled amino acids and initial steps

The amino acids studied were phenylalanine and tyrosine. These were selected because they undergo relatively small dilutions, are unequally distributed in the proteins of interest, and because abridged methods could be devised for their isolation in pure form from small amounts of protein. Rabbit-A, 3.7 kg, received 0.03 millicuries of DL-phenylalanine-3-^{14}C (Tracerlab, 0.5 mc per m*M*) intravenously and was given a lethal dose of nembutal 3 hours later. Rabbit-B, 3.3 kg, received 0.06 mc of labelled phenylalanine 50 minutes before it was killed. Tyrosine in these experiments was labelled by metabolic conversion from phenylalanine. Heparinized plasma was prepared from the blood and chilled for subsequent fractionation. Leg and back muscles were excised from the chilled carcass and weighed. All solutions and equipment were prepared in advance. A strict time schedule had to be followed for 48 to 72 hours in order to obtain satisfactory preparations of all of the proteins desired.

Muscle proteins from rabbit-A

The various proteins could be prepared from 500 g of muscle from one rabbit by application of different fractionation schemes to separate aliquots of the ground muscle.

Myosin. A muscle sample weighing 300 g was used for the preparation of myosin by the method of MIHALYI[5]. The myosin was reprecipitated three times by dilution and was obtained

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in the characteristic semicrystalline form. Special attention was paid to the removal of components precipitated in the early stages of dilution. The protein was then subjected to the ammonium sulfate fractionation treatment of TSAO[6]. Analysis in the analytical ultracentrifuge in 0.5 *M* potassium chloride and 0.05 *M* potassium phosphate, pH 7.0, showed the typical single ultra-sharp boundary with no base line aberrations.

Crystalline L-meromyosin. This protein was prepared from an 800 mg sample of the purified myosin by controlled digestion with trypsin according to the method of SZENT GYÖRGYI[7]. Three times recrystallized trypsin was used with a recrystallized soy bean trypsin inhibitor to stop the reaction. It was found that a preliminary 10 day storage of the inhibited reaction mixture was not necessary if the L-meromyosin was crystallized by dialysis against 6 l of 0.003 *M* potassium phosphate, pH 7.0, in 0.04 *M* potassium chloride. Two recrystallizations were then carried out in the same manner with about 10 ml of solution in the dialysis sac. The crystals were sharp and uniform with no amorphous background material. Sedimentation diagrams revealed no trace of unchanged myosin or of H-meromyosin.

H-meromyosin. The mother liquor from the first L-meromyosin crystals was fractionated with ammonium sulfate by SZENT-GYÖRGYI's method to obtain H-meromyosin. The protein had the properties described by him and behaved as a single substance by the criteria of electrophoresis and sedimentation. In this case as in the others yields were sacrificed in order to facilitate purification.

Actin. The insoluble muscle residues from the myosin extraction were used for the preparation of actin by the method of MOMMAERTS[8]. The actin was purified by three cycles of "polymerization" to the fibrous F-actin form, isolation of F-actin by ultracentrifugation, and partial depolymerization. This removed low molecular weight impurities. High molecular weight impurities were removed by complete conversion of the F-actin to the globular G-actin form and ultracentrifugation. Because of strong interactions solutions of F-actin are not amenable to simple physical analysis by conventional methods. In order to examine G-actin electrophoretically without induction by the supporting electrolyte of F-actin formation 20 mole equivalents of *p*-chlormercuribenzoate were added. The G-actin so treated was not converted to the F-form in 0.05 *M* potassium phosphate and migrated with a single electrophoretic boundary at pH 6.5 and 7.8. The mercurial reagent was not effective at high salt concentrations and thus did not facilitate the application of ammonium sulfate fractionation procedures to the further purification of actin.

Aldolase. For the preparation of aldolase and tropomyosin from the same 200 g sample of muscle the initial extraction was carried out with distilled water. Aldolase was obtained from the extract by the method of TAYLOR, GREEN AND CORI[9] and was recrystallized five times. When distilled water instead of 0.03 *M* potassium hydroxide is used for extraction the extract becomes acidic and glyceraldehyde-3-phosphate cannot be obtained. Water was used in this case so that tropomyosin could subsequently be obtained from the muscle residue. Tropomyosin is lost if the initial extract is alkaline.

Tropomyosin. The complete purification method of BAILEY[10] was employed in the preparation of tropomyosin. A detectable trace of a second component was present in electrophoretic diagrams and so the drying, redissolving, and heating steps later recommended[11] were also employed. In trial runs the crystallization was found to be reproducible but was abandoned because it gave no detectable purification and because it entailed considerable loss of material. No impurities could be detected in the final tropomyosin solution by electrophoretic or sedimentation analysis.

Muscle proteins of rabbit-B

Crude myosin can be obtained in initial precipitates produced by dialysis against water or dilute buffer instead of by direct dilution. By this method aldolase is not lost in a large volume of diluent and can be crystallized from the supernatant fluid after centrifugation of the myosin precipitate. The undissolved muscle residue, as before, serves for the preparation of actin. Although satisfactory myosin was obtained from the precipitate formed on dialysis in trial runs by this method, essentially that of MOMMAERTS AND PARRISH[12], the myosin from labelled rabbit-B was demonstrably impure, anomalous in physical properties, and had to be discarded. The aldolase and actin were good preparations and satisfied the criteria which have been discussed. Separate 150 g aliquots of the ground muscle were used for the preparation of glyceraldehyde-3-phosphate dehydrogenase, four times recrystallized, by the method of CORI, SLEIN AND CORI[13], and tropomyosin by the method of BAILEY[10].

Purification of serum albumin and fibrin

The metal salt alcohol method, number 10, developed for human plasma by COHN and co-workers[14] was applied to 25 ml of the heparinized rabbit plasma. The albumin fraction, extracted from the precipitated zinc salts with barium acetate solution, was dialysed against a large volume of water and lyophylized. This material was later redissolved and subjected to the high salt high alcohol conditions for crystallization, as described by COHN, HUGHES AND WEARE[15] for human

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serum albumin. After removal of a less soluble pigmented fraction the albumin came out in a fine granular form which could not definitely be characterized as crystalline. The precipitate was redissolved and subjected to ammonium sulfate fractionation. Virtually all of the protein was precipitated between 0.57 and 0.61 saturation. A 1% solution of the protein dialysed against 0.1 ionic strength potassium phosphate, pH 7.6, migrated with a single symmetrical boundary during 3 hours of electrophoresis. The globulin fraction obtained in the course of the fractionation contained about 10% of a second component which was not removed in further fractionation attempts. The globulins were therefore discarded.

Fibrin. The residual protein precipitate, after removing all proteins extractable under the controlled conditions of method 10 was dissolved in 5 ml of sodium citrate, 0.1 *M* pH 6.5, and was treated with a small amount of active thrombin for 1.5 hours at 28°. The fibrin precipitate was collected on a stirring rod, homogenized, washed with 0.1 *M* mercaptoethanol and water, and dried.

Isolation of amino acids and specific activity measurements

The amounts of protein available after final purification and removal of samples varied between 80 and 150 mg with the exception of myosin of which there was an excess. These were all dialysed against 0.1 *M* mercaptoethanol in the presence or absence of salts depending upon the properties of the protein and then against water or salt solutions. Each protein was precipitated, washed with water or organic solvents, and then hydrolysed in 6 *N* hydrochloric acid under an atmosphere of nitrogen for 20 hours at 110°. The hydrolysates were taken to dryness two or three times *in vacuo*.

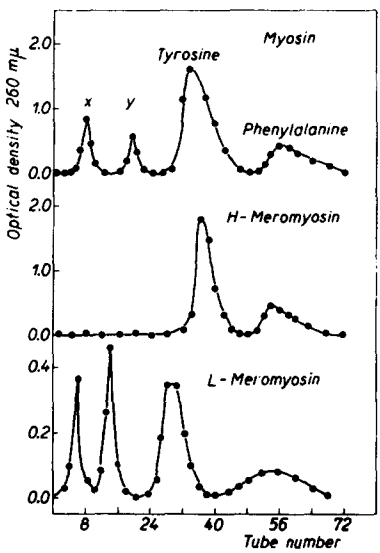

Fig. 1. Elution curves of the tyrosine phenylalanine fraction of hydrolysates of myosin and the meromyosins: ion exchange chromatography on Dowex-50 developed with 2.5 *N* hydrochloric acid and followed by optical density measurements at 260 mμ. The preliminary isolation was by adsorption on charcoal and elution. Peaks X and Y are components of myosin which appear to be associated exclusively with the L-meromyosin sub-unit.

Separation of tyrosine and phenylalanine. The amino acids were dissolved in water and poured on a 3 × 1 cm column of acid-washed charcoal, Darco G-60. The column was then washed by suction with 400 ml of water. Tyrosine and phenylalanine were eluted with 150 ml of 5% ethyl acetate in water. This step was modified from the method of WACHTEL AND CASSIDY[16] and yields tyrosine and phenylalanine in a mixture containing very little of the other amino acids. Separation of tyrosine and phenylalanine from each other can then be effected on a relatively short ion exchange column. For this purpose Dowex-50, 200 to 400 mesh, extensively washed and preequilibrated with 2.5 *N* hydrochloric acid was used. The phenylalanine tyrosine mixture was taken to dryness *in vacuo*, dissolved in 1.0 ml of 2.5 *N* hydrochloric acid, and applied to a 24 × 1 cm Dowex column. Developement was carried out with 2.5 *N* hydrochloric acid at a rate of 6 ml per hour and 1.5 ml fractions were taken on an automatic fraction collector. Eluate peaks were followed by the ninhydrin method[17] or by direct measurements of optical densities at 260 mμ in the Beckman spectrophotometer. Both amino acids absorb at this wavelength and they can be distinguished from each other by the optical density ratios at 260 and 280 mμ. The separation is effected in 30 hours or less. The elution curves shown in Fig. 1 were selected because they illustrate that two peaks, rapidly eluted from the Dowex column and showing 260 mμ absorption maxima, are found in hydrolysates of whole myosin but are associated exclusively with the L-meromyosin moiety. Nucleotides reported to be associated with myosin might have been assumed to be involved in the adenosine triphosphatase activity. However this activity is carried exclusively by the H-meromyosin.

Tubes corresponding to the central portions of each peak were pooled and complete spectra and ninhydrin values were checked against standard solutions. After taking the solutions to dryness the tyrosine was crystallized twice by adjusting acid or alkaline solutions to the isoelectric point. The phenylalanine was converted to the *p*-iodophenylsulfonyl derivative and crystallized as the free acid and as the ammonium salt. Plating, determination of the quantity of each sample, and radioactivity measurement in a windowless flow counter were carried out as previously described[3,4].

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Limits of error

Trial experiments at the radioactivity levels encountered have indicated a precision of about ±4% in a specific activity measurement. The uncertainty arising from the state of purity of the protein is more difficult to evaluate. Physical analyses of the type employed in this work may fail to detect sizable impurities if these are heterogeneous or happen to have the same mobilities as the protein in question. Depending upon the amounts and specific activities of the amino acids in the contaminant the error may be much less or much greater than the percent contamination by weight. An overall accuracy greater than ±8% is not assumed although the results in many cases may be much better than this. Observed differences of this magnitude or smaller might be significant and pertinent to the interpretation but would have to be overlooked.

Method of analysis of data

If the specific activity ratios in a series of labelled amino acids in two proteins of the same animal are the same within experimental limits the proteins may be considered to be labelled from the same pool of amino acid precursors without detectable dilution by intermediates specific for one protein. By this criterion intermediates of any type are themselves newly synthesized. The entrance of a labelled amino acid into a protein under these conditions is a sign of synthesis and the specific activity is proportional to a *fractional change* in the pool of that protein. A knowledge of the specific activity of the precursors is needed for a direct evaluation of the *fractional change* whether it be turnover or growth or a combination of the two. However when two proteins are formed from the same precursor pool the fractional changes may be obtained with respect to each other by comparing specific activities even though the precursor pool is not further characterized. Specific activities and specific activity ratios thus provide a certain amount of information about precursor relationships and also about rates. In a short term tracer experiment the fractional changes observed are small and correction of specific activities for simultaneous breakdown or redistribution are negligible.

RESULTS

Specific activity ratios and precursor relationships

The specific activities of tyrosine and phenylalanine in the various proteins are summarized in Table I. It is observed that the specific activities of phenylalanine and tyrosine in aldolase and the dehydrogenase of rabbit-B are in the ratio 6.8 and 6.7 respectively. These results are in accord with other experiments with a larger number of amino acids and indicate that the two proteins are formed from the same pool of precursors. The ratios in actin in the two animals show some deviation from those in

TABLE I

THE SPECIFIC RADIOACTIVITIES OF TYROSINE AND PHENYLALANINE ISOLATED FROM INDIVIDUAL PROTEINS OF THE SAME RABBIT AFTER AN INTRAVENOUS DOSE OF PHENYLALANINE-3-^{14}C

The specific activities are expressed as counts per minute per μM and are extrapolated from standard curves to correspond to layers of infinite thinness.

Protein	*Rabbit-A*			*Rabbit-B*		
	Tyrosine	*Phenylalanine*	*Ratio***	*Tyrosine*	*Phenylalanine*	*Ratio***
Dehydrogenase*	—	—	—	1.57	10.5	6.7
H-meromyosin	1.0	4.44	4.4	—	—	—
Actin	1.2	5.7	4.7	2.13	13.4	6.4
Aldolase	1.67	7.0	4.2	3.23	21.9	6.8
Tropomyosin	2.6	—	—	6.4	—	—
Myosin	2.9	7.4	2.5	—	—	—
L-meromyosin	—	17.9	—	—	—	—
Serum albumin	50.8	104	2.0	80	204	2.5
Fibrin	361	513	1.4	—	—	—

* Glyceraldehyde-3-phosphate dehydrogenase.
** Specific activity of phenylalanine/Specific activity of tyrosine.

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aldolase but these may not be significant since the criteria for the homogeneity of actin are not yet satisfactory. No test for the precursor relationship with tropomyosin is provided since the phenylalanine content of this protein was too low to yield an adequate derivative sample by the method attempted.

The specific activity ratio of phenylalanine to tyrosine in myosin differs from that in aldolase to an extent which is beyond the probable errors of measurement. An explanation for this deviation is provided by an examination of the L- and H-meromyosins. These two proteins account for most of the weight of the myosin molecule. The specific activities in the H-meromyosin are one half to one third of those in whole myosin and the phenylalanine to tyrosine specific activity ratio, unlike that in whole myosin, now approaches that of aldolase. Insofar as can be determined from the behavior of these amino acids H-meromyosin, actin, and aldolase are formed the same pool of amino acid precursors.

MIHALYI AND SZENT-GYÖRGYI[18] from area analyses of sedimentation diagrams arrived at figures of 0.57 and 0.43 for the weight fractions of myosin contributed by the H- and L-meromyosins respectively. KOMINZ, HOUGH, SYMONDS, AND LAKI[19] reported 6.60 and 1.58% phenylalanine respectively in the H- and L-meromyosins and 4.46% in myosin. These results are consistent with the area analyses and indicate that essentially all of the phenylalanine of myosin is accounted for in the meromyosins. The fraction of the total phenylalanine of myosin contributed by H-meromyosin is found from these figures to be 0.847 and the fraction contributed by L-meromyosin is found to be 0.153, and the respective specific activities are 4.44 and 17.9. Accordingly one may compute the extent to which the specific activities of the phenylalanine in the meromyosins together account for that observed in myosin. The calculated value is $(0.847 \times 4.44) + (0.153 \times 17.9) = 6.5$ counts min^{-1} μM^{-1} and the observed value is 7.4. In view of the combination of analyses involved the agreement is reasonable but the results suggest that the phenylalanine distribution in myosin and the meromyosins may not yet be properly accounted for. In view of the drastic refractionation of the meromyosins the degree to which the balance of specific activities is achieved indicates that the specific activity ratio in myosin is not due to an impurity but to non-uniform labelling between the meromyosins.

Tropomyosin was so named by BAILEY[10] because he considered it a form of myosin, possibly a precursor. On the basis of additivity of amino acid analyses KOMINZ *et al.*[19] suggested that myosin might actually be composed of actin and tropomyosin in a one to one ratio. However a consideration of the specific activities of tyrosine shown in Table I reveals that no combination of actin and tropomyosin can give tyrosine of the specific activity observed in myosin. A simple precursor relationship based upon utilization of actin and tropomyosin from a uniform pool is thus unlikely.

Specific activities and rates of fractional change

As shown in Table I the specific activities of the amino acids in the dehydrogenase are about one half those in aldolase. This is in agreement with previous finding and, under conditions of identical specific activity ratios, it means that the dehydrogenase pool is turning over or increasing at half the rate of the aldolase pool. It seems likely that the process that is actually occurring is *turnover* since if the fractional change represented *growth* the concentration of the dehydrogenase would be decreasing

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constantly with respect to the concentration of aldolase. The relative rates of fractional change with respect to phenylalanine differ by a factor of five in the muscle proteins, the slowest being the dehydrogenase and the most rapid L-meromyosin. The relative positions of the various proteins are the same in the two rabbits. The position of whole myosin determined here with phenylalanine and tyrosine in only one rabbit is in agreement with the relation of myosin to glycolytic enzymes when ^{35}S labelled methionine was followed[3].

In comparing turnover rates of individual muscle proteins the need for first establishing the identity of the precursor pools was emphasized. This is particularly important when the qualitative fact is being considered as to whether the rates actually differ at all. When the turnover rates are known to differ by a large factor this condition may be somewhat relaxed to facilitate comparisons, but it may never be ignored. The turnover rates of the blood proteins are much greater than those of the muscle proteins as first shown by SCHOENHEIMER and co-workers[1,2] and confirmed by many subsequent studies including the present one. From time studies of labelled amino acid incorporation and release the half-life of serum albumin in rabbits has been estimated to be about 4 days[20]. Fibrinogen under various conditions in different small animals has been estimated to have a half-life of 0.5 to 4 days[21,22]. The serum albumin provides a basis of reference for making a rough estimate of the half-lives of the individual muscle proteins from the present results.

In these experiments the labelled tyrosine was made, chiefly in the liver[23], from the labelled phenylalanine. Serum albumin, also made in the liver[24], would therefore be expected to draw upon a free tyrosine pool of higher specific activity than occurs in muscle. That this is actually the situation is indicated by the fact that in rabbit-B the tyrosine in serum albumin has already attained four tenths of the specific activity of the phenyalalanine while in the glycolytic enzymes the specific activity ratio, tyrosine to phenylalanine, is only 0.15. On the other hand the specific activities of the free phenylalanine which is rapidly distributed throughout the body may be nearly the same in liver and muscle. The half-lives of the muscle proteins, as a first approximation, are calculated on the assumption that the specific activities of the free phenylalanine are the same in liver and muscle. If the specific activity is higher in the liver than in the muscle the calculated half-lives of the muscle proteins will be too long.

The results of the comparisons are shown in Table II. Relative specific activities referred for rabbit-A to aldolase as 2.0 and for rabbit-B to the dehydrogenase as 1.0 are listed as relative turnover rates in the first four columns. The results are consistent in the two animals and are averaged in column 5. For reasons which have been discussed the relative specific activities in the proteins in a short term traces experiment have some of the properties of first order rate constants and are proportional to turnover rates in a steady state. The half-life, $t_{\frac{1}{2}}$, of phenylalanine in each protein, and its relative specific activity, S, are related to the corresponding quantities for serum albumin, 4 and 25 respectively, as: $(t_{\frac{1}{2}}) = (4 \times 25)/S$. The calculated half-lives are shown in column 6.

The approximate concentrations of the individual proteins in rabbit skeletal muscle are known from activity measurements and isolation yields and are listed in column 7. With this information the half-lives may be translated into rates of synthesis and breakdown in the steady state system. These values, g per 100 g of muscle

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per day, are shown in column 8. The rates are seen to be small. Justification for equating phenylalanine turnover to turnover of the whole protein has been previously established for the three glycolytic enzymes. In the case of the other proteins this is an extrapolation that can only be justified by further experiment. For this reason, and others, any extended discussion of the meaning and numerous implications of the different turnover rates of individual muscle proteins in terms of the physiology of the muscle cell and the mechanisms of synthesis and breakdown or loss of muscle proteins should be deferred.

TABLE II

RELATIVE TURNOVER RATES OF TYROSINE AND PHENYLALANINE IN INDIVIDUAL PROTEINS OF THE RABBIT

The relative turnover rates are in the same ratios to each other as are the specific activities in Table I. They are arranged with aldolase arbitrarily taken as 2.0 in rabbit-A and glyceraldehyde-3-phosphate dehydrogenase taken as 1.0 in rabbit-B. The half-lives are calculated on the assumption that the specific acitvity of free phenylalanine is approximately the same in liver and muscle and that the half life of serum albumin in the rabbit is 4 days. Phosphorylase is included from data of a previous experiment where it exhibited the same turnover rate as aldolase.

Protein	*Relative turnover rates*				*Average*	*Half-life Days*	*Protein concentration g per 100 g of muscle*	*Synthesis g per 100 g of muscle per day*
	Rabbit-A		*Rabbit-B*					
	Tyrosine	*Phenyl-alanine*	*Tyrosine*	*Phenyl-alanine*				
Dehydrogenase	—	—	1.0	1.0	1.0	100	0.4	0.002
H-meromyosin	1.2	1.3	—	—	1.25	80	2.3	0.014
Actin	1.4	1.6	1.35	1.39	1.5	67	1.5	0.011
Aldolase	2.0	2.0	2.05	2.09	2.0	50	0.3	0.003
Phosphorylase	—	—	—	—	2.0	50	0.04	0.0004
Tropomyosin	3.1	—	4.1	—	3.6	27	0.5	0.009
L-meromyosin	—	5.1	—	—	5.1	20	1.7	0.042
Serum albumin	—	30	—	19	25	4	—	—
Fibrin	—	148	—	—	148	0.7	—	—

DISCUSSION

Isotopic experiments by ASKONAS, CAMPBELL, GODIN, AND WORK[25] on casein and β-lactoglobulin secreted by the lactating goat, studies by HOGNESS, COHN, AND MONOD[26] on the production of an induced enzyme in *E. coli*, and the experiments which have been discussed on the formation of three intracellular glycolytic enzymes of skeletal muscle[3,4] have all indicated that protein synthesis begins with free amino acids and procedes through an unknown number of steps to the functional protein molecule with no detectable utilization of preformed peptide intermediates. This is a kinetic condition in a physiological steady state. It does not exclude the utilization of newly formed peptide intermediates if these do not accumulate or are never in the free form. Non-uniform labelling in single protein chains has been observed by STEINBERG AND ANFINSEN[27] and by VAUGHAN AND ANFINSEN[28] in isolated systems. Although this type of experiment can be criticized on the grounds that side reactions may be evoked in damaged cells it should also be kept in mind that abnormal conditions may reveal potentialities of a complex mechanism that are not normally expressed.

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Myosin appears to belong to a class of *compound proteins* composed of subunits of more than one kind. The labelling of phenylalanine and tyrosine in myosin and the meromyosins suggests that the meromyosins have some biological autonomy in the sense that thay are independently synthesized and broken down. Free subunits of myosin however have not yet been recognized as such in muscle extracts. There is no assurance that the full order of complexity in this system is yet known.

C. F. AND G. T. CORI and co-workers have established that rabbit muscle phosphorylase contains two subunits of phosphorylase-b[29,30]. The separation of the subunits is catalysed by a muscle enzyme[31] in a reaction which is simulated by brief exposure to trypsin and thus resembles the formation of the meromyosins from myosin. Unlike myosin however the subunits of phosphorylase in enzymic and physical tests appear to be of only one kind. Recent observations of MADSEN AND CORI[32] indicate that dissociation of the protein into subunits can also be induced by the action of *p*-chlormercuribenzoate. In accord with the behavior of phosphorylase as a compound or complex of identical or nearly identical subunits, the protein in a multiple labelling experiment of the present type behaves as though all parts turn over metabolically at the same rate[4].

SUMMARY

1. Two rabbits were injected with an intravenous dose of phenylalanine-3-^{14}C. From these rabbits the following proteins were crystallized or isolated in forms which appeared physically homogeneous: (rabbit-A), aldolase, myosin, L-meromyosin, H-meromyosin, actin, tropomyosin, and serum albumin: (rabbit-B) glyceraldehyde-3-phosphate dehydrogenase, aldolase, actin, tropomyosin, and serum albumin.

2. Pure phenylalanine and tyrosine, labelled metabolically, were isolated from hydrolysates of these proteins and the specific radioactivities of the amino acids were determined.

3. From the specific activity ratios, phenylalanine to tyrosine, it seemed likely that H-meromyosin, actin, and the glycolytic enzymes were synthesized from the same amino acid precursor pools.

4. The specific activities of amino acids in L- and H-meromyosins were significantly different. It is suggested that subunits of more than one kind in myosin are synthesized independently and turn over metabolically at different rates.

5. The apparent half-lives of phenylalanine in the individual muscle proteins were estimated by comparison with serum albumin the approximate half-life of which is known. The validity of the assumptions involved in making such comparisons is discussed.

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