Biochem. J. (1978) **176**, 573–582
Printed in Great Britain

Growth and Muscle Protein Turnover in the Chick

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(*Received 5 May* 1978)

The growth rates of young chicks were varied from 0 to 10% per day by manipulation of the adequacy of the amino acid and energy supply. The rates of protein synthesis in the white breast (pectoralis thoracica) muscle and the dark leg (gastrocnemius and peronaeus longus) muscles were estimated by feeding L-[U-^{14}C]tyrosine in amino acid/agar-gel diets ('dietary infusion'). This treatment rapidly and consistently produced an isotopic equilibrium in the expired CO_2 and in the free tyrosine of plasma and the muscles. Whole-body protein synthesis in 2-week-old chicks was estimated from the tyrosine flux and was 6.4g/day per 100g body wt. In 1-week-old chicks the rate of protein synthesis was more rapid in the breast muscles than in the leg muscles, but decreased until the rates were similar in 2-week-old birds. Synthesis was also more rapid in fast-growing Rock Cornish broilers than in medium-slow-growing New Hampshire×Single Comb White Leghorn chicks. No or barely significant decrease in the high rates of protein synthesis, in the protein/RNA ratio and in the activity of RNA for protein synthesis occurred in non- or slow-growing chicks fed on diets deficient in lysine, total nitrogen or energy. Thus the machinery of protein synthesis in the young chick seems to be relatively insensitive to dietary manipulation. In the leg muscles, there was a small but significant correlation between the fractional rate of growth and protein synthesis. A decrease in the fractional rate of degradation, however, appeared to account for much of the accumulation of muscle protein in rapidly growing birds. In addition, the rapid accumulation of breast-muscle protein in rapidly growing chicks appeared to be achieved almost entirely by a marked decrease in the fractional rate of degradation.

It is now well established that the rates of protein synthesis and protein degradation in the muscles of young animals are quite rapid. Growth, which occurs at a much slower rate, is simply the result of a small difference between the rates of synthesis and breakdown. Increased rates of growth may be achieved by increasing this difference, either by increasing the rate of synthesis, which is energetically expensive, or by decreasing the rate of breakdown, a process that probably is energetically less expensive.

It was not unexpected when earlier experiments showed that changes in the amount of protein that established a new equilibrium were accomplished by reciprocal changes in the rates of synthesis and degradation. For example, in the liver of the rat, changes in enzyme activity occur via transient alterations in the breakdown rates and permanent changes in the synthesis rates, always in the appropriate direction (Schimke, 1964; Chee & Swick, 1976). Also, during liver regeneration there was a marked decrease in degradation and a small increase in synthesis (Swick & Ip, 1974; Scornik, 1974; Scornik & Botbol, 1976; Augustine & Swick, 1977).

Similar responses appear to occur in muscle. Goldberg (1969) reported that during work-induced hypertrophy protein synthesis increased, whereas protein breakdown decreased. Young *et al.* (1971) found that protein breakdown appeared to cease immediately on restoration of protein to the diet of depleted rats, whereas the rate of protein synthesis increased later. These latter conclusions have been reached solely from the pattern of disappearance of tracer amino acids. Because the interpretation is complicated by the problems of tracer reutilization, particularly in muscle, Laurent & Sparrow (1977) have repeated some of the experiments and reached a different conclusion. Thus it is important that all such experiments be confirmed by independent methods. When the rate of protein synthesis was estimated from the continuous infusion of tracer amino acid, Turner & Garlick (1974) observed that an increased fractional rate of synthesis and of degradation was associated with the hypertrophy that occurred in the denervated hemidiaphragm of the rat. Furthermore, Millward *et al.* (1975) showed that the rapid growth of young rats was correlated with a rapid rate of muscle protein synthesis as well as a concomitant rapid rate of breakdown. As the animals aged, both rates fell as

the growth rate decreased. Finally, Laurent *et al.* (1978) found that work-induced hypertrophy of the wing muscles of the cockerel involved an increase in both the rate of synthesis and of degradation.

The rate of growth is thus limited by a nearly parallel increase in the rate of protein degradation. The newly hatched chick was chosen to study these phenomena because genetic selection has provided an animal with a rate of weight gain almost double that of the rat, thus giving a broader range within which these processes can be studied. Therefore, the whole-body growth rate of young chicks was varied by manipulation of the diet in such a way as to provide fractional growth rates ranging from 0 to 10% per day. Because at this stage of development the muscles of the leg and particularly of the breast are increasing at a rate greater than that of the rest of the body, the nutritional effects are amplified.

Because of a marked tendency of the blood of the young chick to clot around the end of an intravenous cannula and because of the presence of the crop, which serves as a large reservoir, we have chosen to use the technique of 'dietary infusion' by using agar-gel diets containing a radioactive amino acid (Harney *et al.*, 1976). When this technique was compared with constant intravenous infusion, similar estimates were obtained for rates of protein synthesis in the rat. Some of our early observations have been presented in a preliminary communication (Maruyama *et al.*, 1976).

Table 1. *Composition of the amino acid diet for young chicks*

The mineral mixture, vitamin mixture and antacid used have been described by Maruyama *et al.* (1975*a*).

	Composition (g/kg)	
Amino acid premix		200.25
L-Lysine hydrochloride	14.0	
L-Arginine (free base)	12.0	
L-Histidine hydrochloride monohydrate	4.5	
L-Phenylalanine	7.0	
L-Tyrosine	7.0	
L-Tryptophan	2.25	
L-Proline	3.0	
Glycine	10.0	
L-Valine	10.0	
L-Leucine	14.0	
L-Isoleucine	8.0	
L-Threonine	7.0	
L-Methionine	7.0	
L-Cystine	3.5	
L-Glutamic acid	100.0	
Corn oil		90.0
Mineral mixture		60.0
Vitamin mixture		10.0
Choline chloride		4.0
α-Tocopheryl acetate (10mg/g)		2.0
Cellulose		30.0
Sodium acetate		10.0
Antacid [$Mg_2Si_3O_8/Al(OH)_3$ (3:1, w/w)]		10.0
Dextrin		575.0

Materials and Methods

Animals, diets and experimental design

The chicks were from the mating of New Hampshire males × Single Comb White Leghorn females. In some experiments, sex-linked feathering (McGibbon, 1977) was incorporated in the mating to identify males at 1 day of age. Broiler-type chicks (Rock Cornish) were also used in one experiment. All were wing-banded, weighed and placed in conventional electrically heated chick starting batteries in a room lighted 24h a day. A group of 12 chicks was randomly assigned to each treatment; food and water were provided *ad libitum*.

The growth rate of the chicks was controlled by the feeding of synthetic diets with different amino acid compositions. In the basal diet the indispensable amino acids were fed at an optimal amount (100%) (Table 1). Other diets contained 75% of the amino acid mixture, and/or L-lysine hydrochloride at 75, 50 or 35% of the optimum amount. Another diet contained 200% of the amino acid mixture. In each of these diets the dextrin content was altered in an appropriate manner. Generally, on day 8 the chicks were divided and given the diet either in powder form or combined with agar gel. The latter was incorporated by mixing 1 part of the powdered diet with 2 parts of a boiling 1.875% agar-gel solution. The growth rates of chicks fed on these two diet forms were noted to ensure that the same performance was achieved. Groups of chicks given the several agar-gel diets were killed 3 days before and 3 days after the tracer experiments to measure muscle size and protein content. Regression equations of the muscle data on body weight were used to estimate the rates of muscle growth and muscle protein accumulation at a given body weight and growth rate. On day 14±1, fed chicks that had been given the agar-gel diet were transferred to a metabolism chamber and given 15g (dry wt.) of the appropriate agar-gel diet containing 2μCi of L-[U-^{14}C]tyrosine (sp. radioactivity 420mCi/mmol; Amersham/Searle, Des Plaines, IL, U.S.A.). The tracer had been dissolved and incorporated with the agar-gel solution.

Tissue preparation and analysis

During the feeding of the radioactive diet, the expired CO_2 was trapped in 15ml of ethanolamine/methylCellosolve (1:2, v/v) solution. At 30min intervals for the first 2h and at 1h intervals over the next 4h the traps were changed; the radioactivity was sampled by removing 3ml of the trapping solution

and measured by liquid-scintillation spectrometry by using a toluene/methylCellosolve-based scintillation fluid (Jeffay & Alvarez, 1961). Any chicks (about one in ten) that failed to show a plateau in the rate of $^{14}CO_2$ expiration were discarded.

After 6h blood was taken by heart puncture into a heparinized syringe, and immediately thereafter tissue samples were quickly taken and frozen in either liquid N_2 or solid CO_2. White muscle was represented by the entire pectoralis thoracica muscle and red muscle by the gastrocnemius muscle and peronaeus longus muscle. The proportion of the label that was absorbed during the 6h period was determined by subtracting from the amount of radioactivity added to the diet, the radioactivity recovered in the gastrointestinal contents, spilled or uneaten food, excreta and rinsings from the diet syringe and chamber. These were combined, preserved with phenol and homogenized. Duplicate 0.5ml portions were solubilized in 4ml of NCS solubilizer (Amersham/Searle) by incubation at 37°C in capped scintillation vials; they were counted for radioactivity after the addition of 15ml of scintillation fluid.

Plasma proteins were precipitated by the addition of an equal volume of 10% (w/v) trichloroacetic acid and sedimented; the supernatant fractions were stored at −20°C until analysed for free tyrosine. In the first experiments, frozen tissues were homogenized in 5vol. of 5% trichloroacetic acid with a Polytron homogenizer. The precipitate was collected by centrifugation and washed with 3×1vol. of 5% trichloroacetic acid; the washings were combined with the supernatant solutions. After removal of trichloroacetic acid by extraction with diethyl ether, plasma and tissue supernatant, which contained free tyrosine, were evaporated to dryness and dissolved in 0.5M-citrate buffer, pH5.5. The protein precipitates were sequentially extracted with hot 5% trichloroacetic acid, ethanol/diethyl ether (3:1, v/v) and diethyl ether. In the last experiment, to measure RNA, protein-bound tyrosine (and DNA) in the same sample, the method of Shibko *et al.* (1967) was slightly modified. Frozen tissues were homogenized in 10vol. of 0.2M-$HClO_4$. A sample of the homogenate was reserved for the determination of the total protein content and the precipitate was collected by centrifugation (600*g* for 10min). $HClO_4$ was removed from the supernatant solution by neutralization with 5M-K_2CO_3 and the resulting supernatant, which contained free tyrosine, was treated as described above. The protein precipitate was suspended in 0.3M-NaOH and incubated at 37°C for 1h to solubilize RNA. DNA was then extracted from the pellet with 0.09M-$HClO_4$ at 93°C for 20min. The resulting protein pellet was sequentially extracted with ethanol/0.3% $HClO_4$, ethanol/chloroform (3:1, v/v), ethanol/diethyl ether (3:1, v/v) at 37°C for 15min followed by 0.5vol. of light petroleum (b.p. 37–55°C) before centrifugation, and diethyl ether. The protein pellets obtained by both methods were hydrolysed in 10vol. of 6M-HCl at 110°C for 18h in sealed vessels, evaporated to dryness and dissolved in 0.5M-citrate buffer, pH5.5. The specific radioactivity of tyrosine in the supernatant and protein hydrolysates was determined as described by Garlick & Marshall (1972). L-Tyrosine decarboxylase (Sigma Chemical Co., St. Louis, MO, U.S.A.) was used to convert tyrosine into tyramine. Tyramine was assayed by the nitrosonaphthol fluorimetric method of Waalkes & Udenfriend (1957). Samples from 1g of tissue or approx. 1.5ml of plasma were added to Aquasol (New England Nuclear Corp., Boston, MA, U.S.A.) and counted for radioactivity for 50min or until 5000 counts were accumulated. Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard. RNA was measured by the orcinol reaction with purified yeast RNA (Sigma) as a standard (Schneider, 1957).

Calculations

The fractional synthesis rate was calculated as previously described (Harney *et al.*, 1976) from the specific radioactivity of the tissue free and bound tyrosine with the use of eqn. (8) of Garlick *et al.* (1973), solved by repeated approximations with a computer. The value of *R*, the ratio of bound tyrosine/free tyrosine, was measured in the two sets of muscles in each chick and ranged from 50 to 250 depending on the dietary treatment. The fractional degradation rate was estimated from the fractional rate of protein synthesis, protein content and protein accumulation in the muscle. Protein accumulation in individual muscles was estimated from the fractional whole-body growth rate, the regression of muscle size on body size, and the muscle protein content observed in a sub-group of chicks. The activity or efficiency of RNA utilization for protein synthesis, in g of protein/g of RNA per day, is given (Millward *et al.*, 1975) by:

$$\frac{\text{fractional synthesis rate}}{100} \cdot \frac{\text{protein}}{\text{RNA}}$$

Statistical analyses were done by Duncan's Multiple Range Test (Steel & Torrie, 1960). The 0.05 level of probability was accepted as the criterion for statistical evaluation of significance.

Results

Plasma specific radioactivity and $^{14}CO_2$ release

Estimation of the rate of protein synthesis by the continuous intravenous-infusion method requires that the specific radioactivity of the 'precursor' free amino acid pool reaches a plateau during the infusion

and that the kinetics of the rise to plateau be known also (Waterlow & Stephen, 1968). To this end, the rise in the specific radioactivity of the precursor pool during dietary infusion was examined by monitoring three related parameters.

Fig. 1(*a*) shows the rise in the release of $^{14}CO_2$ in chicks fed on the agar-gel diet containing [^{14}C]-tyrosine. During preliminary experiments a casein/agar-gel diet was fed (Maruyama *et al.*, 1976) and often produced an 'overshoot' in $^{14}CO_2$ release, perhaps because the absorption of labelled free tyrosine was initially faster than that of bound tyrosine (Canolty & Nasset, 1975). The use of the amino acid diets eliminated this problem and resulted in a rapid, smooth rise to a relatively flat plateau in 2h, which persisted for at least 4h more (Fig. 1*a*). From this curve it is reasonable to conclude that the free [^{14}C]tyrosine pool in the liver, where most of the oxidation of this amino acid takes place (Miller, 1962), reached an equilibrium at most 2h after initiation of feeding. The rate constant for the time course of the rise in ^{14}C radioactivity in expired CO_2 was 31 ± 4 days^{-1}, a value smaller than that observed in the rat in the 'dietary infusion', but larger than that observed in the rat infused intravenously (Harney *et al.*, 1976).

Another indicator of isotopic equilibrium is the specific radioactivity of the free tyrosine in the plasma. Fig. 1(*b*) shows that the specific radioactivity rose rapidly and reached a plateau in about 2h. A similar but shorter time course was observed when the diet contained casein, perhaps related to the overshoot phenomenon cited above.

In another experiment, the expiration of $^{14}CO_2$ was monitored and groups of four birds were killed at intervals. The specific radioactivity of free tyrosine was measured in both the red and white muscles. In this trial, the specific radioactivity of the plasma appears to have reached a plateau in 4h, as did the specific radioactivity of free tyrosine in the muscles (Fig. 1*c*).

From these results, then, it appears that the dietary infusion produces an isotopic equilibrium in the plasma, in the expired CO_2, and in the muscles at least as rapidly and as consistently as that resulting from an intravenous infusion. From this we assume that the specific radioactivity of the pool of amino acids that serves as the immediate precursor for protein synthesis is also at equilibrium for much of the experimental period. As evidence, the rates of protein synthesis in the muscles of the birds fed for 4h were identical with those treated for 6h. Although it is essential that the time course of the rise to a plateau be characterized when liver protein synthesis is being estimated, Garlick *et al.* (1973) have argued that for the estimation of protein synthesis in muscle a knowledge of the ratio of free to bound tyrosine is more important.

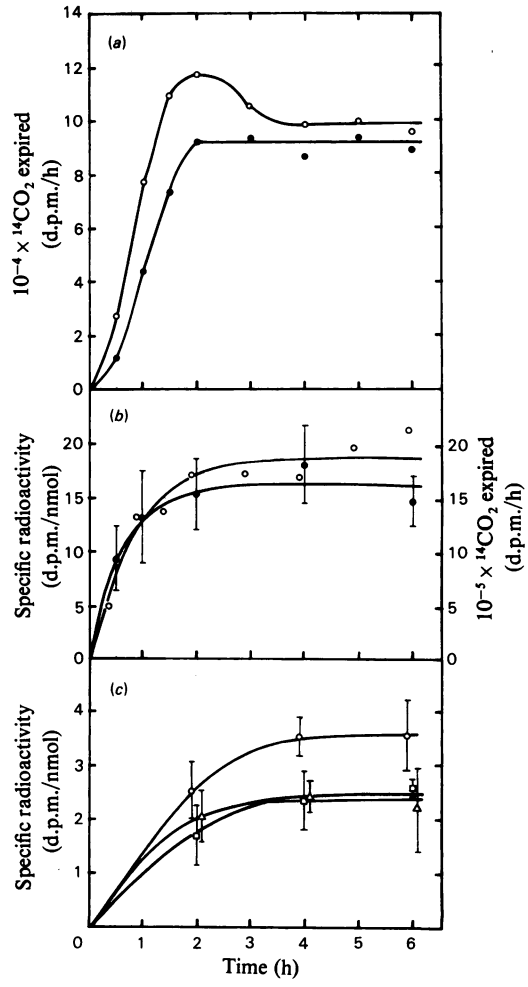

Fig. 1. *Isotopic equilibria during dietary infusion*
(*a*) $^{14}CO_2$ expired over a 6h period by one chick fed on the agar-gel diet containing 2μCi of L-[U-^{14}C]-tyrosine/15g and casein (○) or an amino acid mixture (●) as a protein source. The same diets without tracer were available to the chicks beforehand. (*b*) $^{14}CO_2$ expired (○) and plasma free [^{14}C]tyrosine (●) in chicks fed on the amino acid/agar-gel diet containing 10μCi of L-[U-^{14}C]tyrosine/15g. Blood samples (0.2ml) were taken periodically from the brachial vein into a heparinized syringe. Values are the mean for four chicks. (*c*) Specific radioactivity of free [^{14}C]tyrosine in plasma (○), breast muscle (□) and leg muscle (△) in chicks fed on the amino acid/agar-gel diet containing 2μCi of L-[U-^{14}C]tyrosine/15g. Blood samples were taken from the heart. Values are the mean $\pm$ S.D. for three chicks killed at 2, 4 and 6h.

Tyrosine flux and whole-body protein synthesis

Tyrosine flux, Q (Garlick *et al.*, 1973), in 2-week-old chicks was estimated from the radioactive diet absorbed (I), which amounted to 30–65% of the

amount offered, and the plasma free tyrosine specific radioactivity (Sp) where Q = I/Sp. The amount of tyrosine oxidized, represented by the $^{14}CO_2$ in the expired air, ranged from 25% of the absorbed dose in well-fed controls to 40% in the deficiency groups. Subtraction of this value from Q gives a minimum estimate of the rate of incorporation of tyrosine into total body protein; the average value was 47±2μmol/h per 100g body wt. The average tyrosine content of whole-body proteins, including feathers, was estimated by the method of Waalkes & Udenfriend (1957) and was 3.2±0.4%. From these values the rate of whole-body protein synthesis was calculated to be 6.4g/day per 100g body wt., a value higher than that observed in rats of the same weight (4.2g/day; Garlick *et al.*, 1975), but similar to that seen in smaller rats who were at about the same point in their development (D. J. Millward, personal communication).

Effect of age and strain on the rates of growth and protein synthesis

The growth rate and fractional synthesis rates of two different strains and two different ages of chicks are shown in Table 2. The whole-body growth rate is expressed as the fractional growth rate calculated from the average daily percentage body-weight gain of chicks measured during the 3-day period before the determination of the fractional synthesis rate. The latter was determined in medium-slow-growing chicks (New Hampshire × Single Comb White Leghorn) at 1 and 2 weeks of age and in fast-growing broiler-type chicks (Rock Cornish) at 2 weeks of age. There was no significant difference in the fractional synthesis rate of leg muscle proteins between species or age groups. It appeared, however, that the calculated value of fractional degradation rate decreased as the fractional whole-body growth rate increased, ranging from 11.6% per day in the rapidly growing broiler chicks to 18.0% per day in the 1-week-old New Hampshire × Single Comb White Leghorn birds. On the other hand, the rate of protein synthesis in the breast muscles of the 1-week-old birds was nearly double that in the same species at 2 weeks of age. Protein-synthesis rates in white muscle appear to be subject to a marked developmental change during this period. The decrease in fractional synthesis rate between 1 and 2 weeks was accompanied by an even greater fall in the fraction degradation rate, resulting in a small increase in the fractional whole-body growth rate. At 2 weeks of age the inherent character of a faster rate of growth in the Rock Cornish birds (fractional whole-body growth rate = 10.1% per day compared with 6.4% per day in the New Hampshire × Single Comb White Leghorn) was paralleled by a larger fractional synthesis rate in their breast-muscle proteins.

Effects of lysine and/or total nitrogen deficiency on protein synthesis and degradation in muscles

An inadequate supply of dietary lysine (Edwards *et al.*, 1956; Maruyama *et al.*, 1975*b*) results in a substantial decrease in the growth rate of chicks. In the first experiment, only male chicks were used and the amount of L-lysine hydrochloride in the amino acid diet was decreased from 1.4 to 0.7%, making it lysine-deficient. The feeding of this diet for 4 or 5 days resulted in a 30% decrease in the consumption of the diet and a near cessation of growth (Table 3, Expt. 1). As the fractional whole-body growth rate was decreased the fractional muscle growth rate declined from 16% per day to 2% per day in breast muscles and from 12% per day to 1% per day in leg muscles. Because, however, the fractional muscle growth rate must be estimated from other groups of chicks on the same dietary regime, only the fractional whole-body growth-rate values were used for comparisons with the tracer data from the same chicks. Despite the marked lessening of growth, there was no significant decrease in the fractional synthesis rate in

Table 2. *Effect of strain and age on rates of synthesis and degradation of muscle proteins*

Chicks of both sexes were used. The values are the mean±S.E.M. for four chicks/group. Values without common letter superscripts in a given row are significantly different ($P<0.05$).

	New Hampshire × Single Comb White Leghorn		Rock Cornish
Age ...	1 week old	2 weeks old	2 weeks old
Body weight (g)	66	123	146
Fractional whole-body growth rate (%/day)	5.4	6.4	10.1
Leg			
Muscle size (g)	1.8	5.0	6.3
Fractional synthesis rate (%/day)	24.8±1.0[c]	23.4±0.9[c]	25.3±2.2[c]
Fractional degradation rate (%/day)	18.0	14.2	11.6
Breast			
Muscle size (g)	1.9	6.5	8.6
Fractional synthesis rate (%/day)	38.0±1.7[a]	21.5±1.3[b]	25.8±2.0[b]
Fractional degradation rate (%/day)	26.5	10.0	9.7

Table 3. *Effect of suboptimal dietary amino acid intake on growth rate and several aspects of muscle protein metabolism in chicks*

Chicks used were New Hampshire × Single Comb White Leghorn males. The values are the mean ± S.E.M. of five chicks/group. Values without common letter superscripts in a given row are significantly different ($P < 0.05$).

	Dietary treatment				
	Expt. 1		Expt. 2		
	Control	50% lysine	Control	75% amino acids	75% amino acids, 50% lysine
Body weight (g)	118	69	131	107	100
Fractional whole-body growth rate (%/day)	8.0	0.6	10.3	6.4	5.1
Leg					
Fractional synthesis rate (%/day)	21.6 ± 2.5	20.3 ± 1.5	26.5 ± 1.0[a]	23.5 ± 1.2[b]	22.1 ± 0.9[b]
Fractional degradation rate (%/day)	9.0	19.1	12.0	13.6	13.8
Protein/RNA	58 ± 2	63 ± 2	62 ± 1[c]	77 ± 6[d]	70 ± 1[d]
Activity of RNA (g of protein/g of RNA per day)	12.5	12.8	16.5	18.0	15.5
R	126	83	85	80	75
Breast					
Fractional synthesis rate (%/day)	25.6 ± 2.5	24.3 ± 2.2	25.4 ± 2.3[e]	26.8 ± 1.0[e]	26.1 ± 3.0[e]
Fractional degradation rate (%/day)	9.9	22.0	7.5	13.8	14.7
Protein/RNA	58 ± 5	63 ± 2	68 ± 5[fg]	73 ± 3[g]	62 ± 5[f]
Activity of RNA (g of protein/g of RNA per day)	14.8	15.3	17.1	19.6	16.2
R	110	74	109	121	75

the leg muscles of chicks as estimated from the incorporation of [^{14}C]tyrosine (Table 3, Expt. 1). The calculated fractional degradation rate, however, was doubled in the deficient birds. A similar response was observed for breast muscle, where no significant difference in the fractional synthesis rate was measured between well-fed, growing chicks and deficient, non-growing chicks. Again the calculated fractional degradation rate was more than double in the malnourished birds.

When the total dietary amino acid supply was not sufficient to meet the requirement, the growth rate of chicks was also decreased; 75% of the amino acid mixture supplied in the control diet decreased growth by 28% over a period of 4–5 days (Table 3, Expt. 2). A further decrease in the amount of L-lysine hydrochloride to 50% (0.7% L-lysine hydrochloride) in this low-amino acid diet resulted in the growth decrease of 35%, compared with 92% observed previously when an adequate supply of the other amino acid was provided (Expt. 1).

In this experiment the fractional whole-body growth rate and the fractional synthesis rate of the leg-muscle proteins of chicks in the control groups were significantly larger ($P < 0.05$) than those in the depleted groups. There was only a small increase in the fractional degradation rate in the leg muscles of the chicks fed on the diets low in lysine and/or amino acids. On the other hand, the fractional synthesis rates of the breast muscles were quite similar in all groups, as was observed above. In addition, the calculated fractional degradation rate in the deficient chicks was almost twice as high as that in the control chicks.

Millward *et al.* (1975) showed that a fall in the growth rate and fractional synthesis rate, whether a result of aging or malnourishment, was accompanied by a fall in the concentration and activity of RNA. Akinwande & Bragg (1974), however, failed to find a decrease in muscle RNA concentration in 2-week-old chicks fed on diets inadequate in lysine. In the present experiments, although the rates of growth were decreased by feeding suboptimal amounts of amino acids, the amounts of RNA did not decrease with respect to protein (Table 3). Furthermore, the efficiency of protein synthesis remained high. Therefore the growth decrease produced by the deficient diets was apparently not accompanied by a fall in the efficiency of or the capacity for protein synthesis.

Effect of lysine or energy deficiency on protein synthesis

In the experiments described above, protein synthesis appeared to be relatively insensitive to dietary limitations and to carry on in the face of a lack of either lysine or total amino acids. This

Table 4. *Effect of a lysine, energy, or energy/protein deficiency on the growth rate and several aspects of muscle protein metabolism in chicks*

New Hampshire × Single Comb White Leghorn chicks of both sexes were used. The values are the mean ± S.E.M. of six chicks per group. Values without common letter superscripts in a given row are significantly different ($P<0.05$). Chicks in the 'energy restriction' group were fed on a diet that contained 200% of the amino acid content of the control diet, but received only 50% as much food as that consumed by the control group. Chicks in the 'protein/energy restriction' group were fed on the control diet in an amount equal to that consumed by the lysine-deficient birds, which was about 40% of that eaten by the control group.

	1 week old	2 weeks old			
	Control	Control	Energy restriction	35% lysine	Protein/energy restriction
Body weight (g)	64	103	78	69	77
Fractional whole-body growth rate (%/day)	5.9	9.4	1.6	0.7	1.3
Leg					
Fractional synthesis rate (%/day)	18.2 ± 1.4^{ef}	22.3 ± 2.2^{e}	16.4 ± 1.3^{f}	17.7 ± 0.9^{ef}	19.6 ± 1.2^{ef}
Fractional degradation rate (%/day)	11.4	12.2	14.6	16.9	18.1
Protein/RNA	34 ± 2^{h}	56 ± 4^{g}	65 ± 6^{g}	59 ± 7^{g}	56 ± 4^{g}
Activity of RNA (g of protein/g of RNA per day)	6.1	12.7	10.3	10.3	11.0
R	179	121	187	84	184
Breast					
Fractional synthesis rate (%/day)	33.9 ± 2.0^{a}	18.5 ± 1.7^{b}	17.6 ± 2.1^{b}	17.6 ± 1.6^{b}	23.4 ± 1.7^{b}
Fractional degradation rate (%/day)	23.8	5.9	15.1	16.4	21.5
Protein/RNA	21 ± 1^{d}	61 ± 3^{c}	60 ± 7^{c}	65 ± 3^{c}	55 ± 4^{c}
Activity of RNA (g of protein/g of RNA per day)	7.2	11.4	10.3	11.3	13.0
R	234	120	168	67	183

activity may lead to a depletion of the free amino acid pool. The lower values for *R*, the ratio of bound to free amino acid, seen in the deficient birds (Table 3) suggest a smaller free pool, at least for tyrosine. As a result, protein breakdown may be enhanced to the point that little or no growth occurs. Thus in a fourth experiment one group of birds was fed on a protein-rich, energy-deficient diet and another was given a lesser amount of the complete diet.

Again there was a fall in the fractional synthesis rate of breast muscle between 1 and 2 weeks of age, although the fractional synthesis rate of leg-muscle proteins was somewhat higher at 2 weeks than it was at 1 week of age (Table 4). The fractional synthesis rate of leg muscle was also lower in all of the restricted groups, whether growth was decreased by a lack of lysine, energy, or protein and energy. The fractional degradation rate appeared to be higher in the malnourished birds. In breast muscle the fractional synthesis rate of all groups was similar. Thus all showed the same decrease from the fractional synthesis rate observed in 1-week-old birds. The calculated fractional degradation rate, however, was markedly higher in the non-growing, malnourished chicks than in the well-fed controls, just as was observed in the earlier experiments.

In this experiment, the value of *R* for the lysine-deficient chicks was again low, but that for the energy- or energy/protein-restricted chicks was

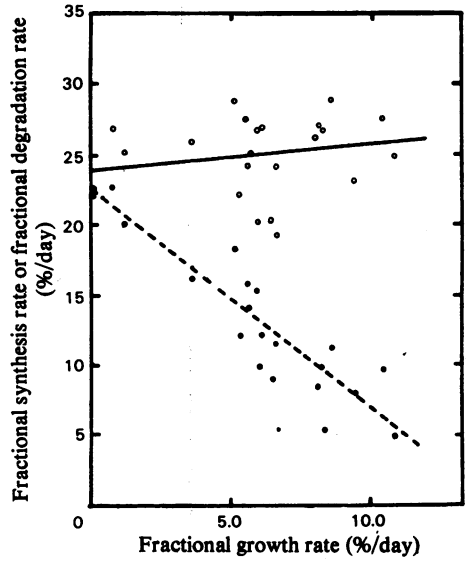

Fig. 2. *Regression of the observed fractional synthesis rate* (○) *and calculated fractional degradation rate* (●) *in breast muscle on the whole-body growth rate*

The regression equations are as follows: fractional synthesis rate = 24.0+(0.19 × fractional whole-body growth rate); $r = 0.20$; $s_b = 0.20$; fractional degradation rate = 22.6−(1.57 × fractional whole-body growth rate); $r = -0.92$; $s_b = 0.15$.

larger than that observed in the well-fed group (Table 4). Thus the increase in the calculated fractional degradation rate probably cannot be attributed to a depletion of the free pool.

As in the previous experiment, the activity of RNA was similar in both muscle preparations and in all treatment groups (Table 4). The absolute values are lower than previously observed and this may be attributed to the change in precipitating agents. The differences appear to be in the protein/RNA ratios where the protein values were lower, whereas the RNA concentrations were similar. Of interest are the rather low values for RNA activity in the 1-week-old birds. This is again due to a low protein/RNA ratio, but in this case it is attributable to the very high concentration of RNA at this time. Perhaps it reflects a preparation for the large growth spurt, particularly in the breast muscle, which is about to occur.

Regression of the fractional synthesis rate and the fractional degradation rate on the fractional whole-body growth rate

Individual values from the 14- and 15-day-old male New Hampshire × Single Comb White Leghorn chicks, summarized in Table 3, were pooled for the regression analysis. A wide range of growth rates was produced by the various dietary treatments. The 1-week-old birds were not included because of the developmental fall in breast-muscle fractional synthesis rate between 7 and 14 days. Chicks in the last experiment were excluded because the growth rates were either very high or very low. The regression of the fractional synthesis rate and the fractional degradation rate on the fractional whole-body growth rate were computed for the breast muscle (Fig. 2) and the leg muscles (Fig. 3). In the breast muscle, the regression coefficient of the fractional synthesis rate on the fractional whole-body growth rate was 0.19; the deviation from zero was not statistically significant. This indicated a high degree of probability that protein-synthesis rate in the breast muscles of chicks was independent of the growth rate. On the other hand, fractional degradation rate showed a negative correlation with the fractional whole-body growth rate; the correlation coefficient of −0.92 was significantly different from zero ($P<0.001$). Thus, in the breast muscle, the process of protein degradation seems to play an important role in the regulation of protein accumulation and to undergo a substantial change during alteration in the growth rate. From the intercept on the ordinate obtained by this regression, it appeared that the fractional synthesis rate and the fractional degradation rate in the breast muscles were still 20–25% per day when the chicks had ceased to grow. The regression coefficient of the

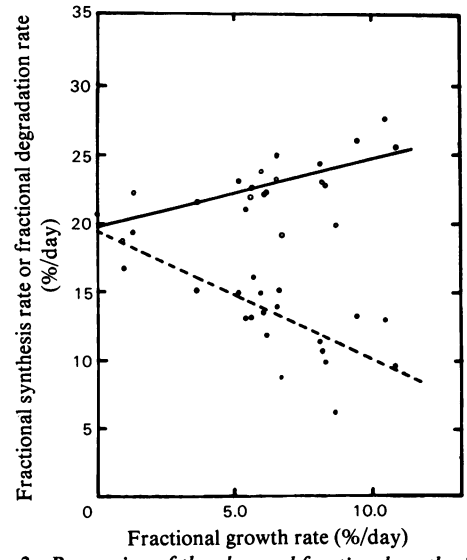

Fig. 3. *Regression of the observed fractional synthesis rate* (○) *and the calculated fractional degradation rate* (●) *in leg muscles on the whole-body growth rate*

The regression equations are as follows: fractional synthesis rate = 19.8 + (0.49 × fractional whole-body growth rate); $r = 0.66$; $s_b = 0.13$; fractional degradation rate = 19.4 − (0.93 × fractional whole-body growth rate); $r = -0.84$; $s_b = 0.14$.

fractional synthesis rate of the leg muscles on the fractional whole-body growth rate was 0.49 (Fig. 3). This value was considerably larger than that in the breast muscles and was significantly different from zero ($P<0.001$). A highly negative correlation of the fractional degradation rate on the fractional whole-body growth rate was nevertheless also noted in the leg muscles. The change in the decreasing fractional degradation rate was approximately twice the change in the increasing fractional synthesis rate for a given change in growth rate. The regression of the fractional synthesis rate and fractional degradation rate on the calculated values of fractional muscle growth rate led to the same conclusions with only minor changes in the regression coefficients.

Discussion

The breast and leg muscles of the newly hatched chick are fully differentiated muscles with a ratio of sarcoplasmic proteins to myofibrillar proteins that is in the normal range found in fully grown birds (George & Berger, 1966). Thus in the present study we are not using as our model embryonic or undifferentiated muscle that is in any morphological way unique to the young chick. The fractional rate of protein synthesis in these muscles was very high in 1-week-old birds, higher than that previously re-

ported for any species: 35–38% per day in breast muscle. The breast muscle is enlarging at this stage at a rate almost twice that of the total body weight of the chick: the proportion of breast muscle observed in the New Hampshire × Single Comb White Leghorn chicks was 4.4% of the whole body weight in 105g chicks and increased to 5.6% in 130g chicks. In commercial Rock Cornish broilers, the breast muscle may constitute 18.5% of the total body weight (Moran *et al.*, 1970). This high proportion of breast muscle is a heritable trait and is a product of genetic selection.

The ratios of fractional synthesis rate to fractional degradation rate were higher in breast muscles than in the leg muscles and in the Rock Cornish chicks than in the New Hampshire × Single Comb White Leghorn birds. The highest ratio, 2.67, was found in the breast muscles of the Rock Cornish chicks; this indicates that 62.5% of protein synthesized was retained in these muscles. On the other hand, in the malnourished chicks the fractional synthesis rate/fractional degradation rate ratio was only about 1.1, which indicates that more than 90% of the protein synthesized in the breast muscles was also degraded. Similar results were observed in the leg muscles.

The rate of protein synthesis was very high in rapidly growing birds, but surprisingly remained very high in non-growing birds as well. This was particularly true of the breast muscle, where the differences in synthesis rates were never statistically significant. In the leg muscles, the fractional synthesis rate increased somewhat as the growth rate increased, but in both muscle groups a decrease in degradation appears to have played a far more important role in the achievement of a high fractional whole-body growth rate. Because the deficient birds did not grow and weighed the same as the 1-week-old chicks it might be reasonable to assume that development was arrested and to compare their fractional synthesis rate with that of the younger birds. If this is done, it appears that there was indeed a decrease in the fractional synthesis rate and little change in the fractional degradation rate in breast muscle. In the leg muscle no developmental fall in the fractional synthesis rate was seen, and a small decrease, which is statistically significant ($P<0.05$) in only about half of the comparisons, in the fractional synthesis rate did occur, which perhaps may be attributed to the deficiencies. But because the fractional synthesis rates of breast muscle of well-fed and of malnourished birds were not different at 2 weeks of age, it seems more likely that the change in fractional synthesis rate from 1 to 2 weeks in all groups may be attributed to development rather than to development in the well-fed chicks and to the deficiency in the non-growing birds. The mildly deficient chicks, which grew at about half the rate of the control birds, gave similar values for fractional synthesis rate as well. The results are clearly in contrast with those reported by Millward *et al.* (1975) for the rat and by G. J. Laurent, M. P. Sparrow & D. J. Millward (personal communication) for the adult cockerel. They seem to be much more consistent with the cited reports of compensatory alterations in synthesis and degradation.

That the RNA concentration with respect to protein remained high and that its activity, as defined here, remained unchanged in slowly growing deficient birds suggests that the control of protein synthesis in the young chick is different from that in the rat, where it is clear that protein synthesis is very sensitive to nutritional status. Indeed, the young chick appears to be unique in this regard and requires further study for this reason. The values for the activity of RNA [(fractional synthesis rate × protein)/(RNA × 100)] are similar to those observed in well-nourished rats (Millward *et al.*, 1975), but 10–20% less than those observed in the adult cockerel (G. J. Laurent, M. P. Sparrow & D. J. Millward, personal communication). This may be related to body temperature, which in the chick rises by 0.7°C in the first week and then more slowly for another 2 weeks. Thus the lower RNA activity values may be associated with these lower body temperatures in the rat and young chick.

One weakness of the dietary-infusion method may be that protein synthesis is obviously being measured in fed animals. Garlick *et al.* (1973) showed that muscle protein synthesis in the rat was decreased by starvation for 24h. Therefore perhaps the presence of food, whether nutritionally adequate or not, produces in the chick a maximal synthesis rate. In the malnourished groups, synthesis may be so decreased when food is absent from the tract that no net growth occurs. This might be true for the groups pair-fed to the lysine-deficient group or given half the amount consumed by the well-fed birds; however, when chicks are fed *ad libitum*, food can always be found in the crop and tract, even when the diet is deficient in some nutrient. Therefore it seems unlikely that feeding the chicks during the measurement of protein-synthesis rates compromises the results.

One feature of this apparent dependence on a low fractional degradation rate for rapid growth requires further comment. The fractional degradation rate was calculated from the estimated protein accumulation and the fractional synthesis rate. Before we can state unequivocally that a marked decrease in the fractional degradation rate did indeed occur with increases in growth, the fractional degradation rate should be measured directly. Attempts to do so have been frustrated by the problem of intensive reutilization of labelled amino acids in rapidly growing chicks. Little or no decay of radioactivity in the amino acids labelled by the administration of

[^{14}C]carbonate was observed (M. L. MacDonald & R. W. Swick, unpublished work), suggesting that in addition to the marked recycling of labelled amino acids, the fractional degradation rate may indeed be very low.

This research was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin–Madison, and the U.S. Public Health Service grant no. AM 14704. We thank Mr. Terry Burk for excellent technical assistance.

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