PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **176,** 292–296 (1984)

Changes in Protein Degradation in Chickens Due to an Inflammatory Challenge (41873)

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Abstract. Tissue-specific changes in protein catabolism were examined in chicks 16 hr following an inflammatory challenge. It was determined that tyrosine was not catabolized or converted to phenylalanine in muscle, thymus, bursa, or spleen. Therefore, rates of tyrosine release from protein were used to estimate rates of protein catabolism in these tissues. Arginine was not catabolized to urea by chick liver; consequently, arginine release from liver protein was used to measure protein catabolism in this tissue. An injection of sheep red blood cells (SRBC) or *Escherichia coli* did not change rates of protein catabolism in liver or bursa as compared to saline-injected controls. SRBC significantly increased protein catabolism in muscle and spleen by 29 and 15%, respectively. *E. coli* resulted in significant increases in muscle, spleen, and thymus of 43, 30, and 34%, respectively. These changes in protein catabolism, together with known changes in protein synthesis, suggest that an inflammatory response to SRBC and *E. coli* result in increased protein accretion in the bursa and liver, and net protein loss from muscle.

Net protein accretion or liberation in a tissue is determined by the balance between protein synthesis and degradation. The rates of protein synthesis in various tissues and the effect of inflammatory agents on these rates were described previously (1). In this report the relative rates of protein degradation were examined. Tissue-specific changes in protein accretion or liberation following stimulation of the immune system may represent a homeostatic response involved in the support of the immune response.

Investigations of protein degradation have been limited by methodological difficulties. Most studies have utilized intact animals and followed the disappearance of radioactively labeled proteins. These studies are subject to a number of artifacts such as those due to reutilization of the labeled amino acids (2). In order to analyze relative rates of protein degradation under carefully controlled conditions, the *in vitro* method of Fulks *et al.* (3) was employed. With this technique, the rate of liberation of an amino acid which is neither synthesized nor catabolized in the tissue being considered is determined.

Materials and Methods. *General.* All experiments utilized 600-g male, Single-Comb White Leghorn chicks (Cornell K-strain) except in experiments examining muscle where 350-g chicks were utilized. Chicks were reared and inflammatory agents were prepared as described previously (4). Chicks were injected with either 1% *Escherichia coli* (263,08: K87,88ab:H19), 1% sheep red blood cells (SRBC), or phosphate-buffered saline (control). Feed was removed at the time of injection and the chicks were killed after 16 hr.

Tissue preparation. Thymus, bursa, and spleen cells were separated from connective tissue as described previously (4). Liver slices were prepared by the method of Stadie and Riggs (5), which employs a razor blade microtome and results in slices which are about 0.5 mm thick. The extensor digitus longus muscle (EDL) was dissected from the lower leg without damage to the fibers and with the tendons intact at each end of the muscle. This was accomplished by separating the EDL from surrounding muscles (tibialis anterior) by blunt dissection and severing the tendons from the points of origin and insertion with a scapel. Tissue samples approximately 100 mg were utilized. The EDL from 350-g chicks weighed about 100 mg. It was important to limit muscle size to 100 mg in order to maintain viability, which is limited by O_2 and nutrient diffusion (6).

Conditions for incubation. Tissues were incubated in a manner similar to that described by Fulks *et al.* (3). Immediately after collection (muscle), slicing (liver), or isolation of cells

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0037-9727/84 $1.50

(thymus, bursa, and spleen), tissues were placed in 25-ml Erlenmeyer flasks. The flasks contained 5 ml of Krebs–Ringer bicarbonate (KRB) buffer (104 m*M* NaCl, 5 m*M* KCl, 3 m*M* $CaCl_2$, 1 m*M* $MgSO_4$, 1 m*M* KH_2PO_4, and 25 m*M* $NaHCO_3$, pH 7.4, at 37°C) with glucose (10 m*M*), amino acids (Table I), and chloramphenicol (0.3 mg/liter) and saturated with a 95% O_2–5% CO_2 gas mixture. The flasks were stoppered and re-equilibrated with the gas mixture. The O_2-charged flasks were preincubated for 30 min at 37°C with shaking. The tissues were then transferred to flasks containing fresh O_2-saturated KRB, chloramphenicol, glucose, amino acids, and 0.5 m*M* cycloheximide. Tissues were incubated an additional 3 hr at 37°C with shaking, and the reaction was terminated by the injection of 1 ml of 30% trichloroacetic acid solution. The concentration of tyrosine in the media was determined by the method of Waalkes and Udenfriend (7). Arginine concentrations were determined by automated amino acid analysis.

Amino acid catabolism. To measure protein catabolism by amino acid release, it is assumed that the marker amino acid is not catabolized to an unmeasured form after its release from protein. This was confirmed for the amino acids used in this study by the addition of ^{14}C-labeled amino acid to the incubation mixture and examining ^{14}C in the metabolic end products.

TABLE I. AMINO ACID COMPOSITION OF BUFFER

Amino acid	Concentration (μmole/liter)[a]
Asp	100
Thr	400
Ser	600
Glu	300
Gln	250
Gly	500
Ala	450
Cys	100
Val	220
Met	80
Ile	180
Leu	250
Tyr	180[b]
Phe	130
Pro	400
Lys	450
His	120
Arg	300[c]
Trp	80

[a] Concentrations reflect the normal plasma amino acid values of 600-g chicks fasted for 16 hr.

[b] Tyr was omitted in experiments examining Tyr catabolism and protein catabolism (except in liver).

[c] Arg was omitted in experiments examining Arg or protein catabolism in the liver.

Tyrosine catabolism. Muscle, thymus, bursa, and spleen were incubated wth 0.06 μCi/ml L-[U-^{14}C]tyrosine. The incubation was terminated by the injection of 1 ml 30% TCA into the incubation media. CO_2 was collected by injecting 90 ml of air into the flask while collecting the displaced gas via a hypodermic needle placed through the serum stopper. $^{14}CO_2$ was trapped in 25 ml of a 1:2 solution of 2-aminoethanol (ethanolamine):ethylene glycol monomethyl ether. Three milliliters of the trapping solution was counted in 12 ml of scintillation cocktail (Amersham, Arlington Heights, Ill.).

Arginine catabolism. Livers from chicks and rats (positive control) were incubated with 0.1 μCi/ml L-guanidino[^{14}C]arginine. The incubation was terminated with 1 ml 30% sulfosalicylic acid (SSA). Urea and arginine were isolated by collecting the effluent from an automated amino acid analyzer (Technicon Instruments Co., Terrytown, N.Y.). The [C^{14}]urea and [^{14}]arginine were determined by scintillation counting.

Conversion of phenylalanine to tyrosine. Tissues were incubated with 0.1 μCi/ml L-phenylalanine (side chain-^3H; ICN Pharmaceuticals, Inc., Irvine, Calif.). The incubation was terminated with 1 ml 30% SSA. Initial phenylalanine and final tyrosine concentrations were determined by automated amino acid analysis and the radioactivity of fractions containing the isolated amino acid was determined.

Results and Discussion. It is known that the kidney and liver can catabolize tyrosine; however, the tissue distribution of the enzymes of tyrosine catabolism has not been examined in detail (8). It was found that the spleen, bursa, and thymus catabolize very little tyrosine as compared to liver (Table II). The extent of tyrosine catabolism in these tissues was similar to that in muscle.

In comparison to rat liver, chick liver catabolized very little arginine. Since chick liver does not possess a functional urea cycle and

TABLE II. TISSUE AMINO ACID CATABOLISM

Tissue	Amino acid	Percentage catabolized per hour[a]
Liver	Tyr	0.92[b]
Muscle	Tyr	0.04
Bursa	Tyr	0.08
Thymus	Tyr	0.01
Spleen	Tyr	0.04
Liver	Leu	0.26
Liver	Arg	0.01
Liver (rat)	Arg	0.97

[a] cpm CO_2 or urea/initial cpm buffer.
[b] Means of two determinations.

has only trace arginase activity (9), arginine can be neither synthesized nor catabolized in chick liver. In this study and the study by Tamir and Ratner, chick arginase activity was about 1% of that in the rat.

In rats, the conversion of phenylalanine into tyrosine by phenylalanine hydroxylase occurs only in the liver and the adrenal medulla (10, 11). The inability of chick muscle, spleen, thymus, and bursa to synthesize tyrosine from phenylalanine is demonstrated in Table III. The chick liver exhibited active phenylalanine hydroxylase activity.

When protein synthesis is blocked and a tissue can neither synthesize nor catabolize a particular amino acid, the rate of increase in the concentration of that amino acid is indicative of this liberation due to protein catabolism. Tyrosine meets these requirements in the muscle, thymus, spleen, or bursa, and arginine meets the requirements in the liver. The release of these amino acids can be used to determine the rates of protein catabolism.

In this study, SRBC and *E. coli* did not affect protein catabolism in the liver or bursa as compared to controls (Table IV). SRBC significantly increased protein catabolism in the muscle and spleen by 29 and 15%, respectively. *E. coli* resulted in significant increased in the muscle, spleen, and thymus of 43, 30, and 34%, respectively.

Since rates of protein degradation were not affected by treatments in the liver and bursa, increases in protein synthesis reported previously (1) indicate that these tissues undergo net protein accretion. In muscle, rates of protein degradation and synthesis were changed in opposite directions after exposure to inflammatory agents. Consequently, protein is catabolized in muscle and amino acids are liberated.

Interpretation of the net change in protein metabolism in the thymus and spleen is difficult since the rates of synthesis (1) and degradation are both increased. The *in vitro* measurement of protein degradation by amino acid release is useful for determining qualitative, but not quantitative, changes. The quantitative limitations of this technique have been explored in muscle (3). When skeletal muscles from growing rats are incubated in unsupplemented KRB, they undergo net protein breakdown. Glucose and amino acids inhibit protein degradation, but the balance between protein synthesis and degradation is still very negative. Insulin also inhibits protein degradation and improves the nitrogen balance of incubated muscles; however, all of these factors together do not prevent net protein catabolism. Since glucose and amino acids, but not insulin, were provided in the incubation media in this study, it is apparent that the incubating muscle would have rates of protein degradation which are higher than

TABLE III. TISSUE-SPECIFIC CONVERSION OF Phe TO Tyr

Tissue	Percentage converted[a]
Liver	6.70[b]
Muscle	0.00
Spleen	0.35
Thymus	0.01
Bursa	0.01

[a] cpm/μmole Tyr at 3 hr/initial cpm/μmole Phe × 100.
[b] Means of two determinations.

TABLE IV. TISSUE PROTEIN CATABOLISM[a]

Tissue	*N*	Control	SRBC	*E. coli*
Liver	3	382 ± 11[b]	383 ± 17	410 ± 12
Muscle	8	224 ± 9[c]	288 ± 11[d]	321 ± 16[d]
Thymus	4	545 ± 14[c]	612 ± 34[c,d]	732 ± 54[d]
Spleen	4	820 ± 22[c]	942 ± 35[d]	1070 ± 41[d]
Bursa	4	649 ± 44	663 ± 46	790 ± 46

[a] Picomoles tyrosine or arginine (liver) released per gram of tissue in 3 hr of incubation.
[b] Mean ± SEM.
[c,d] Means in the same row with different superscripts are significantly different ($P < 0.05$).

in vivo rates. If the *in vitro* incubation of the spleen and thymus results in similar perturbations in nitrogen balance as that which has been reported in muscle, then changes in tyrosine release due to inflammatory agents cannot be considered as reliable quantitative measurements of protein degradation *in vivo*. For this reason the variable, milligrams protein degraded per unit time, which can be calculated from the moles of tyrosine released per unit time, would have little physiological significance. Therefore, comparison of the fractional synthetic rates reported previously (1) with degradation values in order to determine net protein production or loss is not valid.

From the consideration of the changes in free amino acid concentrations in these tissues after exposure to inflammatory agents (4), it appears that the spleen is in a state of net protein synthesis, whereas the thymus undergoes amino acid liberation and, therefore, net protein catabolism. This may indicate that the protein synthetic rate was greater than the degradative rate in the spleen but less in the thymus.

Most conditions which cause amino acid liberation from the muscle, such as food deprivation, denervation, disuse, or glucocorticoids, are a result of decreased protein synthesis and increased protein degradation (12). Inflammation-induced net protein degradation in muscle appears to be another example of amino acid liberation due to complementary changes in protein synthesis and degradation. Net protein accretion in muscle is also accompanied by increased protein degradation, but protein synthetic rates increase to a greater extent (13). This situation occurred in the spleen. It has been suggested that the high rates of protein degradation which occur simultaneously with growth allow the remodeling of structural proteins to accommodate the growth of the cell (13).

Increased liver protein accumulation is usually a result of decreased degradation with little change in synthesis (14); however, the increase in production of export proteins upon refeeding is a result of increased synthesis with little change in degradation (15). The inflammation-induced increase in liver protein synthesis is predominantly due to increased export protein (1) and has similar basis as an increase due to refeeding.

From the consideration of both protein synthesis (1) and catabolism, it is evident that the bursa, spleen, and liver respond to an ip injection of SRBC or *E. coli* by increasing their net rate of protein synthesis. This is not unexpected since it is the cells of the immune system which respond to antigenic stimuli, and the liver produces many of the immunologically active plasma proteins. It is well recognized that the spleen is an important secondary lymphatic tissue which is active during both systemic and localized infections. The ability of the bursa to process and respond to antigens has been recognized recently (16). It cannot be determined from these data if the increased protein synthesis in the bursa is due to an immune response in this tissue or to an increase in the synthesis of new cells which migrate to secondary lymphatic tissues.

The increased demand for amino acids for protein synthesis in the immune tissue and the liver appears to be satisfied by the release of amino acids from the muscle. The absolute increase in protein catabolism in this tissue due to exposure to inflammatory agents may not be great; however, the large size of this tissue apparently allows sufficient release of amino acids. This is demonstrated by the maintenance of essential amino acid concentrations in plasma after injection of inflammatory agents (4). It is possible that changes in protein degradation observed in this study are induced by mediators released from phagocytes stimulated by the inflammatory agents. Endogenous pyrogen, also known as interleukin-1 and leukocyte endogenous mediator, stimulates protein degradation in rat muscle incubated *in vitro* (17). A leukocyte-derived mediator also stimulates a change in the concentration of the nonmetabolizable amino acid, cycloleucine, in liver, suggesting the utilization of amino acids by liver (18).

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Received September 6, 1983. P.S.E.B.M. 1984, Vol. 176.
Accepted March 30, 1984.