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Changes in Protein Synthesis Due to an Inflammatory Challenge (41872)

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Abstract. Rates of protein synthesis in various chick tissues were examined 16 hr after an inflammatory challenge. Protein synthetic rates were calculated from the rate at which [^{14}C]leucine was incorporated into protein and the specific activity of [^{14}C]leucine in the precursor pool. An injection of either *Escherichia coli* or sheep red blood cells (SRBC) decreased the rate of protein synthesis in the gastrocnemius muscle, and increased the rate in liver, bursa, spleen, and thymus. *E. coli,* but not SRBC, decreased protein synthesis in the pectoralis muscle. *E. coli* significantly decreased the aggregation of pectoralis muscle polysomes and increased the aggregation of polysomes in the thymus, bursa, and spleen. *E. coli* increased the aggregation of free, but not bound, polysomes in liver, suggesting an increase in synthesis of export proteins. SRBC significantly increased polysomal aggregation in bursa and spleen only. A crude preparation of leukocyte endogenous mediator, isolated from peritoneal macrophages, decreased muscle-polysomal aggregation. These studies indicate that tissue-specific changes in protein synthesis occur after a noninfectious inflammatory challenge. These changes may be part of a homeostatic mechanism which supports the immune response.

The tissue-specific changes in protein metabolism induced by an immune response and the homeostatic processes by which these changes occur are not well characterized. Characterization of these changes may help elucidate the mechanisms by which environmental factors such as nutrition and stress, which also affect protein metabolism, exert their effects upon immunocompetence.

Inflammation-induced changes in the concentrations of free amino acids of various tissues, described previously (1), suggest that exposure to antigens causes changes in the relative rates of protein synthesis and degradation in several tissues. That these changes in free amino acid concentrations are due, in part, to changes in protein synthesis was examined in these experiments.

Methods. Chicks were housed, inflammatory agents were prepared, and tissues were collected as described previously (1), unless otherwise noted.

Fractional synthetic rate (FSR). The effect of an inflammatory challenge on the rates of protein synthesis in various tissues was estimated from the incorporation of [^{14}C]leucine into protein after a pulse dose. Nine birds per treatment were injected ip with 1 ml of either PBS, 0.5% SRBC, or 0.5% *Escherichia coli.* At 0.5, 1, or 2 hr before sacrifice, three chicks per treatment were injected iv with 3 μCi/100 g of [1-^{14}C]leucine (Amersham, Arlington Heights, Ill.). Feed was withheld after injection; water was provided *ad libitum.* Birds were killed 16 hr postinjection, and tissues were sampled immediately and frozen in liquid nitrogen. Frozen muscle and liver samples were minced using scissors, and 1-g samples were homogenized in 4 ml cold double-distilled water. To a 1.8-ml aliquot of the homogenate, 0.2 ml cold, 30% sulfosalicylic acid (SSA) was added to precipitate proteins. The supernatant was saved for analysis of the specific activity of the precursor pool.

An additional 1.25-ml aliquot of the homogenate was used to isolate protein and RNA. This was accomplished by the following steps. Macromolecules were precipitated from the homogenate by the addition of 2.5 ml cold, 0.3 *N* $HClO_4$. After standing for 10 min (3°C), the mixture was centrifuged at 2000*g* and 3°C for 15 min. The supernatant was discarded and the precipitate was washed twice with cold 0.2 *N* $HClO_4$. After the final washing, the precipitate was drained over a paper towel. The lipids were separated from the precipitate by extraction for 15 min with each of the following in succession: K-acetate-saturated ab-

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solute ethanol, ethanol–ether 3:1, and anhydrous ether.

RNA was then separated from the precipitate by differential hydrolysis. To accomplish this, the precipitate was resuspended in 4 ml of 0.3 *N* KOH and incubated for 1 hr at 37°C. The suspension was cooled in ice, and the protein was precipitated with 2.5 ml of 1.2 *N* $HClO_4$. After 10 min, the samples were centrifuged at 2000*g* and 3°C for 15 min, and the supernatant was separated and saved. The precipitate was washed twice with 0.5 ml of 0.2 *N* $HClO_4$ and the washing combined with the supernatant. The washings and supernatant were diluted to a known volume and $HClO_4$ concentration with water. The RNA concentrations were determined by absorbance at 260 n*m*. Torula yeast RNA was used as a standard.

The remaining pellet was hydrolyzed in a sealed tube with 4 ml of 6 *N* HCl for 20 hr at 110°C. The hydrolysate was concentrated in a vacuum oven at 50°C. The pH of the final concentrate was raised to 2.0 with sodium hydroxide.

The specific activity of leucine in the SSA-soluble fraction (precursor pool) and the hydrolyzed protein was determined by counting 0.5 ml of the sample in 10 ml of scintillation cocktail and determining the leucine concentration of a separate aliquot by automated amino acid analysis. Cells isolated from thymus, spleen, and bursa as previously described (1) were weighed and treated in the same manner as minced muscle and liver.

FSR can be derived from the simultaneous measurements of radioactivity in the precursor pool and the protein molecules according to the equation

$$dP^*/dt = k_s F^* - k_d P^*$$

where P^* is the specific activity of leucine in the protein molecules, F^* is the specific activity of leucine in the precursor pool, and k_s and k_d represent the rate contant for protein synthesis and degradation, respectively (2). The rate of increase in radioactivity in the protein molecule at any given time is given by the difference between radioactivity entering from the precursor pool ($k_s F^*$) and that leaving due to protein degradation ($k_d P^*$). This equation can be integrated between two times after isotope administration, t_1 and t_2, to give

$$P^*t_2 - P^*t_1 = k_s \int_{t_1}^{t_2} F^*(t)dt - k_d \int_{t_1}^{t_2} P^*(t)dt.$$

In this experiment t_2 = 2 hr and t_1 = 0.5 hr after isotope injection. Since protein synthesis was examined very soon after isotope injection, values for $P^*(t)dt$ were insignificant as compared to $F^*(t)dt$ and the equation becomes

$$\text{FSR} = k_s = \frac{P^*t_2 - P^*t_1}{\int_{t_1}^{t_2} F^*(t)dt}.$$

The calculated FSR value has the units hours^{-1} and can be expressed as percentage per day by the conversion factor of 2400. Since all of the chicks in each treatment were required to estimate $\int_{.5}^{2} F^*(t)$, no estimate of variation is available for statistical analysis of the precursor-pool-specific activity and, therefore, the FSR.

Polysomal aggregation. Protein synthesis was further examined by the use of polysomal profiles. Chicks were injected and killed 16 hr later. Feed was removed at the time of injection. The aggregation of polysomes was determined using fresh tissue from three birds per treatment. Cells from thymus, bursa, and spleen were isolated as described previously (1) except red blood cells were not removed from the spleen.

Muscle polysomes were analyzed by the method of Heywood *et al.* (3). The free and bound polysomes in liver were separated according to the method of Ramsey and Steele (4).

Polysomes from the cells obtained from the thymus, bursa, and spleen were prepared as follows. The tissue sample was homogenized in 3 vol of polysome buffer (4) with 250 m*M* sucrose and 0.25% Triton X-100 by the procedure described for muscle (3). The homogenate was centrifuged at 10,000*g* for 5 min, and the supernatant was decanted. Triton X-100 was added to the supernatant to give a 1% solution. This was layered onto a 6–21% continuous gradient containing 75 m*M* KCl, 5 m*M* $MgCl_2$, and 0.5 m*M* EDTA and centrifuged at 131,000*g* for 105 min at 3°C.

The sucrose gradients were drained by puncturing the bottom of the centrifuge tube utilizing a Buchler gradient siphon. The rate of drainage was limited to 3 ml/min by a polystalic pump. Optical density was continuously recorded at 254 nm using a flow-through spectrophotometer with a 10-in. chart recorder. To prevent interference due to mixing vortex lines, the flow cell and delivery tubes were first equilibrated with the heaviest density sucrose. The absorbance curves were divided into a subunit plus small polysome segment (light) and a polysome segment (heavy).

Preparation of soluble mediators. Polymorphonuclear (PMN) leukocytes and macrophages were isolated from the peritoneal cavity. To accomplish this adult birds were injected ip with 150 ml of 0.4% oyster glycogen in sterile physiologic saline containing 100,000 U/liter of penicillin G and 0.5 g/liter streptomycin. An additional 100 ml of sterile saline was injected 14 hr later, and peritoneal exudates were aspirated into the iced vacuum flask containing heparin (20 IU/100 ml exudate). The exudate was centrifuged at 800*g* for 10 min at 4°C, washed with saline, and resuspended at a concentration of 10^5 cells/ml with sterile saline. The cells were incubated immediately for 4 hr with gentle shaking at 37°C. The supernatant was separated by centrifugation (800*g* for 10 min) and sterilized by membrane filtration. The protein content of this preparation was 0.7 mg/ml as determined by the method of Lowry *et al.* (5). An aliquot was heated at 90°C for 30 min and served as a heat-denatured control. To prevent contamination with pyrogens, all glassware was acid washed and autoclaved at 120°C for 15 min. The preparation of these soluble mediators is similar to the methods used by others (6, 7) to isolate a mediator termed leukocyte endogenous mediator (LEM). For this reason the leukocyte product utilized in this experiment will be referred to as LEM. It must be recognized that in this report LEM does not refer to a purified mediator.

Results. In experiment 1, the incorporation of [^{14}C]leucine into protein between 0.5 and 2 hr after isotope injection was examined. Since leucine-specific activity was examined shortly after isotope injection, F^* was considerably higher than P^* and the proteins in the body were accumulating label. The FSR, absolute increase in P^*, and average F^* are presented in Table I. SRBC and *E. coli* exposure did not appear to affect the specific activity of the precursor pool in the gastrocnemius muscle but did decrease leucine incorporation into protein as compared to the control, resulting in decreased FSR. In the pectoralis muscle, leucine incorporation into protein tended to be equally depressed due to SRBC and *E. coli* exposure, as compared to control birds; however, the changes in the specific activity of the precursor pool resulted in little change in FSR due to SRBC and a 24% decrease due to *E. coli.* In the liver, SRBC and *E. coli* exposure resulted in an increase in the specific activity of the precursor pool but proportionally greater increases in leucine incorporation into protein yielded an increase in the FSR. The FSR of protein in the bursa was increased by 43 and 240% over control values due to SRBC and *E. coli* and by 17 and 51%, respectively, in the spleen. In both the bursa and spleen, an inflammatory challenge resulted in depressed specific activity of the precursor pool, yet increased rates of leucine incorporation into protein were observed. The FSR of protein was also increased in the thymus due to both SRBC and *E. coli* injection.

Tissue RNA concentrations were not affected by treatments except in the spleen where *E. coli* resulted in a significant increase as compared to controls (Table I).

The aggregation of polysomes in various tissues was examined in the second experiment. These results are summarized in Table II. *E. coli* resulted in an increase in the aggregation of polysomes, as indicated by decreased ratios of light versus heavy polysomes, in the thymus, bursa, spleen, and liver (bound) as compared to controls. *E. coli* did not result in a significant change in the aggregation of the free polysomes of liver and resulted in decreased aggregation in muscle. SRBC resulted in a significant change in polysomal aggregation as compared to controls only in the spleen. Polysomes tended to be more aggregated, due to SRBC, in the liver (bound), thymus, and bursa and less aggregated in the muscle; however, these changes were not statistically significant ($P < 0.05$).

Soluble factors (LEM) produced by leukocytes resulted in decreased muscle-poly-

TABLE I. THE EFFECT OF SRBC AND *E. coli* ON TISSUE PROTEIN SYNTHESIS AND RNA

Tissue	Treatment	RNA (mg/g)	Specific activity (dpm/μmole)		Fractional synthetic rate
			Precursor[a]	Protein[b]	
Gastrocnemius muscle	Control	1.37 ± 0.064[c]	35,344	346	23.4
	SRBC	1.44 ± 0.093	34,313	243	17.0
	E. coli	1.35 ± 0.068	35,441	237	16.1
Pectoralis muscle	Control	1.28 ± 0.073	27,191	288	25.4
	SRBC	1.24 ± 0.051	22,939	259	27.1
	E. coli	1.26 ± 0.105	32,538	263	19.4
Liver	Control	9.65 ± 0.221	14,905	256	41.3
	SRBC	10.5 ± 0.364	18,863	424	53.9
	E. coli	10.7 ± 0.168	20,072	411	49.3
Bursa	Control	6.62 ± 0.422	21,429	654	73.2
	SRBC	8.12 ± 0.423	18,273	803	105.0
	E. coli	7.26 ± 0.463	13,286	1380	249.0
Spleen	Control	6.68 ± 0.311	20,259	513	60.8
	SRBC	7.41 ± 0.301	16,941	502	71.1
	E. coli	8.01 ± 0.299[d]	16,910	648	92.1
Thymus	Control	6.90 ± 0.495	8,812	175	47.7
	SRBC	7.17 ± 0.336	8,422	202	57.6
	E. coli	6.72 ± 0.293	7,055	176	59.9

[a] $\int_{0.5}^{2} F^*(t)dt$.
[b] $P_2^* - P_{0.5}^*$.
[c] Means ± SEM of six chicks.
[d] Significantly different from control ($P < 0.05$).

somal aggregation (Table III) as compared to saline- and heat-denatured LEM controls or *E. coli*. Zinc concentrations were decreased by both LEM and *E. coli* in comparison to saline- and heat-denatured LEM controls. *E. coli* probably did not result in reduced aggregation of muscle polysomes because of the short time interval between dosing and sampling (5 hr).

Discussion. The rates of muscle protein synthesis determined by the pulse dose technique in these experiments are similar to the results determined in the same muscles by Maruyama *et al.* (8) in chicks. These authors administered [^{14}C]tyrosine in the diet and found the FSR of gastrocnemius and pectoralis muscles to be 22.3 and 18.5% per day, respectively. Quantitative data on protein syn-

TABLE II. POLYSOMAL PROFILES[a]

Tissue	Treatment		
	Control[b]	SRBC[b]	*E. coli*[b]
Pectoralis	1.67 ± 0.21[c]	2.14 ± 0.41[c,d]	3.28 ± 0.40[d]
Liver (free)	0.640 ± 0.07	0.575 ± 0.09	0.597 ± 0.05
Liver (bound)	1.05 ± 0.10[c]	0.83 ± 0.08[c,d]	0.65 ± 0.11[d]
Thymus	0.680 ± 0.02[c]	0.624 ± 0.02[c,d]	0.550 ± 0.03[d]
Bursa	0.855 ± 0.04[c]	0.760 ± 0.07[c]	0.538 ± 0.04[d]
Spleen	0.661 ± 0.03[c]	0.515 ± 0.02[d]	0.504 ± 0.02[d]

[a] Ratio of light to heavy polysomes.
[b] Means ± SEM of three profiles.
[c,d] Means in the same row with different superscripts are significantly different ($P < 0.05$).

TABLE III. MUSCLE POLYSOMAL PROFILES AND PLASMA ZINC[a]

Treatment	Aggregation (light/heavy)	Zinc (μg/ml)
Control	1.97 ± 0.10[b]	1.55 ± 0.06[c]
LEM	3.11 ± 0.31*	0.86 ± 0.13*
Heated LEM	2.14 ± 0.17	1.64 ± 0.05
E. coli	2.11 ± 0.20	0.57 ± 0.08*

[a] Chicks were killed 5 hr postinjection. Feed was withdrawn after injection.
[b] Means ± SEM of three chicks per treatment.
[c] Means ± SEM of four chicks per treatment.
* Significantly different from Controls ($P < 0.05$).

thetic rates in chick liver, thymus, bursa or spleen have not been reported. The FSR of protein synthesis in the chick liver appears to be slightly less than values reported for the rat utilizing constant infusion techniques (9). The immune tissue, and in particular the bursa and spleen, had very high FSR values. This may reflect increased mitosis in cell populations from these tissues.

In the experiments reported here, the cells of the immune tissue were separated from the connective tissue matrix but were not a homogeneous population. The thymus, bursa, and spleen consist predominantly of lymphocytes but also contain other cell types such as epithelial reticular cells, fibroblasts, and macrophages.

Inflammation-induced decreases in the protein synthetic rate of gastrocenemius muscle suggest a role of muscle in supplying amino acids for use in the liver and immune tissues, which have increased demand for these substrates. The absolute decrease in muscle FSR is relatively small in comparison to the increases in liver and the immune tissue. However, in rats the muscle contributes approximately 40% to the whole body protein pool whereas the immune tissue and liver contribute 2 and 4%, respectively (10). It is possible that the relatively small decrease in muscle FSR is compensated by the large amount of protein in this tissue, allowing the muscle to supply adequate quantities of amino acids to the liver and immune tissue.

Both *E. coli* and SRBC challenge resulted in increased FSR in the liver, spleen, thymus, and bursa. In most cases, the decrease in tissue free amino acid concentrations reported previously (1) corresponded with increased protein synthesis. It is likely that the increased use of amino acids for protein synthesis is responsible for the depressed tissue amino acid concentrations. SRBC did not result in depressed liver free amino acid concentration but did increase the FSR. In addition, both SRBC and *E. coli* resulted in increased thymic FSR accompanied by increased free amino acid concentrations. These discrepancies may be due to either an increase in the rate of amino acid transport to match or exceed the increased demand for protein synthesis or an increase in protein catabolism which is relatively greater than the increase in synthesis.

The determination of polysomal aggregation is a useful method to detect changes in protein synthesis. An increase in aggregation of polysomes represents a greater number of ribosomes translating each individual mRNA and increased protein synthesis whereas a decrease in aggregation represents reduced synthetic activity as a result of fewer translating ribosomes. This interpretation of polysomal aggregation is only valid when changes in the rate of protein synthesis are regulated by initiation. Since the regulation of protein synthesis is often due to or accompanied by changes in the initiation step (11), polysomal aggregation is a good measure of protein synthesis. This method can be misleading when regulation of protein synthesis occurs at transcription or at other steps of translation. However, when the change in rate of protein synthesis is known, the corresponding change in polysomal aggregation indicates the point of control for this change (11).

In these experiments, the general correlation between the rates of protein synthesis, as measured by isotope incorporation, with the extent of polysomal aggregation indicates two points. First, both methods are accurate methods for the detection of changes in protein synthesis resulting from inflammatory agents; however, the isotopic method is quantitative and possibly more sensitive. Second, the changes in rates of protein synthesis are regulated, at least in part, at the initiation step.

RNA concentrations were not affected by treatment in any tissue except the spleen. Since mRNA constitutes 80 to 90% of the total RNA (12, 13), it appears that with the exception of

the spleen, protein synthesis is not controlled by changes in the availability of mRNA.

The control of inflammation-induced changes in protein synthesis in the thymus, bursa, and spleen appears to be similar to the control of protein synthesis after lymphocyte stimulation. Mitogen-induced increases in protein synthesis do not appear to be dependent upon the synthesis of new ribosomes, and there is no change in the rate at which individual protein molecules are synthesized (14). There is, however, a large increase in the proportion of lymphocyte ribosomes translating mRNA, indicating an increase in the rate of initiation (15, 16). Furthermore, it appears that unstimulated lymphocytes contain a translation inhibitor which reduces protein synthesis by inhibiting initiation factors (17).

Young *et al.* (18) have demonstrated depressed incorporation of [^{14}C]leucine into protein by muscle ribosomes *in vitro,* together with a disaggregation of polysomes after *Salmonella typhimurium* injection in rats. This, in conjunction with the present studies, demonstrates reduced protein synthesis in muscle due to decreased initiation after both infectious and noninfectious bacterial challenges as well as SRBC.

The increase in FSR in the liver is a result of an increase in the synthesis of protein synthesized on membrane-bound polysomes. This suggests that there is an increase in the synthesis of proteins destined for export from this tissue (i.e., plasma proteins) and/or membrane or organelle proteins. After experimental infections in rats, labeled amino acids are incorporated into plasma proteins at a much faster rate than in noninfected controls (19). Infection as well as LEM from stimulated leukocytes appeared to induce similar biochemical changes in liver (7). These include increases in the rate of amino acid transport and amino acid incorporation into protein. These changes occur in adrenalectomized, thyroidectomized, hypophysectomized, or diabetic rats. Interpretation of these experiments is difficult because the specific activity of the precursor pool was not determined.

LEM, also known as endogenous pyrogen and interleukin-1, has been shown to reduce plasma iron and zinc concentrations in several species including mouse, rat, and man (20, 21). These results suggest that LEM also lowers the plasma zinc concentration in the chick. Another possible function of LEM is to reduce the rate of protein synthesis in muscle. This may result in the release of amino acids from muscle for use in other tissues. The reduction in protein synthesis due to LEM occurred quickly, within 5 hr, which was much less than the time for an *E. coli*-induced reduction. This suggests that these changes are caused by a regulatory property of LEM and not an antigenic or inflammatory property.

Since LEM resulted in similar changes in muscle protein synthesis as did *E. coli,* it is possible that this mediator is responsible for the changes induced by *E. coli.* It is known that endotoxin is a potent inducer of LEM release (22). From this study, it cannot be determined if LEM directly interacted with muscles to decrease rates of protein synthesis or if the effects are induced by a hormone or other mediator which is released as a result of LEM. Baracos *et al.* (23) have demonstrated that leukocytic pyrogen (LEM) stimulates protein degradation but does not influence rates of protein synthesis in muscles incubated *in vitro.*

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