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Changes in Plasma, Tissue, and Urinary Nitrogen Metabolites Due to an Inflammatory Challenge (41871)

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Abstract. Studies were undertaken to define the changes in protein metabolism that result from stimulation of the immune system by noninfectious inflammatory agents. Chicks were injected with inflammatory agents and metabolite concentrations were determined between 4 and 48 hr postchallenge. Inflammatory agents resulted in a generalized decrease in the concentration of plasma nitrogen metabolites, including ammonia, uric acid, urea, and several amino acids. *Escherichia coli* and sheep red blood cell (SRBC) injections induced changes in the concentrations of tissue-free amino acids at 16 hr postchallenge. After *E. coli* injections, free amino acid concentrations were increased by 175% in muscle and decreased by approximately 25% in liver, spleen, and bursa. A SRBC challenge resulted in similar decreases in free amino acid concentrations in the spleen and bursa as did *E. coli;* however, muscle and liver free amino acid concentrations were mostly unchanged. Urinary ammonia was increased, urinary uric acid was decreased, and urinary amino acids were not affected by *E. coli* injection. These findings indicate that stimulation of the immune system by noninfectious inflammatory agents induces tissue-specific changes in nitrogen metabolism. Changes in amino acid pool sizes in various tissues suggest alterations in rates of protein synthesis or degradation.

The immune system is dependent upon protein synthesis and, therefore, amino acids for the turnover of existing proteins and the synthesis of new proteins involved in an immune response. Nucleotides are required for the RNA and DNA synthesis which accompanies cell division. The nitrogen requirement for an optimal immune response and the homeostatic mechanisms which come into play to meet these demands have received little attention.

It is possible that changes in protein metabolism, which occur when the immune system is stimulated, are homeostatic in nature and serve to meet the new demands of the cells involved in the immune response. Infection-induced changes in the protein metabolism of muscle and liver have been examined and suggest that sizable changes occur. For example, infection results in decreased protein synthesis in muscle (1) and an increase in the utilization of amino acids in liver for synthesis of acute phase plasma globulins (2, 3). In studies which have examined infection, the metabolic changes due to stimulation of the immune system are confounded by changes due to the pathological invasiveness of the infectious organism and its toxins. The present study was undertaken to define the changes in protein metabolism due to stimulation of the immune system using noninfectious inflammatory agents.

Materials and Methods. *Animals.* Male Single-Comb White Leghorn chicks of the Cornell K strain were used in all experiments. Chicks were raised in environmentally controlled battery brooders and exposed to 15 hr of light daily from 0700 to 2200 hr. Chicks were fed a commercial chick starter (Beacon Milling Co., Cayuga, N.Y.) until they were used for experimentation at 600–900 g. Chicks were allowed to adjust to their cages and cage mates for at least 48 hr before experiments were initiated.

Inflammatory agents. Sheep red blood cells (SRBC) and *Escherichia coli* 263 (08:K87, 88ab, H19) were washed three times with and then suspended in phosphate-buffered saline (PBS). Keyhole limpet hemocyanin (KLH) was obtained from Sigma Chemical Company (St. Louis, Mo.). Inflammatory agents were prepared to give a 1% suspension of SRBC or *E. coli* and a 0.05% suspension of KLH in PBS. Chicks were injected ip with 1 ml of

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either inflammatory agent or PBS (control) at a time which would allow tissue sampling to begin at 1000 hr. In experiments which included secondary injections, the priming dose was given 10 days prior to the experiment. Except in the first experiment, chicks were fasted or pair-fed the amount of feed consumed by the *E. coli*-treated group after injection.

Tissue collection. Blood was collected by heart puncture. Proteins were precipitated from plasma by adding 0.5 ml of 6% sulfosalicylic acid per milliliter plasma. The deproteinized plasma was frozen until analysis. After bleeding, chicks were killed by cervical dislocation. Tissues were immediately removed and frozen. Before analysis, the tissues were thawed, and the thymus, bursa, and spleen were placed in 5 ml cold PBS. The capsule and connective tissue stroma were removed by teasing with a scalpel and forceps. The cellular mixtures were then filtered through a 200-mesh nylon screen into cold centrifuge tubes. Red blood cells were removed from the spleen by the method of Rao and Glick (4). Pectoralis muscle and liver samples were scissor-minced. One-gram tissue samples were homogenized in 3 ml PBS utilizing a tightly fitting Potter–Elvehjem tissue homogenizer submerged in ice. Proteins were precipitated from the homogenate with 3 ml of 8% sulfosalicylic acid. Deproteinized tissue and plasma samples from two birds per treatment were pooled for amino acid analysis, resulting in five pooled samples per treatment per tissue.

Urine collection. The collection of urine separate from feces was accomplished by exteriorizing the section of the cloaca in which the ureters terminate, utilizing blunt forceps. A suture (size: OO) was inserted into the cloacal epithelium immediately distal to the ureters. The ends of the suture were threaded through a 1-ml disposable pipet tip. The pipet tip was pulled snugly against the cloaca and acted as a funnel to collect the urine as it was excreted. Two 1.5-ml samples were collected from each bird; one sample was collected into an empty centrifuge tube and analyzed for urea and uric acid, and one sample was collected into a centrifuge tube containing 0.1 ml of 0.1 *N* HCl and analyzed for ammonia.

Metabolite concentrations. An automated amino acid analyzer (Technicon Instruments, Tarrytown, N.Y.) was used to resolve amino acids in 50–200 μl of diluted deproteinized plasma, urine, or tissues. Urea was determined by the method of Skeggs (5). Plasma uric acid was determined by phosphotungstic acid reduction (6). Ammonia and urinary uric acid concentrations were determined enzymatically (Sigma, St. Louis, Mo.).

Sterility test. Whole blood was collected by heart puncture and peritoneal washings were collected after first injecting 20 ml sterile PBS into the peritoneal cavity. These fluids were spread onto nutrient agar plates. After 24 hr incubation at 37°C, bacterial colonies were counted.

Analysis of variance followed by Duncan's multiple-range test was used for data analysis.

Results. The injection of inflammatory agents resulted in a reduction of feed intake (Fig. 1). In the case of SRBC, this reduction was significant at 4 hr postinjection and returned to normal between 8 and 16 hr. *E. coli* injection resulted in a reduction in feed intake which was longer in duration and quantitatively greater than that due to SRBC. Feed intake was less than 20% of control levels at 4 hr and did not return to normal until 24 hr after *E. coli* injection. In this experiment, the control birds were sham-injected with PBS and otherwise handled in the same manner as treatment-injected birds. For this reason the depressed feed intake resulting from SRBC or *E. coli* injection cannot be attributed to the injection process or related stress.

The injection of inflammatory agents resulted in a generalized depression in the concentration of plasma nitrogen metabolites (Table I). The extent of change was dependent

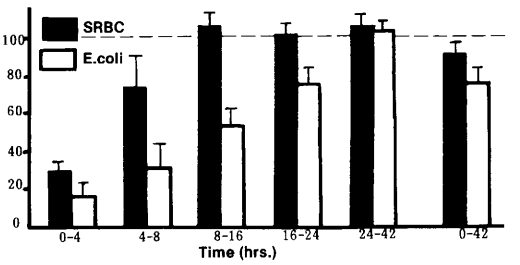

FIG. 1. The effect of antigenic challenge on feed consumption (% of controls fed *ad libitum*). Bars represent four replications of four birds ±SEM.

TABLE I. PLASMA UREA, URIC ACID, AND AMMONIA[a,b]

Metabolite	Treatment	Hours postinjection					
		4	8	12	24	36	48
Uric acid (mg/100 ml)	Control	5.70 ± 0.88	4.60 ± 0.51[c]	5.23 ± 0.62[c]	5.98 ± 0.61[c]	5.74 ± 0.58[c,d]	6.26 ± 0.86[c]
	SRBC 1	4.38 ± 0.54	5.23 ± 0.79[c]	4.20 ± 0.48[c,d]	3.23 ± 0.42[d]	7.60 ± 0.88[d]	6.63 ± 1.0[c]
	SRBC 2	6.00 ± 0.83	5.33 ± 0.42[c]	3.86 ± 0.37[d]	3.13 ± 0.23[d]	4.70 ± 0.67[c]	4.88 ± 0.61[c,d]
	E. coli 1	5.17 ± 0.98	3.07 ± 0.43[d]	6.22 ± 7.4[c]	3.81 ± 0.49[d]	6.93 ± 1.0[c,d]	3.38 ± 0.55[d]
	E. coli 2	5.57 ± 0.33	3.35 ± 0.41[d]	6.00 ± 1.2[c]	6.32 ± 1.0[c]	5.77 ± 0.41[c,d]	5.17 ± 0.92[c,d]
	KLH	5.95 ± 0.89	5.05 ± 0.91[c]	4.55 ± 0.34[c,d]	4.27 ± 0.11[c,d]	4.86 ± 0.73[c]	3.65 ± 0.77[d]
Urea (mg/100 ml)	Control	1.21 ± 0.16	1.25 ± 0.13[c]	1.37 ± 0.11[c,d]	1.22 ± 0.10[c]	1.30 ± 0.17	1.28 ± 0.10
	SRBC 1	0.833 ± 0.18	1.16 ± 0.23[c,d]	1.18 ± 0.18[c]	0.780 ± 0.07[d]	1.17 ± 0.19	1.21 ± 0.11
	SRBC 2	1.09 ± 0.16	1.23 ± 0.19[c]	1.34 ± 0.18[c,d]	1.28 ± 0.18[c]	1.52 ± 0.27	1.32 ± 0.16
	E. coli 1	1.29 ± 0.19	0.908 ± 0.06[d]	1.58 ± 0.09[c,d]	1.28 ± 0.15[c]	1.18 ± 0.10	1.24 ± 0.10
	E. coli 2	0.975 ± 0.11	0.675 ± 0.15[d]	1.72 ± 0.21[d]	1.16 ± 0.19[c,d]	1.23 ± 0.14	1.36 ± 0.12
	KLH	1.36 ± 0.24	1.37 ± 0.33[c]	1.60 ± 0.25[d]	1.17 ± 0.21[c,d]	1.16 ± 0.14	1.42 ± 0.17
Ammonia (μg/ml)	Control	2.88 ± 0.28[c,e]	0.837 ± 0.33[c]	2.27 ± 0.33[c]	3.68 ± 0.28[c]	2.59 ± 0.19[c]	1.43 ± 0.49[c]
	SRBC 1	1.21 ± 0.05[c,d]	0.720 ± 0.12[c]	1.86 ± 0.55[c,d]	1.05 ± 0.23[d]	0.881 ± 0.21[d]	0.300 ± 0.06[d]
	E. coli 1	0.638 ± 0.31[d]	0.301 ± 0.01[c]	1.99 ± 0.42[c]	1.11 ± 0.36[d]	0.567 ± 0.17[d]	0.339 ± 0.05[d]
	KLH	4.13 ± 0.37[e]	2.24 ± 0.26[d]	0.399 ± 0.10[d]	0.984 ± 0.24[d]	0.611 ± 0.15[d]	0.361 ± 0.09[d]

[a] Feed intake was equalized by pair feeding all chicks to the amount consumed by the chicks receiving *E. coli* 1 during each 4-hr period.

[b] Values represent means ± SEM of six chicks.

[c,d,e] Means in column within metabolites with different superscripts are significantly different ($P < 0.05$).

upon the stimulus, time period, and metabolite considered. Plasma ammonia concentrations were drastically affected by inflammatory agents. As compared to controls, a primary injection of either SRBC (SRBC 1) or *E. coli* (*E. coli* 1) resulted in depressed ammonia concentrations. These decreases were significant at 4, 24, 36, and 48 hr for *E. coli* 1 and at 24, 36, and 48 hr for SRBC 1; at each of these time periods, *E. coli* 1 and SRBC 1 injection resulted in plasma ammonia concentrations which were less than 40% of control values. KLH resulted in an initial increase (8 hr) followed by a significant decrease in ammonia concentrations at 12, 24, 36, and 48 hr.

Uric acid concentrations were depressed maximally to 54 and 52% of controls at 24 hr for SRBC 1 and a secondary injection of SRBC (SRBC 2), respectively, and to 54% at 48 hr and 73% of controls at 8 hr for *E. coli* 1 and a secondary injection of *E. coli* (*E. coli* 2), respectively. KLH resulted in a significant decrease in uric acid (58% of control) 48 hr postinjection.

Changes in plasma urea were variable; however, urea concentrations were significantly decreased from control values at 8 hr due to *E. coli* 1 and *E. coli* 2 and at 24 hr due to SRBC 1.

Glu and Gln concentrations were also affected by inflammatory agents (Table II). SRBC 2 decreased Glu at 12 and 24 hr postinjection while SRBC 1 significantly decreased Glu only at 36 hr. A similar pattern was observed after *E. coli* injection; *E. coli* 2 decreased Glu at 12 and 24 hr, whereas *E. coli* 1 did not result in decreased values until 24 hr postinjection. All five antigen treatments decreased Gln concentrations at 24 hr, relative to control values.

It is known that plasma Zn concentrations are decreased by infection (7). In this experiment all four treatments significantly depressed ($P < 0.05$) Zn concentrations as compared to controls (data not shown).

The *in vivo* viability of the *E. coli* used to challenge the chicks was also determined in this experiment. At 4, 8, and 12 hr postinjection, whole blood and peritoneal washings were tested for sterility. *E. coli* 1- and *E. coli* 2-injected birds as well as control birds had no detectable bacterial populations in their body fluids, demonstrating the lack of viability

TABLE II. PLASMA Glu AND Gln CONCENTRATIONS[a,b]

	Hours postinjection					
	12		24		36	
Treatment	Glu	Gln	Glu	Gln	Glu	Gln
Control	31.0 ± 1.2[c]	56.6 ± 8.6	37.2 ± 2.0[c]	40.7 ± 2.0[c]	34.2 ± 1.5[c]	55.6 ± 4.1[c]
SRBC 1	32.1 ± 2.2[c]	48.7 ± 5.9	30.6 ± 2.1[c,d]	30.1 ± 1.7[d]	21.6 ± 1.3[d]	35.1 ± 2.0[d]
SRBC 2	26.2 ± 1.4[d]	38.1 ± 7.9	25.1 ± 1.2[d]	32.7 ± 1.5[d]	29.7 ± 2.6[c]	49.5 ± 7.3[c]
E. coli 1	30.6 ± 2.7[c,d]	37.3 ± 4.6	25.3 ± 1.5[d]	27.6 ± 1.8[d]	30.3 ± 2.1[c]	47.5 ± 5.1[c]
E. coli 2	26.2 ± 1.1[d]	40.1 ± 7.2	27.4 ± 1.1[d]	25.0 ± 2.2[d]	29.7 ± 1.9[c]	63.5 ± 2.9[c]
KLH	36.4 ± 2.4[c]	35.2 ± 5.5	29.1 ± 2.2[c,d]	31.1 ± 1.6[d]	34.6 ± 3.2[c]	39.0 ± 4.7[c]

[a] Feed intake was equalized by pair feeding all chicks to the amount consumed by the chicks receiving *E. coli* during each 4-hr period.

[b] Values are expressed in μmol/dl and are the mean ± SEM of six chicks.

[c,d] Means in a colum with different superscripts are significantly different ($P < 0.05$).

of the injected *E. coli* and the lack of contamination due to the injection procedure.

The effect of SRBC and *E. coli* on free amino acid concentrations 16 hr postinjection are summarized in Tables III and IV. *E. coli* injection resulted in decreased concentrations of many of the nonessential amino acids; Asp, Ser, Glu, Gln, and Tyr were significantly decreased in concentration as compared to values from PBS-injected controls. Of the essential amino acids, Met was decreased while the branched chain amino acids, Ile, Leu, and Val, as well as Phe were elevated as a result of *E. coli* injection. SRBC injection resulted in decreased Glu, Gln, and Tyr and increased Met concentrations as compared to controls.

Changes in muscle-free amino acids did not reflect changes in plasma. *E. coli* injection resulted in a substantial increase in the concentration of most of the free amino acids in the muscle. This represents an increase in total amino acid concentrations of 175% over control values. SRBC injection caused an increase in only ASP concentrations; no other amino acid was affected significantly.

The concentrations of many of the free amino acids in the liver were decreased due to *E. coli* injection, whereas SRBC injection did not result in any significant changes. *E. coli* injection resulted in a 24% decrease in the total concentration of free amino acids as compared to controls.

The concentration of free amino acids in the tissues involved in the immune response were also affected by the injection of inflammatory agents. Both SRBC and *E. coli* exerted their largest effect on the bursa. SRBC and *E. coli* injection resulted in a significant depression in the concentrations of 10 and 7 amino acids, respectively. In this tissue, none of the amino acids were increased in concentration 16 hr after the injection of either SRBC or *E. coli*. Both treatments resulted in an approximately 25% reduction in total free amino acids in the bursa.

The results observed in the spleen were similar to those in the bursa. Six and seven amino acids were decreased in concentration as a result of *E. coli* and SRBC, respectively. Again, both treatments resulted in an approximately 25% depression in total free amino acids when compared to controls.

The thymus responded to the injection of inflammatory agents differently than the bursa and spleen. In this tissue, several amino acids were increased in concentration due to *E. coli* or SRBC injection. No amino acid was significantly decreased. Total free amino acid concentrations were not significantly elevated by either treatment; however, they tended to be increased by about 12% over control values.

The urinary concentrations of ammonia, urea, and uric acid are shown in Table V. *E. coli* injection resulted in increased urinary ammonia concentrations as compared to controls. This increase was statistically significant ($P < 0.05$) at 12 hr. Urinary urea concentra-

TABLE III. FREE AMINO ACID CONCENTRATIONS[a]

Tissue	Treatment	Amino acid																	
		Asp	Thr	Ser	Glu	Gln	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Total
Plasma[b]	Control	5.7	43.5	78.0	30.8	26.2	34.4	50.9	54.1	22.0	9.5	17.3	23.0	23.5	12.1	47.7	9.5	36.2	524
	SRBC	5.5	43.0	79.2	23.3*	17.2*	42.9	46.2	47.1	28.6	6.0*	17.1	28.5	17.0*	15.1	34.3	11.1	29.0	491
	E. coli	3.7*	43.8	47.2*	21.4*	13.4*	27.3	38.1	36.6	47.3*	5.4*	30.1*	44.9*	11.4*	20.0*	37.9	9.6	29.9	468
	SEM	0.5	2.7	6.3	1.5	1.3	7.4	4.5	5.6	4.6	0.8	2.8	3.2	0.8	1.3	3.7	1.6	1.2	16.7
Muscle[c]	Control	48.3	32.1	55.0	66.5	42.4	17.5	56.0	64.7	24.8	10.5	20.9	25.5	23.8	15.0	17.9	10.8	16.8	549
	SRBC	64.2*	33.3	66.1	57.1	44.4	20.1	43.5	63.1	24.3	7.62	15.3	24.1	18.8	11.7	20.4	8.92	17.2	550
	E. coli	41.5	61.8*	80.6*	73.8*	96.4*	30.1*	122**	110**	58.2**	20.7**	42.2**	58.4**	29.2	28.6*	48.2**	26.4**	32.0**	960*
	SEM	3.1	2.3	2.6	2.6	8.4	2.8	6.0	5.7	1.7	1.0	1.5	1.9	2.2	1.4	2.6	1.2	1.8	30.3
Liver[c]	Control	65.6	34.7	58.7	86.3	31.6	26.3	73.1	64.3	22.2	14.3	16.1	35.3	19.4	12.0	34.9	14.6	31.5	640
	SRBC	72.3	38.7	56.8	91.4	40.9	28.6	76.9	71.1	26.2	16.9	19.4	39.3	20.2	14.5	37.9	16.6	36.7	704
	E. coli	54.4	26.0*	40.1	89.7	23.8*	21.3	50.6*	47.6	17.9	9.32	15.1	23.7*	12.3*	7.69*	21.3*	11.4	19.9*	491*
	SEM	5.6	2.0	5.4	4.7	2.1	2.1	5.4	6.2	1.9	1.4	2.0	2.8	1.3	1.0	3.0	4.4	2.3	26.7

[a] Chicks were fasted postinjection and killed after 16 hr. [b] Values expressed as μmol/100 ml. [c] Values expressed as μmol/10 g tissue.
* Significantly different from controls ($P < 0.05$). ** Significantly different from controls ($P < 0.01$).

TABLE IV. FREE AMINO ACID CONCENTRATIONS[a]

Tissue	Treatment	Amino acid																
		Asp	Thr	Ser	Glu	Gln	Gly	Ala	Val	Met	Ile	Leu	Try	Phe	Lys	His	Arg	Total
Thymus	Control	50.9	13.7	22.6	90.4	10.3	47.2	24.2	7.22	4.63	5.07	11.0	4.66	4.43	6.69	2.76	5.64	311
	SRBC	62.8*	16.4*	24.6	99.0	14.4	48.9	29.1*	7.22	4.91	5.62	12.1	5.06	4.97	6.42	4.48	6.41	352
	E. coli	59.7	15.9	21.5	95.6	11.6	48.9	25.9	9.44	6.96**	6.07	12.6	5.13	5.82*	9.41*	3.56	8.00*	346
	SEM	3.2	0.9	1.1	4.9	2.3	1.9	1.0	1.0	0.4	0.4	0.5	0.2	0.3	0.5	0.8	0.5	14.7
Bursa	Control	32.9	14.3	28.2	89.4	15.6	68.0	30.1	10.9	6.38	6.96	14.2	6.89	6.32	10.8	4.29	10.9	377
	SRBC	26.2*	11.5	21.9*	81.2	7.81*	56.3*	21.4	8.27	4.27*	5.57	9.58*	4.24	4.04*	7.16*	3.12*	6.52*	279*
	E. coli	22.9**	13.7	20.6*	85.4	11.0	54.1*	24.4	10.7	4.44*	4.98*	10.8	4.23	4.68	7.55*	2.90*	7.26	290*
	SEM	0.8	0.8	1.2	4.8	1.7	2.4	2.7	1.0	0.5	0.4	0.9	0.7	0.6	0.5	0.2	0.7	12.0
Spleen	Control	61.2	22.6	36.9	107.7	26.0	71.9	37.5	19.3	11.8	9.81	20.0	10.5	8.22	27.2	6.01	16.6	493
	SRBC	41.0*	20.6	30.2	88.4	12.6**	62.6	24.2*	15.0	11.8	5.67*	15.1	5.11*	4.79*	20.1	6.44	12.8*	376
	E. coli	30.2*	21.8	27.9	93.5	20.0	43.8*	29.1	11.6*	10.1	5.25*	14.8	5.99*	6.79	21.3	7.30	11.7*	361*
	SEM	2.9	2.0	3.1	7.4	1.8	4.3	2.6	1.1	1.2	0.4	1.4	0.5	0.6	1.7	0.5	1.0	25.3

[a] Values expressed as μmole/10 g. Chicks were fasted postinjection and killed after 16 hr.
* Significantly different from controls ($P < 0.05$). ** Significantly different from controls ($P < 0.01$).

TABLE V. URINARY NITROGEN[a]

Hours postinjection	Treatment	Ammonia (μg/ml)	Urea (mg/100 ml)	Uric acid (mg/ml)	Total amino acids[b] μmole/ml
12	Control	102 ± 14[c,d]	14.2± 1.4	7.11 ± 0.58	0.514
	SRBC	138 ± 9.1[d]	14.6 ± 0.51	7.16 ± 0.26	0.501
	E. coli	184 ± 13[e]	13.6 ± 1.1	5.74 ± 0.23	0.513
24	Control	85.3 ± 13	10.7 ± 1.2	5.85 ± 0.26[d]	0.477
	SRBC	98.0 ± 13	11.2 ± 0.91	5.11 ± 1.07[d]	0.508
	E. coli	113 ± 22	8.0 ± 1.1	3.06 ± 0.59[e]	0.461

[a] Chicks were fasted after injection.
[b] From analysis of a sample pooled from all four chicks in each treatment. Individual amino acid concentrations were similar among treatments.
[c] Means ± SEM of four chicks.
[d,e] Means in a column within the same time period followed by different superscripts are significantly different ($P< 0.05$).

tions were not significantly affected at either 12 or 24 hr postinjection. Urinary uric acid concentrations tended to be depressed at 12 hr and were significantly decreased at 24 hr postinjection by *E. coli* but not SRBC. Total amino acid concentrations in the urine were not affected by either SRBC or *E. coli* injection.

Discussion. Inflammatory agents alter feed intake and many aspects of nitrogen metabolism, the changes occurring relatively quickly after injection. The extent of change is largely influenced by the type of stimulus. *E. coli* resulted in larger perturbations in most parameters than the nonmicrobial agents SRBC and KLH.

It is well documented that infection results in depressed appetite and consequently depressed feed intake (8). Berry and Smythe (9) observed a depression in feed intake after the injection of either viable or heat-killed *S. typhimurium*. Their work demonstrates that the development of an infectious process is not an obligatory prerequisite for a reduction in feed intake. The present studies demonstrate that a noninfectious and rapidly cleared strain of *E. coli* also results in reduced feed intake; in addition, SRBC, which contains no toxins but are rapidly phagocytized, depress feed intake. It is apparent that feed intake must be equalized between treatments so that effects on metabolism resulting from the inflammatory agents are not confounded by diet. This was accomplished in the present studies by withholding feed or pair feeding after injection.

The presence of foreign organisms during infection stimulates the immune system. Consequently, studies investigating changes in nitrogen metabolism during infection may be compared to this present study. In making these comparisons, several factors should be considered. First, measurements of nitrogen metabolism during infection are typically made several days after the organism is introduced. In this present study observations were made closer to the time of challenge. Second, pathological changes resulting from the infectious organism may induce alterations in nitrogen metabolism which are unrelated to those changes induced by the immune response to those organisms. Third, this study utilizes chickens which differ from mammals in several important aspects of nitrogen metabolism, including uric acid excretion, lack of a functional urea cycle, and tissue distributions of amino acid catabolizing enzymes.

Studies of changes in nitrogen metabolism due to noninfectious bacteria or nonmicrobial antigens have not been reported.

Infection in humans and experimental animals results in a negative nitrogen balance as evidenced by increased nitrogen excretion as urea ammonia, creatine, and uric acid (10, 11). This increase begins about 4 days postinfection. In experiments reported here, urinary nitrogen concentrations were reduced during the first day after *E. coli* injection; uric

acid concentrations in particular were depressed. The increase in ammonia concentration due to *E. coli* (64 and 22 μg N/ml urine at 12 and 24 hr, respectively) is a relatively small change compared to the decrease in nitrogen concentrations as uric acid (375 and 764 μg N/ml at 12 and 24 hr, respectively). Some of the decrease in urinary uric acid concentrations may be a result of increased excretion of nitrogen as ammonia. Most of the decrease in urinary and plasma uric acid concentrations may represent an increase in utilization of nitrogen for synthesis of nucleic acids and/or a decrease in nitrogen production by amino acid catabolism.

The increase in urinary ammonia together with decreased plasma glutamine may be indicative of increased renal glutaminase activity. Since plasma ammonia levels are also decreased, ammonia secretion into the kidney tubules also may be increased. Regardless of the mechanism, the increased urinary ammonia is indicative of acidification of the urine. It is not clear if this is due to an *E. coli*-induced acidosis or if the increased ammonia excretion reflects a renal alkalosis.

Due to the lack of a functioning urea cycle, arginine is not synthesized in the chick (12). Urea is a reflection of arginase activity in kidney. These experiments indicate that arginine degradation was unaffected by treatment.

Plasma nonessential amino acid concentrations represent a balance between the influx of amino acids into plasma due to dietary intake and amino acid synthesis in the tissues, and the loss of amino acids from plasma due to catabolism, excretion, or use for protein synthesis. In the present experiments, dietary intake was equalized between treatments, and urinary amino acid concentrations were not increased. Therefore, the changes in plasma nonessential amino acids may be due to a relative increase in the rate of deamination versus amino acid synthesis in the tissues or to an increased utilization for nucleic acid or protein synthesis. A net increase in protein synthesis is unlikely because it would result in a depression in the essential amino acids as well. Essential amino acids are not synthesized *in vivo,* so their plasma concentrations must reflect the balance between whole body protein synthesis and catabolism. Since the plasma concentrations of most of the essential amino acids were unchanged, except the branched chain amino acids which were increased, it is doubtful that the antigen-challenged chicks were in a net protein anabolic state. The depressed nonessential amino acid concentrations may be a result of deamination coupled with increased shunting of nitrogen into another pool. The fate of the nitrogen cannot be determined from these data but may consist of nucleotide synthesis and ammonia excretion.

The change in concentrations of amino acids in various tissues in response to an inflammatory challenge may explain some of the changes in plasma. Changes in tissue free-amino acid concentrations reflect changes in transport, amino acid catabolism, amino acid synthesis, and the relative rates of protein synthesis and protein catabolism. In this latter case, an increase in tissue free-amino acid concentrations would represent net protein catabolism or amino acid liberation, and a decrease would represent net protein synthesis or amino acid utilization. This is particularly true for those amino acids which are neither synthesized nor catabolized in the tissue being considered, i.e., phenylalanine in muscle or arginine in liver. Based on this interpretation of tissue amino acid concentrations and assuming that variations in amino acids are not mediated by transport phenomenon, *E. coli* resulted in a net liberation of amino acids in the muscle and possibly the thymus and a net utilization by the spleen, liver, and bursa. SRBC resulted in similar effects in the spleen and bursa but little, if any, change in the muscle and liver.

The increased plasma branched chain amino acids after *E. coli* injection may be due to release of these amino acids from muscle at a faster rate than they are used for protein synthesis in the liver, bursa, and spleen.

It should be emphasized that assigning metabolic significance to static measurements of amino acid concentrations or pool sizes is difficult because of the complexity of factors and magnitude of the fluxes involved.

Squibb (13) has reported a depression in liver lysine, histidine, arginine, aspartic acid, alanine, valine, and leucine concentrations in the chick liver due to the Newcastle disease

virus. This was attributed to increased protein synthesis as demonstrated by increased total liver RNA and protein. Depressions of plasma amino acids occur in experimental infection of humans, regardless of etiology (14). This depression occurs on the first day after infection before the onset of clinical signs such as fever or anorexia.

In the experiments reported here, it is not clear whether the alterations in nitrogen metabolism are due to a specific immune response. The antibody titer of chicks to *E. coli* 263 should be high due to cross-reactivity with the normal indiginous microbial flora. Chicks, however, have very low, if any, antibody titers to SRBC or KLH at the time of their first exposure. It was demonstrated that prior exposure to antigens did not increase the perturbations in nitrogen metabolism. This indicates that nonspecific factors mediate these changes. Phagocytosis by macrophages and polymorphonuclear leukocytes is a nonspecific immune response to particulate antigens such as those utilized in this study. These phagocytic cells may elaborate a substance which induces the wide-ranging changes in nitrogen metabolism. Leukocyte endogenous mediator (LEM), one such substance, has been shown to induce a change in the transport of nonmetabolizable amino acids in the liver and muscle (15). LEM, also referred to as interleukin 1, stimulates muscle protein degradation (16).

E. coli resulted in quantitatively greater perturbations in most of the parameters measured than did the nonmicrobial agents, SRBC and KLH. This may be due to several factors. First, the onset of metabolic changes occurs later after KLH or SRBC as compared to *E. coli* injection. This is particularly true for Zn, ammonia, and uric acid. The lower response of plasma and tissue amino acids to SRBC as compared to *E. coli* may be due to the relatively early time periods during which measurements were taken.

After injection, both SRBC and *E. coli* would be eliminated by phagocytosis; however, microbial antigens, particularly *E. coli* endotoxin (present in strain 263), potentiate the phagocytic process and initiate other immune responses. These include an increase in neutrophil and macrophage chemotaxis to the site of injection, macrophage production of macrophage colony-stimulating factor, lymphocyte-stimulating factor, and endogenous pyrogen and the stimulation of polyclonal B-lymphocyte proliferation (17). One of these mechanisms may be responsible for the quicker onset and quantitatively greater change in nitrogen metabolism induced by *E. coli* as compared to SRBC.

This study demonstrates that stimulation of the immune system by noninfectious inflammatory agents induces tissue-specific changes in nitrogen metabolism. There is a change in the amino acid pool size of various tissues, suggesting an alteration in protein synthesis or degradation. It is possible that some of these changes represent a homeostatic mechanism responsible for the shunting of amino acids between tissues to support an immune response.

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