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Effects of Chronic and Acute Infections on Tissue Levels of Carnosine, Anserine, and Free Histidine in Rats and Chickens[1] (40995)

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Abstract. The tissue concentrations of the imidazole-containing compounds, anserine, carnosine, and free histidine, were determined in male chickens and rats suffering from acute or chronic infections. Either type of infection resulted in a decrease in tissue carnosine concentration. The tissue anserine concentration was unaltered by either the acute or the chronic infection. The tissue free-histidine concentration increased significantly during chronic infections, but remained unaffected during the acute infections. These results were consistent with the hypothesis that carnosine acts as a tissue reservoir for histidine, ultimately serving, when called upon by the stress of an infection, as a precursor of histamine.

Carnosine (β-alanyl-L-histidine) and anserine (β-alanyl-L-methylhistidine) are two of the most abundant compounds in the nonprotein nitrogen fraction of vertebrate muscle tissue (1). These histidine-containing dipeptides have been thought to be involved in a variety of physiological processes (2–4); however, no definite metabolic role has been ascribed to either compound. Nagai (5) recently reported that carnosine "treatment" enhanced tissue repair. Histological examination (6) correlated with increased development and faster maturation of granular tissue to carnosine treatment. Other evidence suggests that the histidine-containing dipeptides may act indirectly in the response to trauma (7), as a histidine reservoir (8) for the synthesis of histamine, for which an involvement in trauma and wound healing has been long documented (9).

Nagai (5) implicated carnosine as a physiological agent concerned with *in vivo* bacterial resistance. Therapy with omega amino acids and related compounds has been shown to reduce the mortality rate due to infection and, in some cases, prevent the infection (10, 11). Fujii (12) demonstrated antistaphylococcal and antifibrinolytic activities for the histidine-containing dipeptides.

The purpose of this study was to examine the relationship between infection and the tissue concentration of the histidine-containing dipeptides. Initial observations were made on cockerels (male chickens) that had developed spontaneous *Staphylococcus aureus* infection of the foot pad, and on rats suffering from a respiratory infection. Additional experiments were carried out under controlled conditions involving infection of rats with *Salmonella typhimurium* and of cockerels with *Mycobacterium avium*.

Materials and methods. Twenty-eight-week old adult, white Leghorn cockerels were fed, *ad libitum*, a purified diet adequate in all nutrients (13). During the course of the study, which is not of relevance here, a *Staphylococcus aureus* infection of the foot pad developed in several animals. Three weeks after the onset of the infection, four infected cockerels and six control birds were killed by decapitation. Blood was drawn just prior to killing for spectrophotometric hemoglobin determination using the cyanmethemoglobin method (13). Tissue was excised from the pectoralis major muscle, and from leg muscle (gastrocnemius, soleus, and tibialis complex); the cerebral cortex was also removed for analysis. Carnosine, anserine, and free-

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histidine concentrations were determined on the protein-free extracts of the tissue homogenates by amino acid analysis according to procedures previously described (14).

Ninety-day-old male, Sprague–Dawley rats were maintained on a similar, nutritionally complete, purified diet as the cockerels. During the course of the primary experiments, four animals were found to be suffering from a respiratory disorder. These animals and four control rats were killed and tissue was excised from the pectoralis major muscle and from leg muscle (gastrocnemius, soleus, and tibialis). The cerebral cortex was also excised and analyzed for the imidazole-containing compounds (14). Blood was also drawn prior to killing for the spectrophotometric determination of hemoglobin.

Day-old white Leghorn males were injected via the right jugular vein and the peritoneum with a 0.5-ml suspension of *Mycobacterium avium* (tuberculosis), strain Kirchberg. This suspension was a 1:10 dilution of a 10- to 14-day-old culture in Tween albumin medium, adjusted to an optical density of 0.50 at 550 nm. The presence of disease was confirmed by noting an increase in liver size, a target organ for this organism. The chicks were fed a standard chick diet *ad libitum* (445 g/chick/21 days) and were killed 21 days postinoculation. Tissue from the pectoralis major muscle was excised and analyzed for free histidine as well as for anserine and carnosine (14).

Twenty-one-day old male, Sprague–Dawley rats were maintained on a standard laboratory diet. During the course of the experimental period the infected and the control animals were pair-fed 80% of their normal, *ad libitum* intake (132 g/rat/9 days). Rats were inoculated intraperitoneally with 0.2 ml of a suspension containing 1×10^7 *Salmonella typhimurium* organisms. The infection was confirmed by noting an increase in spleen size, a target organ for this organism. Groups of rats were killed 1 and 9 days postinoculation and tissue from the leg muscle was analyzed for imidazole-containing amino acids and dipeptides (14).

Results. Gross manifestations of the chronic staphylococcal infection in cockerels and the respiratory-infected rats were similar. In both cases there was a significant decrease in food consumption, a weight loss, and a significant reduction in blood hemoglobin concentration (Table I).

The chronic staphylococcally infected cockerels and the rats suffering from respiratory disease also showed a similar pattern of tissue concentrations for histidine and the dipeptides, anserine and carnosine (Table II). Pectoral muscle, leg muscle, and brain of infected cockerels and rats showed a highly significant decrease in carnosine concentrations, a significant increase in tissue free-histidine concentration, and no change in anserine concentration. In the pectoral muscle of staphylococcus-infected cockerels the carnosine concentration had decreased 85% below that found in healthy,

TABLE I. FOOD INTAKE, BODY WEIGHT CHANGE, AND BLOOD HEMOGLOBIN CONCENTRATION OF RATS WITH A RESPIRATORY INFECTION AND OF COCKERELS WITH A *Staphylococcus aureus* INFECTION

State of infection	Food intake (g/animal/week)	Body weight change[a] (g)	Blood hemoglobin (g/100 ml)
Cockerels			
Noninfected	539 ± 63[b,d]	139 ± 9[d]	14.1 ± 0.4[d]
Infected	336 ± 63[d]	−396 ± 29[d]	13.0 ± 0.55[d]
Rats			
Noninfected	87 ± 6[c,d]	−20[d]	14.9 ± 0.7[d]
Infected	52 ± 9[d]	−79[d]	11.1 ± 0.5[d]

[a] Between time of discovery of the infection and the killing of the animals.
[b] Mean ± SE for six controls (noninfected) and four infected cockerels.
[c] Mean ± SE for four controls and four infected rats.
[d] Difference between infected and noninfected values for the same measurement is significant at $P < 0.01$. (Student's t test).

TABLE II. ANSERINE, CARNOSINE, AND HISTIDINE CONCENTRATIONS IN PECTORAL MUSCLE, LEG MUSCLE, AND BRAIN OF RATS WITH A RESPIRATORY INFECTION, AND OF COCKERELS WITH A *Staphylococcus aureus* INFECTION

Tissue	State of infection	Anserine	Carnosine	Free histidine
		(μmole/g wet tissue)		
Cockerels				
Pectoral muscle	Noninfected	41.45 ± 1.23[a]	11.60 ± 1.03[b]	0.11 ± 0.05[b]
Pectoral muscle	Infected	42.46 ± 1.42	1.83 ± 0.39[b]	0.42 ± 0.11[b]
Leg muscle	Noninfected	11.66 ± 0.93	2.73 ± 0.73[b]	0.08 ± 0.03[b]
Leg muscle	Infected	12.19 ± 1.03	0.36 ± 0.11[b]	0.34 ± 0.07[b]
Brain	Noninfected	0.40 ± 0.11	0.03 ± 0.008[b]	0.13 ± 0.03[b]
Brain	Infected	0.57 ± 0.14	0.002 ± 0.001[b]	0.26 ± 0.05[b]
Rats				
Pectoral muscle	Noninfected	4.96 ± 0.19[c]	1.57 ± 0.29[b]	0.13 ± 0.03[b]
Pectoral muscle	Infected	4.98 ± 0.35	0.06 ± 0.02[b]	1.04 ± 0.11[b]
Leg muscle	Noninfected	5.03 ± 0.25	1.54 ± 0.23[b]	0.11 ± 0.02[b]
Leg muscle	Infected	4.86 ± 0.30	0.07 ± 0.02[b]	1.88 ± 0.19[b]
Brain	Noninfected	n.d.[d]	0.04 ± 0.009[b]	0.13 ± 0.03[b]
Brain	Infected	n.d.	0.001 ± 0.0005[b]	0.30 ± 0.06[b]

[a] Mean ± SE for six controls (noninfected) and four infected cockerels.
[b] Difference between infected and noninfected values for the same tissue is significant at $P < 0.01$. (Student's t test).
[c] Mean ± SE for four controls and four infected rats.
[d] None detected.

control birds. This finding was contrasted by a 400% increase in free histidine. In the leg muscle of the respiratory-infected rats the carnosine concentration had decreased 95% below that of the healthy control animals, while tissue anserine remained unaltered, and tissue free-histidine concentrations increased 10-fold.

Changes in tissue free-histidine and dipeptide concentrations during the acute infections with *Mycobacterium avium* and *S. Typhimurium* were less pronounced than those observed during the naturally occurring chronic infections (Table III). The general pattern during acute infection was similar, however. Infection was accompanied

TABLE III. ANSERINE, CARNOSINE, AND HISTIDINE CONCENTRATIONS IN PECTORAL MUSCLE OF YOUNG COCKERELS INFECTED WITH *Mycobacterium avium* (TUBERCULOSIS) AND IN LEG MUSCLE OF YOUNG RATS INFECTED WITH *Salmonella typhimurium*

Tissue	State of infection	Anserine	Carnosine	Free histidine
		(μmole/g wet tissue)		
Cockerels 21 days after inoculation				
Pectoral muscle	Noninfected	15.99 ± 1.24[a]	14.7 ± 1.38[b]	0.15 ± 0.07
Pectoral muscle	Infected	17.29 ± 1.37	8.60 ± 1.97[b]	0.13 ± 0.08
Rats 1 day after inoculation				
Leg muscle	Noninfected	2.64 ± 0.28	7.05 ± 0.62	0.64 ± 0.07
Leg muscle	Infected	2.97 ± 0.92	6.75 ± 0.96	0.79 ± 0.21
Rats 9 days after inoculation				
Leg muscle	Noninfected	1.24 ± 0.23	3.99 ± 0.41[b]	1.21 ± 0.17
Leg muscle	Infected	0.99 ± 0.09	2.57 ± 0.38[b]	1.15 ± 0.24

[a] Mean ± SE for seven cockerels or rats per treatment.
[b] Difference between infected and noninfected values for the same tissue is significant at $P < 0.01$. (Student's t test).

by a reduction of tissue carnosine concentration, while the tissue concentrations of both anserine and of free histidine did not change. Tuberculous infection of the young cockerels was accompanied by a significant, 45%, decrease in pectoral muscle carnosine concentration while anserine and free-histidine concentrations remained unchanged.

There was no effect of infection noted in rats 1 day postinoculation with *S. typhimurium*. However, it is interesting to note that the variability (standard error) associated with the values for the animals killed 1 day postinfection was much greater than the variability associated with any of the other observations. This may well indicate differences in the initial response of individual animals to the infection. With this level of *S. typhimurium* inoculation there are no visible signs of the infection 24 hr postinfection; such symptoms usually are first observed only about the sixth day.

Nine days after inoculation with *S. typhimurium*, rats showed a 25% decrease in carnosine while both anserine and free-histidine concentrations remained unaltered.

Discussion. The results from this study strongly suggest an involvement of carnosine in infection. In both rats and cockerels infection manifested itself in a decrease in tissue carnosine concentration. In the case of the chronic staphylococcus or respiratory infections, one might wish to invoke reduced food intake as the cause for the lowered carnosine concentrations. However, inanition does not bring about reductions in carnosine (15) and this was clearly also not the case for the animals acutely infected with *S. typhimurium;* the infected and control animals in this instance were pair-fed.

The diminution of carnosine could well be related to the generalized stress phenomenon in which muscle metabolism has been altered as reported by Cuthbertson (16). The carnosine decrease might also be a specific response. Ousterhout (8) reported that chicks could survive longer on a histidine-deficient diet than on a diet devoid of any other essential amino acid because of their ability to mobilize a histidine reserve. During injury or infection the histamine-forming capacity of tissue increases and mast cells rich in histamine are mobilized to the site of injury (9). Thus, there is the distinct possibility that carnosine has no novel physiological role, but may act as a histidine reservoir for histamine synthesis via decarboxylation (7).

Blood hemoglobin concentrations were significantly decreased during the chronic staphylococcus and respiratory infections. Hemoglobin is a protein rich in histidine (17). Thus, hemoglobin degradation, like that of carnosine, may act as a histidine reservoir, to be drawn upon during stress.

Tissue anserine concentrations were unaltered by infection. This confirms the results of previous experiments (13, 15), which have shown that tissue anserine concentration cannot be altered by dietary manipulation. However, following the stress of hind leg fracture, we have observed a decrease in anserine concentration of rat leg muscle of a magnitude similar to that observed for carnosine (7). Anserine would appear not to be an ideal storage form for histidine since there is no specific catabolic enzyme and its degradation results in the liberation of 1-methylhistidine (18). It has been reported that neither anserine nor 1-methylhistidine is demethylated to yield free histidine (1).

There was no apparent increase in tissue free-histidine concentration due to the acute infections in contrast to the larger increase in free histidine due to the chronic infections. Chronic infection resulted in a 90% reduction in tissue carnosine concentration, while the acute infections caused a much lower decreased in carnosine; thus, the net free-histidine accumulation due to the chronic infection may well be due to this greater carnosine mobilization.

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