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Alterations in Plasma Copper, Zinc, Amino Acids, and Seromucoid during Rocky Mountain Spotted Fever in Guinea Pigs[1] (39311)

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Alterations in plasma trace metal concentrations are associated with various bacterial and viral infections in man and experimental animals (1-4). Usually a decrease in plasma zinc precedes the onset of clinical signs and peak illness while a rise in plasma copper, indicative of increased ceruloplasmin synthesis/release normally occurs only during and following peak illness. In this regard Rocky Mountain spotted fever (RMSF) in guinea pigs appears unique in that the rise in plasma copper precedes the onset of clinical signs while plasma zinc depression follows the peak of fever and coincides with the highest titers of rickettsemia. This brief report also details an increase in plasma seromucoid concentration as well as a rise in the plasma phenylalanine/tyrosine ratio, indicating that alterations in host nitrogen metabolism similar to those changes observed during bacterial infections (2, 5) occur during this rickettsial disease.

Materials and methods. Twenty-four Hartley strain albino guinea pigs (250-300 g) were infected by intraperitoneal injection of 1 ml of a 10% suspension of infectious chicken egg yolk sac containing approximately 10^7 *Rickettsia rickettsii,* Sheila Smith strain. In a concurrent study, this inoculum caused a 30% mortality between 10 and 18 days. Twelve more guinea pigs serving as a control group were inoculated with a sterile 10% yolk sac suspension. Throughout the study animals were provided water and standard guinea pig chow *ad libitum.* Four infected and two control animals were sacrificed using halothane anesthesia at selected times. Rectal temperatures were taken just prior to sacrifice. Heparinized blood samples were obtained by intracardiac puncture. Blood samples were quickly frozen at −65°C for rickettsial isolations (6). Tetracycline (250 μg/10 ml blood) was then added prior to obtaining plasma and further laboratory analyses.

Plasma copper and zinc was measured by atomic absorption spectrophotometry (1-4). The seromucoid concentration of plasma was determined by a modification of the procedure of Neuhaus *et al.* (7). Total plasma protein was determined by refractometry using a TS meter (American Optical Co., Buffalo, N.Y.). Plasma amino acid concentration was determined on a Technicon amino acid analyzer (4).

For statistical analyses the data from infected animals were compared to the mean of all the control animals using Student's *t* test.

Results. Four infected and two control guinea pigs were sacrificed on Days 1, 2, 3, 5, 8, and 12 following inoculation with either virulent *R. rickettsii* or sterile yolk sac suspension. Within 2 days a signficant increase in rectal temperature was measurable in infected animals. Body temperature reached a peak on Day 3 and declined thereafter; by Day 12 there was no longer a significant difference between control and infected animals. Some guinea pigs had detectable rickettsemia by Day 1. Peak rickettsemia occurred on Day 5 and although there was some decrease in titer thereafter, all animals had rickettsiae in their blood on Days 8 and 12 (Fig. 1).

Despite the rise in body temperature on Day 2, indicative of overt illness, no significant alteration in plasma zinc concentration

[1] In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

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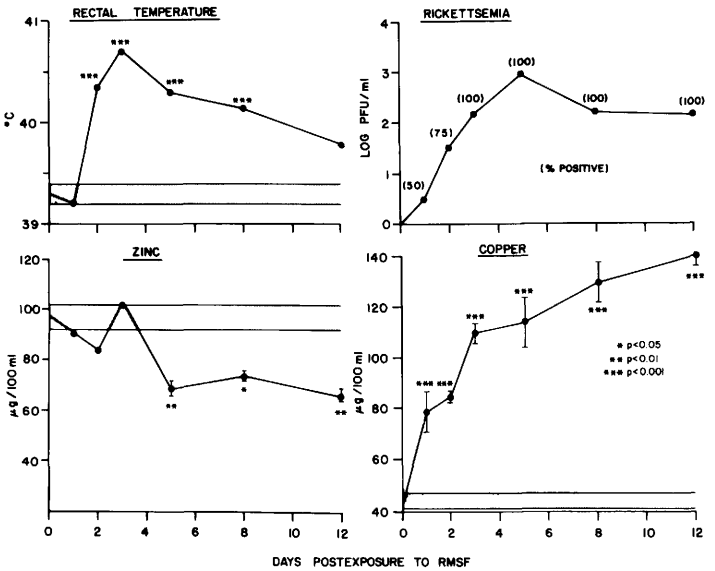

FIG. 1. Rectal temperature, rickettsemia, and plasma zinc and copper concentrations in guinea pigs during RMSF. The shaded bars represent the mean ± SEM of the values from the control animals (n = 12). Standard error bars are shown for experimental animals (n = 4) in each instance where a significant difference exists between control and experimental animals. PFU, plaque forming units.

TABLE I. PLASMA PROTEIN CONCENTRATION (MEAN ± SEM).

Group	Seromucoid (mg/100 ml)	Total protein (g/100 ml)
Controls (n = 12)	160 ± 16	4.24 ± 0.13
Infected (n = 4)		
Day 1	214 ± 35	4.25 ± 0.27
Day 2	353 ± 72[a]	4.28 ± 0.17
Day 3	263 ± 4[a]	4.48 ± 0.06
Day 5	373 ± 76[a]	3.93 ± 0.20
Day 8	390 ± 15[a]	4.05 ± 0.18
Day 12	410 ± 61[a]	4.18 ± 0.21

[a] $P < 0.005$ vs controls.

was noticeable until Day 5, when there was approximately a 25% decrease which persisted until Day 12. The initial decrease in plasma zinc coincided with peak rickettsemia. In contrast, plasma copper concentrations were almost doubled on Day 1, continued to rise throughout the illness, and were treble that of control values on Day 12 (Fig. 1).

A marked increase in plasma seromucoid concentration was observed by Day 2 which persisted until Day 12, but little or no change in total plasma protein concentration was detected (Table I). No significant alterations in total plasma amino acids occurred during this illness at the times studied. However, plasma phenylalanine concentration was increased on Days 3 and 5 which in the absence of a change in tyrosine concentration resulted in a significant increase in the phenylalanine/tyrosine ratio (P/T) at these times (Table II).

Discussion. The early rise in plasma copper was unanticipated; it is of interest since it may aid in elucidating the pathogenesis of this disease. Previous studies of bacterial and viral illnesses in man and experimental animals showed increased plasma copper concentration only during and following peak illness (1–4). An increase in plasma copper is thought to be indicative of increased ceruloplasmin synthesis and/or release (8). If the relationship between plasma copper and ceruloplasmin holds true for the guinea pig during RMSF, then very early in this disease something triggers the synthesis and/or release of a specific plasma protein. Other acute-phase proteins also may be increased during RMSF judging from the rise in plasma seromucoid concentration. However, in the absence of turnover studies, it cannot be conclusively proven that these putative increases in ceruloplasmin and seromucoid represent increased protein synthesis/release and not di-

TABLE II. PLASMA AMINO ACID CONCENTRATION.

Group	Total plasma amino acids (mmoles/liter)	Phenylalanine (μmoles/liter P)	Tyrosine (μmoles/liter T)	P/T
Controls (n = 11)	3.79 ± 0.24	68.2 ± 4.3	71.7 ± 14.9	1.14 ± 0.10
Infected (n = 4)				
Day 1	3.54 ± 0.54	86.3 ± 7.1	73.9 ± 13.4	1.24 ± 0.14
Day 2	2.97 ± 0.36	74.4 ± 3.0	49.3 ± 9.5	1.62 ± 0.20
Day 3	4.17 ± 0.31	105.1 ± 6.2[c]	59.9 ± 6.6	1.79 ± 0.11[c]
Day 5[a]	3.33 ± 0.20	99.2 ± 12.7[b]	55.2 ± 7.7	1.81 ± 0.14[c]
Day 8[a]	3.81 ± 0.46	88.6 ± 7.3	66.9 ± 9.3	1.36 ± 0.13
Day 12	3.66 ± 0.33	92.2 ± 9.6	80.9 ± 11.9	1.18 ± 0.12

[a] n = 3.
[b] $P < 0.01$ vs controls.
[c] $P < 0.005$ vs controls.

minished degradation.

Another indication of altered nitrogen metabolism is the plasma phenylalanine/tyrosine ratio (P/T) which was significantly increased on Days 3 and 5. Increased P/T ratios have been noted in a number of infections (4, 9, 10) and appear to reflect increased amino acid mobilization from muscle tissue rather than merely decreased capacity to metabolize phenylalanine (5). The increase in P/T occurred, however, without a significant decrease in total plasma amino acids usually observed during infection (4).

Alterations in plasma protein, amino acid, and trace metal metabolism/distribution similar to those described in this report have been shown to correlate with tissue damage during tularemia in the rat (2), to be a consequence of phagocytosis (11), and to result from the injection of leukocyte-derived factors (12, 13). RMSF in guinea pigs is characterized by widespread vasculitis, scrotal swelling, and necrosis (14). Vasculitis evident as early as 1 day after exposure becomes progressively more severe and is accompanied by thrombosis and vascular occlusion (J. B. Moe, manuscript in preparation). It thus remains to be determined (a) to what extent the alterations in host metabolism described in this paper correlate with the onset and severity of the vasculitis, (b) whether the early metabolic (and pathologic) changes are the result of a toxic substance said to be associated with the rickettsiae (15), and (c) if leukocyte-derived factors are present and operative.

Summary. Guinea pigs inoculated with virulent *Rickettsia rickettsii* responded with a significant increase in plasma copper concentration within 1 day, preceding fever and detectable rickettsemia by 2 and 4 days, respectively. A decrease in serum zinc concentration coinciding with peak rickettsemia was detectable on Day 5. Evidence of altered host nitrogen metabolism during this illness included a doubling of plasma seromucoid concentration and a significant rise in the plasma phenylalanine/tyrosine ratio.

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