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EFFECTS OF STARVATION AND STREPTOCOCCUS PNEUMONIAE INFECTION ON--ETC(U)
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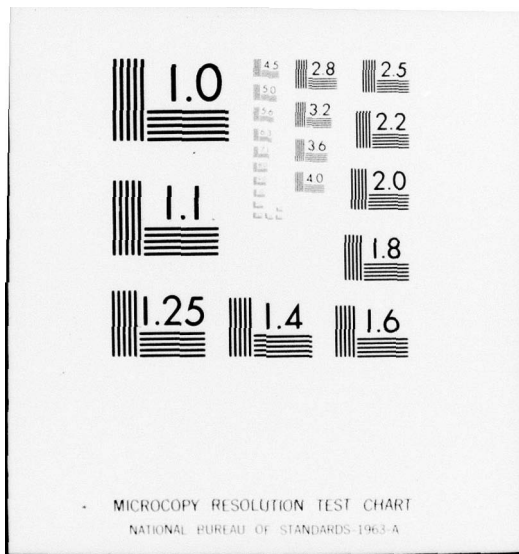


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**EFFECTS OF STARVATION AND STREPTOCOCCUS PNEUMONIAE INFECTION
ON CARNITINE ACYLATION STATES IN THE RAT¹**

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SUMMARY: This study was performed to determine if alterations in carnitine and its acylation states could account for the decreased ketone body production seen during Streptococcus pneumoniae infection in the rat. Despite a lower ketogenic capacity, the hepatic total and free carnitine increases to the same extent in infected rats as in starved control rats. However, variations are seen in the short- and long-chain acylcarnitines of the infected rat. Liver short-chain derivatives were inversely proportional to plasma β -hydroxybutyrate in fed, starved and infected rats.

The involvement of carnitine in the regulation of fatty acid oxidation and ketone body formation is well established (1-3). Previous investigators have suggested that free and total hepatic carnitine concentrations correlate directly with the ketogenic capacity of the liver during starvation and diabetes (3). In an infectious illness, coupled with starvation, there is a diminished "starvation-induced" ketosis (4). Therefore, the present study was initiated to determine if alterations in carnitine acylation states could account for the decrease in ketone body production.

¹In 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 the 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 for Accreditation of Laboratory Animal Care.

The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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MATERIALS AND METHODS: Male, Fisher-Dunning rats (F-344/Mai f, obtained from Microbiological Associates, Walkersville, Md.) weighing 175-200 g were used. Rats were maintained on a commercial diet (Wayne Lab Blox, Allied Inc., Chicago, Ill.) and housed in rooms maintained at $23 \pm 1^\circ$. Rats were inoculated with 10^4 *S. pneumoniae* Ia5 by subcutaneous injection in the nape of the neck. Control rats were inoculated with the heat-killed organisms. Food was withheld from both infected and control rats. The rats were stunned by a blow to the head and killed by cervical dislocation. Liver, plasma and muscle were collected and prepared as described previously (5). Free as well as short- and long-chain acylcarnitines were measured on plasma and neutralized tissue extracts according to the methods of Pace et al. (5). Ketone bodies (β -hydroxybutyrate and acetoacetate) were measured as described by McGarry et al. (6), and α_2 -macroglobulin was determined by immunoprecipitin assay (7). Fed rats, starved controls and infected rats were compared based on one-way analysis of variance (8). Results are presented as mean \pm S.E.M. for from 6 to 12 rats in each group.

RESULTS AND DISCUSSION: In starvation the oxidation of free fatty acids and ketone bodies spares the oxidation of glucose. However, in a bacterial infection coupled with starvation there is a diminished ketone production (4) and an increased flux of amino acids to the liver for the production of glucose. The inability to use endogenous fat stores for energy production appears to be one of the contributing factors responsible for the protein-wasting state found during an infection (9).

A primary objective of this study was to establish base-line values for carnitine and short- and long-chain acylcarnitines in tissues and plasma of the rat. Only 2% of the acylcarnitines present in plasma are long-chain derivatives, therefore, short- and long-chain are reported as one value (Table I). The data presented in Table I show that the total amount of carnitine in liver increases during starvation while plasma and muscle carnitine is not markedly altered by starvation. As expected from the findings of Fraenkel (10), skeletal muscle is rich in carnitine, and plasma has the lowest carnitine concentration. The distribution of carnitine in the fed rat is in agreement with results reported by Böhmer (1). The hepatic long-chain acylcarnitines of the fed rat are significantly higher in our studies than values found in the

literature (1). However, Böhmer (1), also found a 10-fold variation in the level of long-chain derivatives. This difference may be explained by variations in the length of time taken for sample collection and the method of sample preparation. In addition, Pearson and Tubbs (11) have observed that acylation states varied with the method of kill; liver acetylcarnitines fell in ischemia, and long-chain acylcarnitines rose in rats killed by decapitation.

Experiments were performed on rats infected with S. pneumoniae. The progress of the infection was followed by changes in rectal temperature, the appearance of α_2 -macrofetoprotein and the presence of bacteremia. The data reported in Table I show that S. pneumoniae infection coupled with starvation causes increases in liver free and total carnitine and also alters the carnitine acylation state when compared to starved control. Short-chain acylcarnitines increase 2-fold in the liver of the infected rat compared to the starved rat while hepatic long-chain derivatives decrease 30%.

In the plasma, free carnitine was decreased during starvation but was significantly increased after 48 hr of infection. Plasma short-chain acylcarnitines were initially elevated but decreased slightly by 48 hr, while the total carnitine pool (acid soluble) increased during infection. Total muscle carnitine showed little change during infection; however, the long-chain acylcarnitines were significantly decreased.

The ratio of hepatic short-chain to free carnitine increased during infection while the ratio of long-chain to free carnitine decreased. When carnitine and its derivatives in muscle, liver and plasma were expressed as a percent of the total carnitine pool the relative concentrations of carnitine after 48 hr of infection were similar to those of a fed rat rather than its starved control. There was a direct correlation between the amounts of plasma short-chain acylcarnitines and plasma β -hydroxybutyrate concentrations shown

in Figure 1. This agrees with Hoppel and Genuth's data (12). However, Figure 2 shows that an inverse correlation existed between the concentration of liver short-chain acylcarnitines and plasma β -hydroxybutyrate concentrations.

The above data suggest a number of possible explanations for the decreased ketone production accompanying a bacterial infection. The carnitine acetylation may represent a mechanism by which activated acetyl groups can leave the mitochondria (13). Thus, under certain conditions more acetyl-CoA can react to give acetylcarnitine and less is directed to ketone body production. It has been reported that both carnitine and acetylcarnitine are released from liver cells (14). This may explain the initial increase in short-chain derivatives seen in plasma during infection (Table I). Another possibility is that a significant proportion of the short-chain carnitine derivatives may be composed of propionylcarnitine (15). This derivative almost completely disappears during starvation and is a known inhibitor of ketogenesis (16). Table I shows a decrease in liver and muscle long-chain acylcarnitine accompanying the decreased fatty acid oxidation and ketone production during infection (4). This decrease in long-chain acylcarnitines could reflect the decreased availability of long-chain fatty acids (4) and/or a decrease in the ability to form long-chain acylcarnitines, i.e., a decrease in transferase I activity (17).

Hormonal responses have been shown to differ between infected and control rats. Zenser et al. (18) have reported insulin and glucagon to be increased during infection, resulting in a 2-fold decrease in the insulin:glucagon ratio. Insulin has also been reported to reverse the ketosis of fasting rats within 1 hr (19). This information indicates that the concentrations of insulin and glucagon in blood may contribute to the alterations in hepatic metabolism of fatty acids produced by a bacterial infection. The biochemical

mechanisms by which insulin regulates the direction of fatty acids into the various metabolic pathways in the liver are unknown. Mechanisms, such as changes in the levels of certain hepatic enzymes, may be of some significance.

The present studies suggest that, contrary to the findings of McGarry et al. (3), elevated free and total carnitine concentrations do not always correlate with the degree of ketosis. Despite the elevated carnitine concentrations, a rat infected with *S. pneumoniae* has a low ketogenic capacity. The distribution of carnitine during an infection may reaffirm the hypothesis that ketogenesis is not regulated at any one site but that substrate availability and utilization of acetyl-CoA may play a role in the distribution of substrate between the pathways of ketogenesis and lipogenesis.

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Table I. Free carnitine, short-chain and long-chain acylcarnitines, and total carnitine pool in liver, plasma and muscle from rats fed, starved and *S. pneumoniae*-infected for 24 and 48 hr

Carnitine	nmol/g or ml					
	Fed	Starved		Starved + infected		
		24 hr	48 hr	24 hr	48 hr	
Liver						
Free	155 ± 11	237 ± 11 ^{††}	240 ± 19 ^{††}	200 ± 10 [†]	225 ± 16 ^{††}	
Short	82 ± 13	71 ± 12	70 ± 14	49 ± 8	129 ± 15 ^{**} , ^{††}	
Long	77 ± 14	88 ± 8	85 ± 13	45 ± 1 [*]	54 ± 6 [*]	
Total pool	301 ± 27	397 ± 28 [†]	388 ± 38 [†]	294 ± 16 [*]	408 ± 14 [†]	
Plasma						
Free	75 ± 5	45 ± 2 ^{††}	58 ± 4	32 ± 2 ^{††}	93 ± 14 ^{**} , ^{††}	
Short + long	33 ± 5	57 ± 8	70 ± 8 ^{††}	85 ± 16 [*] , ^{††}	60 ± 6 [†]	
Total pool	104 ± 6	102 ± 6	129 ± 9	134 ± 6 [*] , [†]	153 ± 17 ^{††}	
Muscle						
Free	775 ± 57	658 ± 40 [†]	800 ± 86	618 ± 22 [†]	742 ± 34	
Short	523 ± 118	414 ± 51	353 ± 51	411 ± 28	351 ± 98	
Long	189 ± 21	197 ± 14	224 ± 16	146 ± 20 [*]	168 ± 10 [*]	
Total pool	1451 ± 106	1131 ± 50 [*]	1258 ± 75	1175 ± 29 [*]	1249 ± 77	

*P < 0.05 compared to starved controls; **P < 0.01 compared to starved controls.

[†]P < 0.05 compared to fed controls; ^{††}P < 0.01 compared to fed controls.

LEGENDS TO FIGURES

Fig. 1 Effect of infection on plasma β -hydroxybutyrate concentrations. (○) Fasted controls, \pm S.E.M. (●) Rats infected with S. pneumoniae. Each point represents a minimum of 6 rats.

Fig. 2. Relationship between ketogenic capacity and short-chain acylcarnitine content of liver. The best-fit line for the regression was determined by the method of least squares.

