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EFFECTS OF WOUND BACTERIA ON POSTBURN ENERGY METABOLISM

ANNUAL REPORT

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The study identified the hypermetabolic and thermoregulatory effects of burn wound colonization and examined the role of endotoxin (LPS) in these responses. Resting metabolic rates and colonic temperatures (Tc) of 400-600 g, male Sprague-Dawley rats were monitored before and after full-thickness 30% total body surface burns. Fifty-three burn wounds were seeded with 10^8 non-virulent P. aeruginosa (NVP) at the time of injury and 33 wounds were allowed to colonize spontaneously. Seeded wounds contained 10^6 colony forming units/g (CFU/g) from postburn day (PBD) 4 to PBD 15, while the unseeded wounds did not reach this level of colonization until the second week. Resting oxygen consumption ($\dot{V}O_2$) and Tc of the seeded group were above those of the unseeded rats during the first week post burn when the seeded rats had higher wound bacterial counts. The increase in resting $\dot{V}O_2$ of 44 non-bacteremic rats correlated with wound bacterial counts ($r = 0.63$, $p < 0.001$) suggesting that wound bacteria and/or their products contribute to burn hypermetabolism. Changes in Tc were unrelated to wound bacterial count but accompanied changes in $\dot{V}O_2$ ($r = 0.60$, $p < 0.001$).

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Hypermetabolic and Tc changes of 18 NVP seeded rats were comparable to those of 17 rats whose wounds were seeded with 10^8 S. epidermidis (SE). Since SE wounds contained relatively little LPS, and the burned animals were not endotoxemic, LPS is not an obligatory afferent mediator of postburn hypermetabolism. Furthermore, the hypermetabolic response to NVP seeded wounds could not be reproduced by continuous, subcutaneous infusion of NVP LPS beneath unseeded wounds of 30 rats (15 at a rate of 2.6 µg LPS/100 g/h and 15 at 12.6 µg/100 g/h). These LPS doses equal or exceed the estimated maximum rate of LPS release in the NVP seeded wound, indicating that LPS was not the sole contributor of postburn hypermetabolism following gram negative infection. Taken together, these results suggest that wound bacteria act as non-specific stimuli rather than unique afferent mediators of postburn hypermetabolism.

ABSTRACT

This study identified the hypermetabolic and thermoregulatory effects of burn wound colonization and examined the role of endotoxin (LPS) in these responses. Resting metabolic rates and colonic temperatures (Tc) of 400-600 g, male Sprague-Dawley rats were monitored before and after full-thickness 30% total body surface burns. Fifty-three burn wounds were seeded with 10^8 non-virulent P. aeruginosa (NVP) at the time of injury and 33 wounds were allowed to colonize spontaneously. Seeded wounds contained 10^6 colony forming units/g (CFU/g) from postburn day (PBD) 4 to PBD 15, while the unseeded wounds did not reach this level of colonization until the second week. Resting oxygen consumption ($\dot{V}O_2$) and Tc of the seeded group were above those of the unseeded rats during the first week post burn when the seeded rats had higher wound bacterial counts. The increase in resting $\dot{V}O_2$ of 44 non-bacteremic rats correlated with wound bacterial counts ($r = 0.63$, $p<0.001$) suggesting that wound bacteria and/or their products contribute to burn hypermetabolism. Changes in Tc were unrelated to wound bacterial count but accompanied changes in $\dot{V}O_2$ ($r = 0.60$, $p<0.001$). Hypermetabolic and Tc changes of 18 NVP seeded rats were comparable to those of 17 rats whose wounds were seeded with 10^9 S. epidermidis (SE). Since SE wounds contained relatively little LPS, and the burned animals were not endotoxemic, LPS is not an obligatory afferent mediator of postburn hypermetabolism. Furthermore, the hypermetabolic response to NVP seeded wounds could not be reproduced by continuous, subcutaneous infusion of NVP LPS beneath unseeded wounds of 30 rats (15 at a rate of 2.6 µg LPS/100 g/h and 15 at 12.6 µg/100 g/h). These LPS doses equal or exceed the estimated maximum rate of LPS release in the NVP seeded wound, indicating that LPS is not the sole contributor of postburn hypermetabolism following gram negative wound infection. Taken together, these results suggest that wound bacteria act as non-specific stimuli rather than unique afferent mediators of postburn hypermetabolism.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985).

TABLE OF CONTENTS

INTRODUCTION

The rise in energy expenditure following trauma varies with the extent of injury and reaches its greatest magnitude in the burned patient. A young, otherwise healthy patient with a 50% total body surface burn (TBSB) can have a resting metabolic rate twice the normal with every major system in the body working at an accelerated rate. In support of the increased energy turnover is a 3-4 fold increase in cardiac output, a heart rate of 100-120/minute, a respiratory rate of 18-20/minute, and a caloric requirement of 5000 Calories/day.

Catecholamines are the principal efferent mediators of postburn hypermetabolism (1), but the afferent signals which initiate and sustain the increased sympathoadrenal activity are largely undefined. The basic relationship between wound size and the degree of hypermetabolism suggests that these signals originate in the wound, and there is good evidence that they travel via the circulation to affect central nervous system control of metabolism (2,3). Only when we know what these signals are and how they alter metabolic control will we know whether it is appropriate, or even feasible, to consider reducing the energy cost of injury.

Historically, burn patients were thought to be hypermetabolic because they were cold (4-6). The increase in metabolic heat production was believed to compensate for the increased evaporative heat loss from the wound. Heat balance is further complicated by the fact that burn patients are febrile and have to produce even more heat to raise body temperature above normal. Gradually, however, many began to question the relative significance of a thermoregulatory basis for postburn hypermetabolism. The problem with this concept was that postburn hypermetabolism could not be eliminated by either blocking wound evaporation or raising ambient temperature into the thermoneutral zone (7-10). Since the metabolic rate at thermoneutrality is minimal and independent of changes in ambient temperature (11), the extra metabolism at thermoneutrality was considered the energy cost of injury.

In the past, investigators have separated the metabolic responses of burn patients into those occurring before and after bacteremia or sepsis. They do so in order to distinguish between the effects of injury and the superimposed infection. While this is an important clinical distinction, it leads to the concept that non-bacteremic patients are "free of infection" (12) and fails to address any systemic metabolic effects of bacteria prior to their entrance into the bloodstream. Since the burn wound is not sterile during the hypermetabolic phase of injury and infection alone produces metabolic and neuroendocrine adjustments similar to those in thermal injury (13), wound bacteria and/or their products may be important metabolic stimuli in the "uninfected" patient.

Bacterial contamination of the burn wound appears to increase the metabolic rate of rats (14). In one study, variations in the severity of infection were achieved by seeding 30% TBS burn wounds with bacteria and treating some of these wounds with topical antimicrobial agents. The hypermetabolic effects of infection appeared as a continuum, first evident as a 10-30% increase in resting oxygen consumption of non-bacteremic animals and progressing to 40-50% increases with wound invasion and systemic infection. Topical antimicrobial treatments reduced the hypermetabolism of seeded, non-

4

bacteremic burned rats, but without additional bacteriologic studies, it was impossible to know whether the consequences of topical wound therapy were the result of reduced wound colonization or some other systemic effect of the antimicrobial agent.

If bacteria initiate the metabolic response while still confined to the burn wound, they must release, or cause the release, of humoral mediators. These afferent signals may be bacterial products (enzymes, toxins, etc.) or cytokines produced by host inflammatory cells in response to bacteria/products. Endotoxin is a prime candidate in either case. This lipopolysaccharide (LPS) in the cell wall of gram negative bacteria increases metabolic rate upon entering the circulation (15) and is one of the most potent inducers of cytokine production (16). Interleukin-1 (IL-1) and cachectin/tumor necrosis factor (TNF) are two cytokines believed responsible for many systemic responses to injury and infection (17-18).

The hypermetabolic effects of IL-1 and TNF remain uncertain, however. IL-1 production in uninjured humans has caused fever and other acute phase responses but did not make them hypermetabolic (19). Intravenous (IV) infusion of murine recombinant IL-1 (rIL-1) was reported to depress resting energy expenditure of rats (20), while IV administration of partially purified monocytic IL-1 had the opposite effect on another group of uninjured rats (21). There are claims that TNF and IL-1 act synergistically to increase resting energy expenditure, but they are largely unsubstantiated at present (22,23). Another major problem with these two cytokines is that no one has found measurable quantities of either IL-1 or TNF in the serum of non-bacteremic burned animals or humans.

The hypothesis examined in this study was that a major portion of the increase in energy expenditure following thermal injury is initiated by bacteria and/or their products in the burn wound. To test this hypothesis, we began by determining whether postburn hypermetabolism of the non-bacteremic rat was affected by changes in wound bacterial strain or density. Upon finding that the degree of hypermetabolism varied as a function of wound bacterial density, we asked whether different bacteria have different capacities to cause hypermetabolism. Once it was established that the hypermetabolic responses to gram negative and gram positive wound infections were identical, the role of LPS was examined.

MATERIALS AND METHODS

Study Design. The basic protocol involved measuring resting metabolic rate and colonic temperature of rats before and after full-thickness 30% TBS burns. Variations in wound bacterial density were accomplished by seeding some burn wounds with non-virulent organisms while allowing others to colonize spontaneously. Topical antimicrobial agents were not used in order to avoid the potential for systemic drug effects. Quantitative wound cultures were performed to characterize the extent of colonization, and blood and spleen cultures were used to identify bacteremic animals. Since the study addressed the effects of localized wound infection, bacteremic or septic animals were not included in the data analysis.

The metabolic effects of endotoxin were assessed by (a) comparing the responses to gram positive and gram negative wound infections, (b) monitoring plasma endotoxin levels in hypermetabolic, burned rats, and (c) determining whether continuous subcutaneous infusion of LPS under the unseeded burn wound raised the metabolic rate of rats.

Animals. The animals selected for study were 4-7 month old, male Sprague-Dawley rats (Hilltop, Scottdale, PA) weighing 400-600 grams. They were housed in individual cages and had access to food and water throughout the study. A 12-h light/dark cycle was maintained. Ambient temperature ranged from 25-28°C while animals were not under study.

Burn Injury and Wound Seeding. The rat was anesthetized (sodium pentobarbital, 4.5 mg/100 g body weight, ip) and its pelage clipped from the back and flanks. It was then placed in a mold exposing 30% of total body surface, and a full-thickness burn produced over this area by immersion in 98°C water for nine seconds. Burn wounds were either left unseeded or seeded with a non-virulent *P. aeruginosa* (NVP) (24) or *S. epidermidis* (SE) (ATCC12228) at the time of injury. All 18-h seeding cultures contained 10^8 bacteria/ml of tryptic soy broth, and one ml of the broth was spread over the entire wound.

Metabolic and Temperature Measurements. Resting metabolic rate was determined by indirect calorimetry. Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were calculated from respiratory gas exchange measurements conducted over a 3-hour interval. In each study, eight rats were placed in individual, flow-through respiration chambers located in a temperature controlled cabinet, and left undisturbed for at least one hour prior to study (Figure 1). Ambient temperature was maintained at thermoneutrality (30°C before injury and 32°C after injury (14)) by a proportional controller (Model 72, Yellow Springs Instruments, Yellow Springs, OH) which sensed air temperature in the cabinet and varied the output of a 1500 W heater.

Room air was drawn through the respiration chambers by small fish tank pumps. Chamber air passed through a condensor and then through solenoid valves (Series 3 Subminiature Valve, General Valve Corp., Fairfield, NJ) where it was either exhausted into the room or directed into an analytical line. Air flow rate in the analytical line (two liters/minute) was monitored by a turbine flowmeter (Omniflo, Model FTO-N5-GJS, Flow Technology, Inc., Phoenix, AZ), and air temperature measured by a YSI Model 514 thermistor connected to a Model 44 Tele-Thermometer (Yellow Springs Instruments, Yellow Springs, OH).
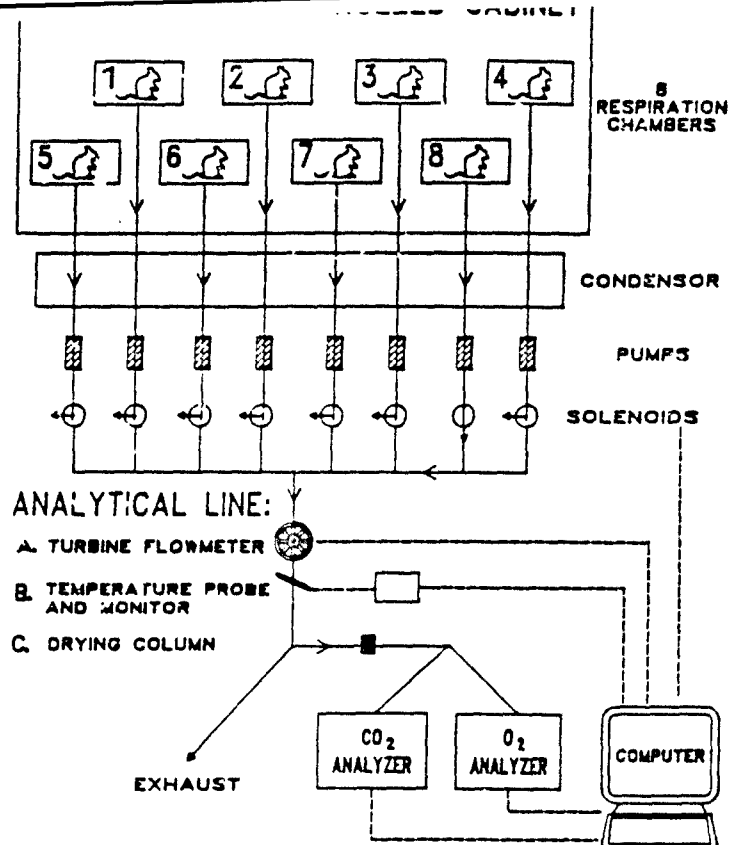
Figure 1. The metabolic measurement system. Solid lines identify air flow. Dashed lines indicate electrical connections.

A portion of the analytical air was drawn through a drying column and then through O_2 and CO_2 analyzers (Model S3A-II Oxygen Analyzer and Model CD-3A Carbon Dioxide Analyzer, Ametek, Pittsburgh, PA). Gas analyzers were calibrated at the beginning of each study and at 30-min intervals thereafter.

$\dot{V}O_2$ and $\dot{V}CO_2$ were determined by multiplying the rate of air flow (STPD) times differences in O_2 and CO_2 concentrations across each chamber. These respiratory gas exchange measurements were performed in a serial fashion until all eight animals had been studied 4-5 times over a 3-h interval. Resting metabolic rate was calculated from the average respiratory exchange over the study interval and expressed in Watts per square meter of body surface (25). Surface area calculations were based on the animal's weight at the end of each study (26). Solenoid operation, data acquisition and metabolic calculations were obtained through the use of an Integrated System for Automated Acquisition and Control (Model 91A, Cyborg Corp., Newton, MA).

Metabolic measurements were performed for 2-3 weeks before injury in order to achieve reproducible resting values. After injury, studies were performed on postburn days (PBD's) 3-4, 7-8, and 14-15, and the data averaged for each two-day block.

Colonic temperature (Tc) was measured six centimeters from the external anal sphincter with a YSI Model 402 thermistor probe connected to the Tele-Thermometer described above. The thermistor was calibrated in a stirred water bath and considered accurate to $\pm$ 0.1°C. Colonic temperatures were determined one hour after each metabolic study. The purpose of this delay was to permit a comparison between Tc of the burned and unburned animals taken at the same time and in the same environment. Since the metabolic studies had to be conducted in the thermal neutral zone for burned animals (32°C) and this is too warm for unburned controls, the burned rats were placed in a cooler room (25-28°C) with the normal animals for one hour prior to temperature measurements.

Bacteriology. Quantitative bacteriologic cultures were performed on blood, spleen and burn wound. The peritoneum was opened under aseptic conditions and 8-10 ml of blood drawn from the abdominal aorta. Four to six drops of blood were placed on a plate of TSA with 5% sheep's blood (Difco) and the rest saved for other analyses. The spleen was removed, dipped in alcohol, and flamed to eliminate surface bacteria. It was then placed in a sterile tissue grinder containing 5 ml of sterile water. The number of viable bacteria in 0.1 ml of the homogenate was determined by serial dilution and back plating on sheep's blood agar. The wound surface was cleaned with an alcohol-impregnated gauze and approximately 60% excised to fascia. This was diced, weighed, homogenized in sterile water, and the homogenate serially diluted as previously described. Plates were read after 24 and 48 hours of incubation at 37°C and the bacterial counts expressed as the number of colony forming units per ml blood, per gram spleen, or per gram wound.

Endotoxin Preparation, Infusion and Analysis. The endotoxin used in this study was a lipopolysaccharide (LPS) extracted from non-virulent _P. aeruginosa_ bacteria by the method of Westphal and Jann (8). An eighteen-hour culture in 1500 ml of tryptic soy broth yielded 7-10 mg LPS (about 1% of dry cell weight). One microgram of LPS equaled 150 endotoxin units (EU) as determined by the Limulus amoebocyte lysate (LAL) test using the _E. coli_ LPS 055:B5 standard (Sigma Chemical, St. Louis, MO).

The LPS was dissolved in sterile, non-pyrogenic saline and placed in small osmotic pumps (Alzet Models 2001 or 2ML1, Alza, Palo Alto, CA). The pumps were inserted into the subcutaneous tissue of the midscapular region (under the burn wound) immediately after injury. Each pump provided continuous subcutaneous infusion of LPS in burned, unseeded animals for seven days following injury. Two doses were used - 2.6 µg LPS/100 g body weight/h (at one µl/h) and 12.6 µg LPS/100 g/h (at 10 µl/h). Burned control rats received sterile, non-pyrogenic saline administered in the same fashion.

Plasma endotoxin concentration was determined by the LAL test. Five ml of aortic blood were obtained aseptically and placed into sterile, pyrogen-free heparin vials. After 10 minutes of centrifugation, the plasma sample was diluted 1:9 with pyrogen-free water and heated at 75°C for five minutes to remove inhibitors. The sample was cooled to 10°C and 0.2 ml added to a single-test vial containing the lysate concentrate (E-toxate 210-8, Sigma Chemical, St. Louis, MO). The plasma was considered to contain LPS whenever this solution formed a solid gel after an one-hour incubation at 37°C. When the _E. coli_ standard LPS was added to normal rat blood and the LAL test

performed as described, we were able to detect 0.02 ng LPS/ml plasma. Since this LPS standard contains 6000 EU/µg, the limit of our sensitivity was 0.1 EU/ml plasma.

Special Diet. While most rats were fed standard rodent laboratory chow (Ralston Purina Co., St. Louis, MO), some received a special diet. The C-21 diet was selected because its paste consistency eliminated spillage and permitted precise measurements of intake (28). It consisted of 21% casein, 4% mineral and vitamin mixture (both from ICN Biochemicals, Cleveland, OH), 25% hydrogenated-soy oil (Crisco, Proctor and Gamble Co., Cincinnati, OH), 15% sucrose, and 35% starch.

Data Analysis. Multiple unpaired t-tests with the Bonferroni adjustment (29) were used to assess differences between groups of burned animals at each postburn interval. Likewise, paired t-tests with the Bonferroni adjustment were used to identify postburn changes within the same group of animals.

9

Animals Studied. One hundred and sixty-five burned rats were divided into six categories for this study. There were 33 unseeded rats, 71 NVP seeded rats, 18 SE seeded rats, 15 unseeded rats receiving low dose LPS infusion, 15 unseeded rats receiving high dose LPS infusion, and 13 unseeded rats receiving a saline infusion.

Effects of Wound Colonization. Fifty three burn wounds were seeded with NVP bacteria, and 33 wounds were not seeded. Prior to injury there was no difference in resting oxygen consumption, core temperature or body weights between these two groups. (Metabolic rate is reported as oxygen consumption in ml O_2/h/g body weight for this set of experiments, because a failure of the CO_2 analyzer prevented $\dot{V}CO_2$ measurements.) Table 1 provides a breakdown of the number of animals studied at each postburn interval and how many in each group were sacrificed for bacteriologic studies.

Table 1. Number of rats studied. (Bacteriologic studies)

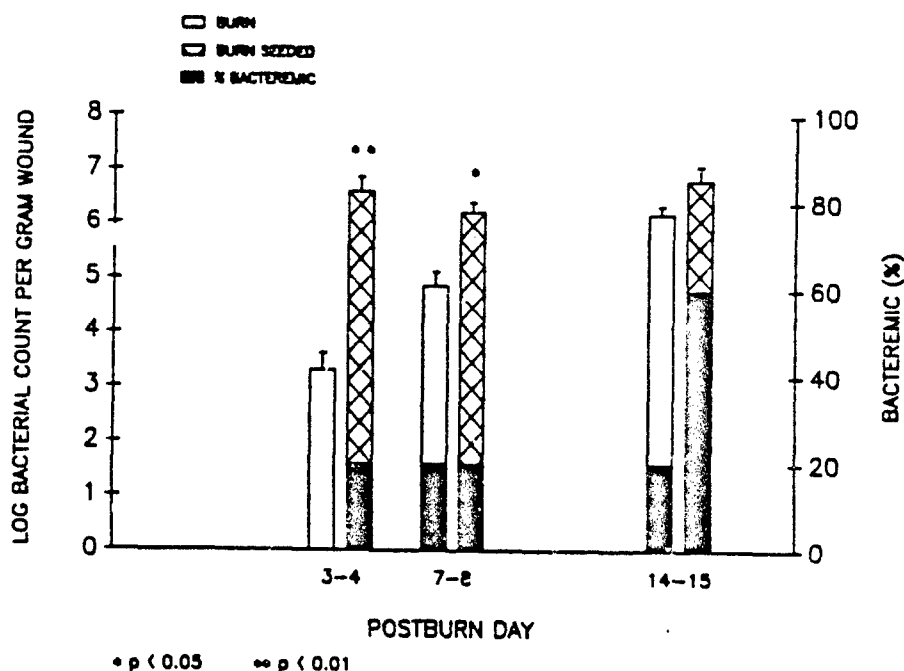| Postburn Day	0	3-4	7-8	14-15
Unseeded	33	25(10)	15(10)	12(12)
NVP Seeded	53	40(14)	36(14)	27(14)

Figure 2. The unseeded rats had less bacteria in their burn wounds (log count/g wound) than NVP seeded animals during the first postburn week. At the end of two weeks, a majority of the seeded animals were bacteremic.

Seeding the wound resulted in a relatively constant level of infection (10^6 to 10^7 colony forming units per gram, CFU/g) over the two week period of observation (Figure 2). The unseeded wounds colonized more slowly; averaging less than 10^4 CFU/g on postburn days 3-4, 10^5 by the 7th and 8th postburn days and 10^6 by the end of the second week. There was a significant difference in log wound bacterial count between the two groups only during the first postburn week. None of the unseeded animals were bacteremic on the 3rd and 4th postburn days, but 20% of the seeded animals were. At one week, 20% of both groups were bacteremic; and, at two weeks, 60% of the seeded animals were bacteremic as compared to only 20% of the unseeded group.

Seeding the burn wound accelerated metabolic and thermoregulatory responses. Oxygen consumption was elevated in both groups by the 4th postburn day, but the resting $\dot{V}O_2$ of the seeded rats (1.07 ± 0.02 ml/h/g, mean $\pm$ SEM) was above that of the unseeded animals (0.88 ± 0.02 ml/h/g, p<0.01, unpaired t-test) (Figure 3). Oxygen uptake remained different between the two groups throughout the first week (0.94 ± 0.02 ml/h/g for unseeded rats versus 1.11 ± 0.02 ml/h/g for seeded animals on PBD's 7-8, p<0.01) but this difference disappeared by the end of the second week due to a marked increase in the $\dot{V}O_2$ of the unseeded animals. At this point, both groups had the same level of wound colonization, but the majority of seeded animals were bacteremic.
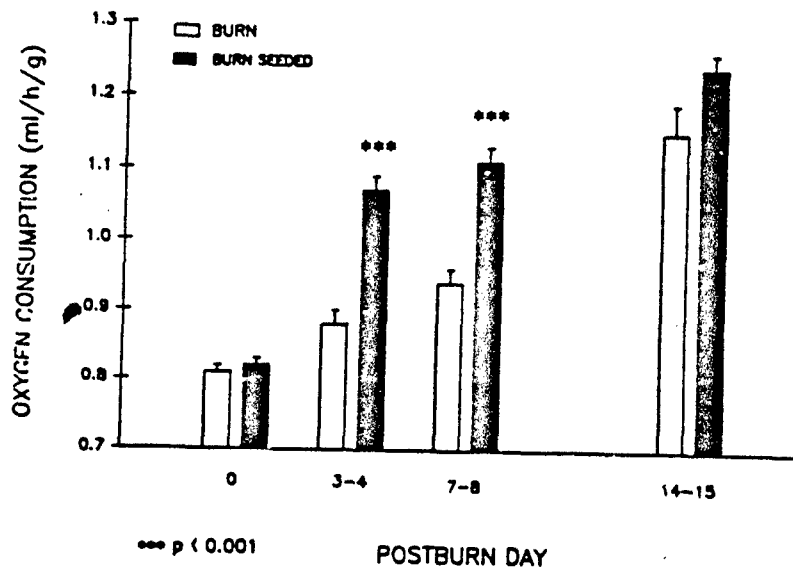

Figure 3. Resting oxygen consumption of the seeded rats exceeded that of the unseeded animals during the first week following injury.

Seeding the wounds accelerated the rate of body weight loss following injury (Figure 4). During the first week, seeded animals lost about 10% of their body weight, compared to a 4% loss in the unseeded group. Since oxygen consumption is expressed per gram body weight, a small portion of the initial differences in O_2 uptake between the two groups may reflect the greater weight loss of the seeded animals.
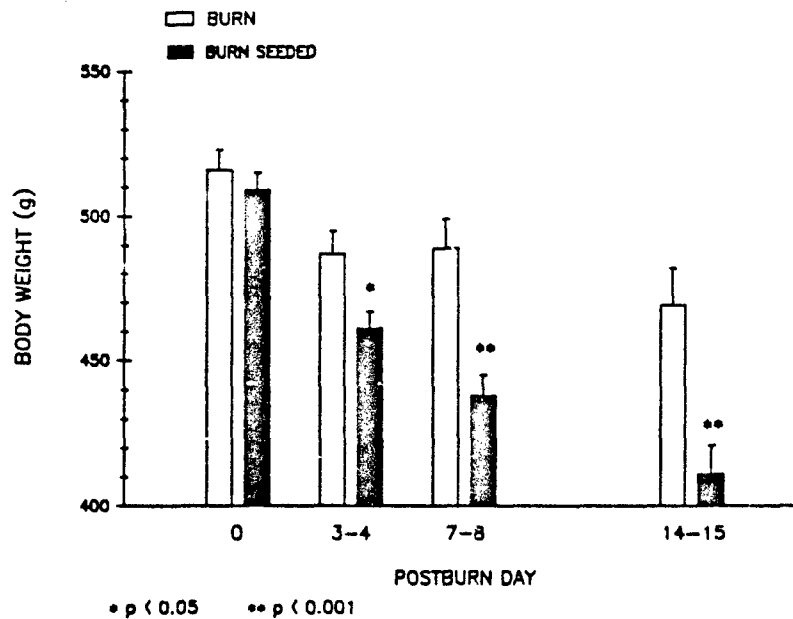

Figure 4. Seeding the burn wound accelerated the rate of weight loss following injury. The p values reflect differences between groups at each postburn interval.

While both burn groups were hypermetabolic during the first week, only the seeded animals were febrile (Figure 5). On postburn days 3-4 and 7-8 colonic temperature averaged $37.0 \pm 0.1^{\circ}C$ (mean $\pm$ SEM) for the unseeded animals as compared to $36.5 \pm 0.1^{\circ}C$ for uninjured controls and $37.4 \pm 0.1^{\circ}C$ for the seeded animals (p<0.01, seeded vs. unseeded on both test intervals). Core temperature of the unseeded animals rose to equal that of the seeded animals by the end of the second week ($37.6 \pm 0.1^{\circ}C$ for the unseeded and $37.8 \pm 0.1^{\circ}C$ for seeded animals).

Since systemic infection will increase oxygen uptake in this model (14), we excluded all bacteremic animals in an effort to emphasize the effects of wound microorganisms. Analyzing the data from 44 non-bacteremic rats (24 unseeded and 20 seeded) revealed that there was a significant linear

correlation between log of the wound bacterial count and the increase in resting O_2 uptake during the first two weeks post burn (r = 0.63, p<0.001) (Figure 6).
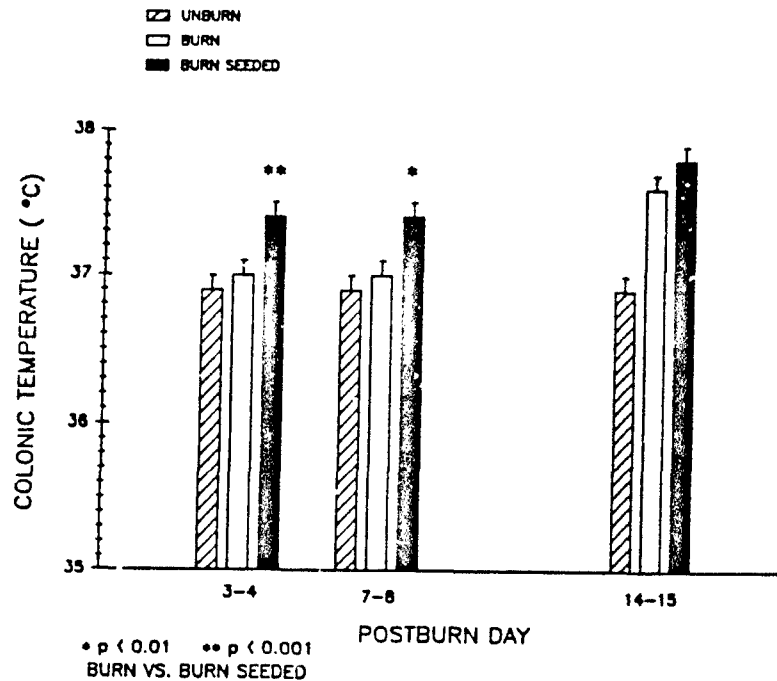

Figure 5. The seeded rats maintained a higher colonic temperature than the unseeded rats during the first week following injury. By the end of the second week both groups of burned animals were febrile.

There was no correlation between log wound colony count and core temperature for the non-bacteremic animals, but colonic temperature was related to the increase in oxygen consumption (r = 0.60, p<0.001) (Figure 7).

Gram Negative verses Gram Positive Wound Infection. This study was performed in two trials. In the first trial, the metabolic and Tc responses of nine rats with gram positive wound infections (SE seeded wounds) were compared to those of nine animals with gram negative burn wound infections (NVP seeded wounds). Both groups became hypermetabolic, and there was no significant difference in the degree of hypermetabolism on PBD's 3-4 or 7-8 (Table 2). The NVP seeded rats lost 16 g/day during the first four days post injury as compared to 7.5 g/day for the SE animals (p<0.05, unpaired t-test). Colonic temperatures of the NVP and SE rats were not significantly above those of unburned controls.

13
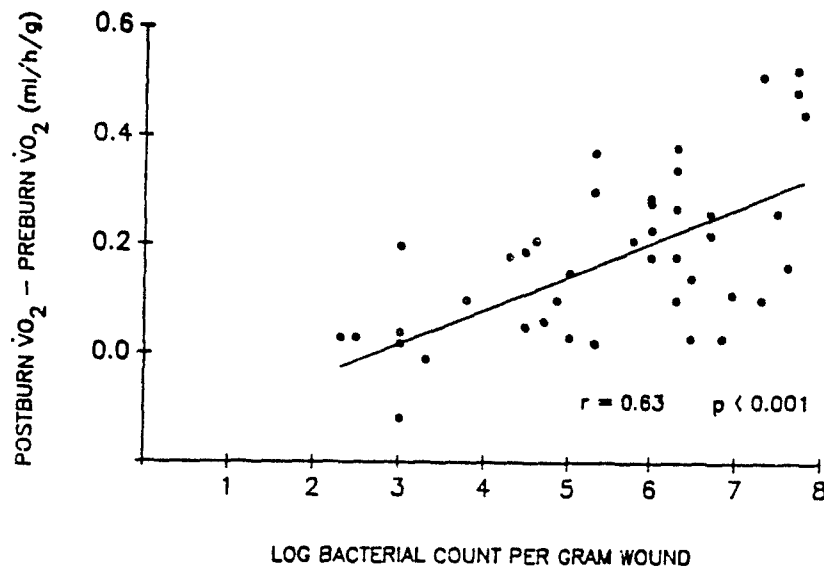
Figure 6. The increase in resting oxygen consumption during the first two weeks after injury (Postburn $\dot{V}O_2$ - Preburn $\dot{V}O_2$) was related to the degree of wound colonization of 44 non-bacteremic burned rats (24 seeded and 20 unseeded).

Figure 7. Colonic temperatures of 44 non-bacteremic burned rats (24 seeded, 20 unseeded) were related to changes in resting oxygen consumption (Postburn $\dot{V}O_2$-Preburn $\dot{V}O_2$) during the first two weeks after injury.

Table 2. Effects of gram negative (NVP) and gram positive (SE) wound infections.

TRIAL 1

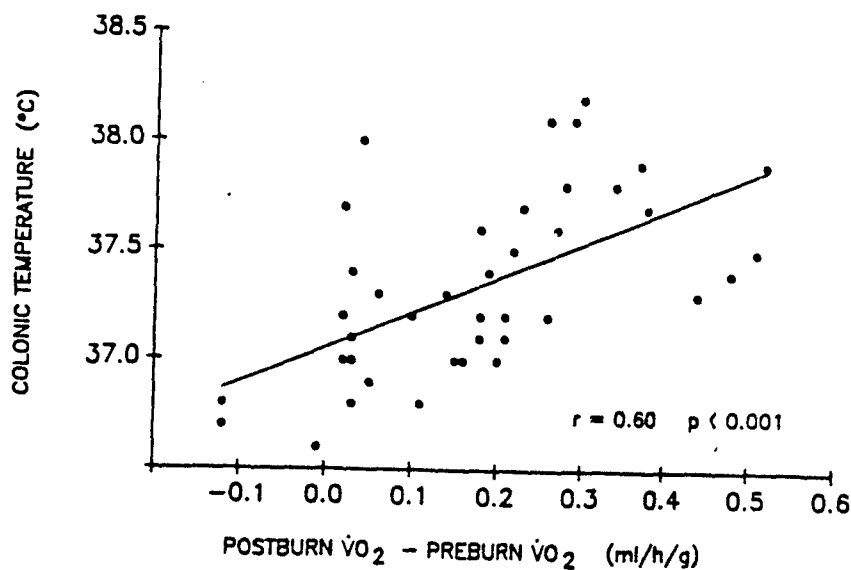| Metabolic Rate (W/m^2)	PBD 0	PBD 3-4	PBD 7-8
NVP	33.1 ± 1.2	38.3 ± 0.9	38.8 ± 0.8
SE	32.5 ± 0.6	38.0 ± 1.1	38.3 ± 0.8
p	NS	NS	NS
Body Weight (g)			
NVP	495 ± 8	430 ± 7	425 ± 8
SE	475 ± 13	445 ± 12	436 ± 14
p	NS	NS	NS
Colonic Temperature (^{o}C)			
NVP		37.8 ± 0.1	37.4 ± 0.1
SE		37.8 ± 0.1	37.3 ± 0.1
Unburned		37.4 ± 0.1	37.2 ± 0.1
p		NS	NS

TRIAL 2

Metabolic Rate (W/m^2)	PBD 0	PBD 3-4	PBD 7-8
NVP	30.9 ± 0.7	38.6 ± 0.9	36.5 ± 0.9
SE	30.5 ± 0.7	37.4 ± 1.1	37.5 ± 1.4
p	NS	NS	NS
Body Weight (g)			
NVP	483 ± 10	438 ± 8	429 ± 8
SE	485 ± 12	445 ± 13	429 ± 13
p	NS	NS	NS
Colonic Temperature (^{o}C)			
NVP		37.5 ± 0.1	35.8 ± 0.1
SE		37.4 ± 0.1	37.2 ± 0.1
Unburned		36.9 ± 0.1	37.2 ± 0.1
p		NS	NS

The second trial was performed to determine whether the initial differences in weight loss between NVP and SE rats resulted from differences in food intake or some unrecognized difference in energy expenditure. When eight SE animals were fed the average intake of nine NVP animals, both groups lost weight at the same rate (Table 2). Once again, the hypermetabolic and thermoregulatory responses to gram positive and gram negative wound infections were not significantly different on PBD's 3-4 or 7-8. As in the previous trial, the burned seeded animals were not febrile.

The NVP and SE seeded wounds averaged 10^6 CFU/g on PBD's 7-8. Eighty to 90% of the viable bacteria were the species placed on the wound at the time of injury. One of 18 NVP animals and 1/17 SE rats were bacteremic on PBD 8. Plasma samples from eight NVP and eight SE rats did not contain measurable amounts of LPS on PBD 8.

Subcutaneous Endotoxin Infusion. In this study, NVP endotoxin was infused into the subcutaneous tissue beneath unseeded wounds for seven days following injury. Fifteen rats were infused at a rate of 2.6 µg LPS/100 g/h, while 15 others received 12.6 µg LPS/100 g/h. Thirteen burned rats received normal saline. All infusates were sterile.

Table 3. Effects of continuous subcutaneous LPS infusion on resting metabolic rate of burned rats.

GROUP	Number	METABOLIC RATE (W/m^2, mean ± SEM)			Incidence of Endotoxemia
		PBD 0	PBD 3-4	PBD 7	PBD 7
Saline	13	34.7 ± 0.4	32.8 ± 0.5	34.7 ± 0.5	1/13
Low dose*	15	33.9 ± 0.6	34.6 ± 0.9	34.4 ± 0.5	1/15
High dose**	15	33.9 ± 0.5	35.3 ± 0.5	35.9 ± 0.7	1/15

*   2.6 µg LPS/100 g/h
** 12.6 µg LPS/100 g/h

Continuous subcutaneous infusion of NVP endotoxin did not significantly raise the metabolic rate of burned unseeded rats above the burned unseeded controls (Table 3). Consequently, within this LPS dose range, subcutaneous infusion of LPS will not result in the hypermetabolism observed following gram negative burn wound infection (Figure 8). LPS infusion had no effect on Tc, and none of these rats were bacteremic on PBD 7.

One rat in each of the three infusion groups was endotoxemic on PBD 7. In each case, the metabolic rate of the endotoxemic animal was above the mean for that group - 35.6 W/m^2 for the rat receiving sterile saline, 37.7 W/m^2 for the animal receiving low dose LPS, and 41.4 W/m^2 for the high dose animal (Table 3).
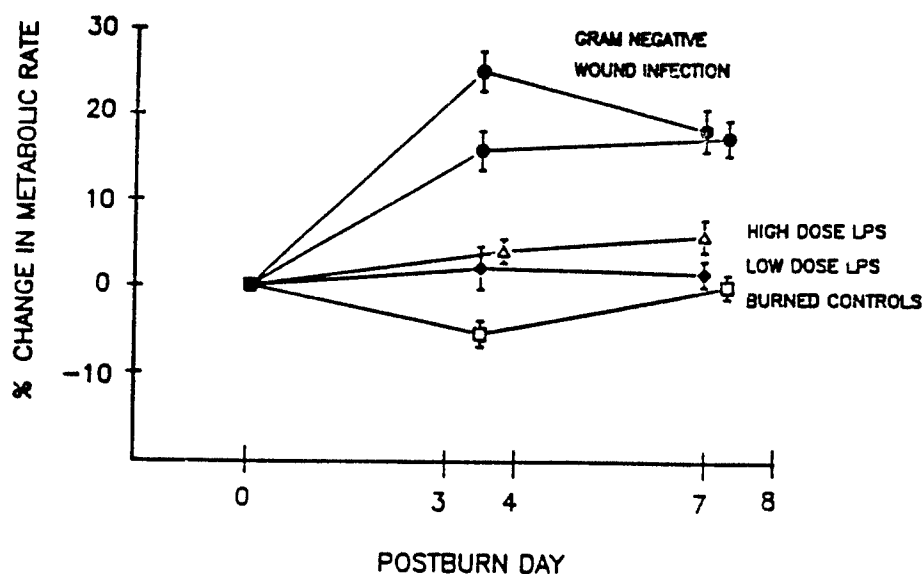
Figure 8. Seeding the burn wound with **P. aeruginosa** resulted in a greater hypermetabolic response than when endotoxin (LPS) from this same bacteria was continuously infused into the subcutaneous tissue beneath the unseeded wound (p<0.01, unpaired t-tests + Bonferroni). Two LPS doses were employed (low = 2.6 μg/100 g/h, high = 12.6 μg/100 g/h).

Effects of Wound Colonization. Temporal differences in resting oxygen consumption and colonic temperature between seeded and unseeded animals are best explained by differences in the degree of localized burn wound colonization. Seeding the burn created an abrupt increase in wound bacterial content (10^6 CFU/g by PBD's 3-4) which then remained relatively constant over the next two weeks. Associated with this level of colonization was an increase in resting $\dot{V}O_2$ and Tc. In contrast, the unseeded wound colonized more slowly and reached a comparable level of colonization sometime during the second week post injury. During the first week, when there was a significant difference in wound colony count between the two groups, the seeded animals had higher resting oxygen consumptions and central temperatures. When the bacterial count in the unseeded wounds reached that of the seeded wounds, there were no longer any measurable differences in $\dot{V}O_2$ or Tc between these two groups of burned animals.

Resting $\dot{V}O_2$ of this rat model varies with the extent of infection and reaches its highest levels following wound invasion and systemic infection (14). For this reason, some of the difference in $\dot{V}O_2$ between the two groups on PBD's 3-4 may reflect the greater incidence of systemic infection in the seeded rats. It does not, however, account for the difference in $\dot{V}O_2$ on PBD's 7-8 when both groups had the same relative number of bacteremic animals. An increase in the incidence of systemic infection will also not explain the marked rise in $\dot{V}O_2$ and Tc of the unseeded group during the second week. So, while systemic infection will increase resting $\dot{V}O_2$ of burned rats, it is not considered responsible for the differences in O_2 uptake observed between these two groups of burned animals.

The correlation between wound colony count and resting $\dot{V}O_2$ of the non-bacteremic animals supports the contention that bacteria begin to exert systemic metabolic effects while confined to the burn wound (Figure 6). Since bacterial contamination of the burn wound begins very soon after injury, it is difficult to assess the hypermetabolic response to the injury alone, but the data suggest that animals with sterile burn wounds would not be very hypermetabolic. At what point wound bacteria begin to affect $\dot{V}O_2$ is equally difficult to establish. The unseeded animals in this study were hypermetabolic on PBD's 3-4 when the average wound bacterial count was 10^4 CFU/g. However, in an earlier study of the same model, we found no increase in $\dot{V}O_2$ during the first week (14). In both this and the earlier study, the major increase in $\dot{V}O_2$ developed during the second week when wound colony count rose from 10^3 to 10^6 CFU/g (Figure 2). Since the delay between injury and hypermetabolism can be reduced by seeding the wound, it appears that bacteria are essential to the metabolic response to thermal injury. The important thing is not so much when the metabolic effects of wound bacteria first become apparent, but rather that they appear in a dose-response manner before microorganisms are discovered in the blood and the animal (or patient) is considered "infected".

While the correlation between wound colony count and $\dot{V}O_2$ of non-bacteremic rats was significant, variations in the data suggest that there are several factors affecting metabolic rate other than the number of viable bacteria in the wound. One factor may be the bacterial strain involved. In a previous study, topical antimicrobial agents appeared to be more effective

in blunting the hypermetabolic response to some bacteria than others (14). Another factor may be variations in the immunocompetence of the rat. The capacity to mount an inflammatory response, phagocytize and kill bacteria and release endogenous mediators all probably contribute to the composition of the afferent limb for postburn hypermetabolism.

The relationship between resting $\dot{V}O_2$ and burn wound bacterial count has not been previously reported. In fact, Demling et al (30) were unable to show any relationship between wound bacterial count and the $\dot{V}O_2$ of burned sheep. There are several explanations for these differences. First, the burn represented 30% of the total body surface area of the rat and only 15% in the sheep. Consequently, any impact of the wound would be more pronounced in the rat. Second, a greater portion of the wound was cultured in the rat (60% of the total wound) than in the sheep (one cubic centimeter) thereby providing a better estimate of the average wound count in the rat. Third, given the variation in the relationship between wound bacterial count and resting $\dot{V}O_2$, as derived in 44 non-bacteremic rats, it is not surprising that the previous investigators were unable to find any relationship in only 15 animals.

The high incidence of positive blood/spleen cultures in the seeded animals at two weeks post burn was unexpected since these rats were not clinically septic. Unlike previous work (14), the increased incidence of bacteremia in the seeded animals was not associated with a major increase in resting metabolic rate above that of the non-bacteremic, unseeded rats (Figure 3). The most likely explanation for this difference is that bacteremic animals represent varying degrees of systemic infection. In both this and the earlier study, animals were sacrificed as soon as they appeared clinically septic. This was a more common problem in the earlier study, especially in the unseeded animals. In fact, one reason we went to the NVP seeded model was because they rarely became septic, and when they were sacrificed at three weeks post burn, they were not bacteremic. Taken together, the results of these two studies suggest that NVP seeded rats may develop low grade bacteremia which rarely progresses to clinical sepsis. Since the metabolic effects of wound bacterial growth appear as a continuum, there may be no abrupt change in the energy expenditure at the transition from localized to systemic infection.

Core temperature of the burned, non-bacteremic rats was correlated with changes in resting oxygen uptake. This relationship probably reflects an increase in metabolic heat production in response to an upward shift in central reference temperature. Since the burned animal (or patient) has a limited capacity to increase heat conservation in order to raise body temperature, it must increase heat production to become febrile. Unlike the increase in $\dot{V}O_2$, there was no apparent relationship between wound colony count and Tc of the non-bacteremic rats. In fact, during the first postburn week, the unseeded animals were hypermetabolic but remained afebrile. Hypermetabolism without fever has been described earlier in a variety of burn models (14,31,32). This separation may reflect (a) different afferent mediators, (b) separate metabolic and temperature control centers, each with different thresholds for the same afferent mediator, or (c) a combination of both. At this point, the issue is not so much why they are different but rather that there appears to be separate metabolic and thermoregulatory drives in burned animals.

Effects of Wound Endotoxin. To determine whether wound endotoxin contributed to the hypermetabolism of the NVP seeded rats we first compared the responses of SE and NVP seeded animals and then attempted to reproduce the NVP response with chronic, subcutaneous LPS infusion. The results of these studies clearly indicate th.•t endotoxin, released in the burn wound, is not an obligatory mediator of the hypermetabolic response. This was demonstrated in several ways, but the most irrefutable was the fact that rats with gram positive wound infections (wounds containing little or no LPS) were as hypermetabolic as animals w.th gram negative infections (Table 2). In two separate trials, metabolic rate ranged from 16 to 25% above preburn levels during the first postburn week. In the first study, the NVP rats lost weight more rapidly, but this proved to be a result of differences in food intake rather than energy expenditure. While the SE seeded animals were as hypermetabolic as their NVP counterparts, this did not indicate that LPS played no role in the hypermetabolic response to gram negative wound infection. It did reveal, however, that gram positive bacteria and/or their products are equally effective hypermetabolic stimuli.

Unlike the NVP seeded rats described above, these NVP (and SE) animals remained afebrile. This difference in the thermoregulatory responses of seeded animals reflects the more limited hypermetabolic response in the latter groups (20-26% increase in $\dot{V}O_2$ by PBD's 7-8 versus the 30-34% increases recorded earlier) and the general relationship between $\dot{V}O_2$ and Tc of non-bacteremic burned rats following injury (Figure 7).

Since endotoxin produced in the burn wound is not essential for the hypermetabolic response, the question is whether LPS plays any measurable role in the hypermetabolic response to gram negative wound infection. If it does, of equal import is whether endotoxin acts within the wound or exerts its effects only after entering the circulation.

None of the eight NVP animals tested were endotoxemic on PBD's 7-8. Likewise, Jones et al (33) found that mice with P. aeruginosa infected burn wounds were not endotoxemic on PBD 2. So, while we may have missed endotoxemic episodes during the first week post injury, the data suggest that the hypermetabolism on PBD's 7-8 was not the product of circulating LPS. Rats are relatively insensitive to LPS and, like humans and other animals, become increasingly tolerant with long-term treatment. Fish and Spitzer (15), for example, have shown that continuous intravenous infusion of E. coli LPS has only transient effects on the metabolic rate of normal rats. Their endotoxemic rats were only hypermetabolic the first two days of a 7-day infusion. Since our NVP seeded animals were not endotoxemic, continuous intravenous LPS infusion is not considered an appropriate way to study the response to burn wound endotoxin. For this reason, we decided to estimate the maximum amount of LPS in the NVP seeded wound and to infuse at least this amount into the subcutaneous tissue subjacent to the wound.

The rate of LPS delivery in the NVP seeded wound is based on estimates of the amount of LPS in each NVP bacterium and the number of NVP cells in the wound. Bacterial weight has been reported to range between 10^{-12} to 10^{-13} g with 75% of the weight being water (34). If 6-9% of the dry cell weight of P. aeruginosa dry cell weight is LPS (35), each NVP cell can contain as much as 22×10^{-15} g LPS.

The 30 gram NVP seeded wound contains 10^6-10^7 viable NVP/g during the first two weeks after injury. If, in this stationary growth phase, 5×10^8 new bacteria are formed and an equal number die every 30 minutes, 2.4×10^{10} dead NVP are deposited into the wound each day. At 22 femtograms of LPS per bacterium, over 500 μg of endotoxin is released into the wound each day. If all of this LPS enters the circulation during the 24-hour period, the maximum rate of LPS delivery would be 4 μg/100 g body weight/hr. This is one-third the intravenous dose necessary to produce even a transient hypermetabolism in unburned rats (15).

Continuous subcutaneous infusion of LPS at 2.6 and 12.6 μg/100 g/h had little, if any, measurable effect on the resting metabolic rate of burned rats (Figure 8, Table 3). Since both doses equal or exceed the estimated maximum rate of LPS production in the NVP seeded wound, the hypermetabolic response to gram negative wound infection appears to involve more than LPS. Others have reported that mice are less responsive to subcutaneous injections of LPS than when the same amount of endotoxin was injected intramuscularly or intravenously (34). The basis for this difference remains undefined, but the data support the contention that any wound endotoxin reaching the underlying subcutaneous tissue may have limited systemic metabolic impact.

CONCLUSIONS

The results of this study demonstrate that wound bacteria contribute to the postburn hypermetabolism of 30% TBSB rats. The hypermetabolic effects of localized burn wound infection develop in a dose-response manner before microorganisms appear in the bloodstream. Conversely, there is little, if any, evidence to suggest that rats with sterile wounds would be hypermetabolic. The highly variable relationship between wound bacterial count and resting $\dot{V}O_2$ indicates that the number of viable bacteria in the wound is not the only determinant of postburn hypermetabolism. Since gram negative and gram positive wound infections produce comparable hypermetabolic responses, wound bacteria appear to act more like non-specific stimuli than unique afferent mediators of postburn hypermetabolism. Since hypermetabolic burned rats are not enuotoxemic and gram negative infected wounds do not appear to contain enough LPS to make them hypermetabolic, LPS cannot be considered an afferent mediator or the sole inducer of afferent mediators of postburn hypermetabolism.

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