Nutrient Modulation of Inflammatory and Immune Function

Frank B. Cerra, MD, FACS, FCCM, Minneapolis, Minnesota

The metabolic response to injury occurs after a diverse group of surgical injuries including major surgical intervention, shock, infection, and sources of inflammation such as pancreatitis. The response is mediated by the macroendocrine system, the autonomic nervous system, and the cell-cell communication system. The clinical manifestations include now well-described clinical, physiologic, and metabolic characteristics.

The approach of aggressive source control, invasive circulatory resuscitation, and nutrition/metabolic support has been associated with an overall reduction in morbidity and mortality. In those patients who do not respond to this approach, the disease process progresses to mutiple organ failure syndrome with its associated high mortality. Altering the route of feeding, preventing single nutrient and generalized nutrient deficiency, and reducing nosocomial infections with selective gut decontamination have not significantly altered the course or outcome of the disease process in this latter group of patients with persistent hypermetabolism.

The available data support the position that this persistent hypermetabolism represents abnormal metabolic regulation resulting in persistence of the inflammatory response with associated suppression of the immune defenses. A number of research approaches are being taken to understand and modulate this abnormal state of regulation. Because of the role of specific nutrients in these regulatory processes, beyond their role in classic nutrition support, nutrients such as arginine, n-3 polyunsaturated fatty acids, and RNA are being evaluated for their ability to modulate inflammation and to improve immune function. Preliminary results are encouraging.

This communication presents an overview of a developing area of surgical research and therapy, that of using nutrients as a means of altering the inflammatory response and immune function. This approach of nutrient pharmacology, or the administration of a nutrient targeted to perform or induce a specific metabolic function that has not usually been associated with nutrition support, is an outgrowth of the nutrition/metabolic research performed in surgical intensive care units in patients with persistent hypermetabolism and multiple organ failure following trauma/burns, surgery, or surgical sepsis.

The initial focus of this research was the application of classic nutrition support principles to this group of patients who manifested a rapidly developing form of malnutrition with associated organ dysfunction, nosocomial infections, and wound failure. After therapy directed at the control of the initiating event and the restoration and maintenance of oxygen transport, nutrition support was added as a potential therapeutic modality. Some reduction in morbidity and mortality was observed, but appeared to be primarily related to the prevention of single nutrient or generalized nutrient deficiencies. The manifestations of the disease process persisted.

The concept evolved that this phase of postresuscitative hypermetabolism represented a pathologic state of persistent inflammation with suppression of immune function. With persistence, this pathologic process could result in parenchyme dysfunction and multiple organ failure with its associated high mortality rate.

The research emphasis became one of learning when and how to modulate the processes of inflammation and repair and immune function. The cells of greatest interest have become the white blood cell, the macrophage, and the lymphocyte, and the focus of research is on the regulation of individual cell and organ function. Because of the known roles of specific nutrients in these regulatory functions, nutrients came to be recognized as potential tools for their modulation. This communication summarizes the current rationale and experience with a few of these nutrients: arginine, n-3 polyunsaturated fatty acids (n-3PUFA), and RNA. Data from human studies serve as the primary database for the discussion.

BACKGROUND TO DEVELOPING TARGETED NUTRIENTS

The group of patients in whom the research has been performed have a number of general characteristics: an event that is known to stimulate an inflammatory response, a period of circulatory resuscitation, a clinical response best described as persistent hypermetabolism, and an increased risk for major complications and organ failures [1]. The initiating events that have been associated with this response include trauma, burns, major

From the Department of Surgery, University of Minnesota, Minneapolis, Minnesota.

Requests for reprints should be addressed to Frank B. Cerra, MD, Department of Surgery, University of Minnesota, 406 Harvard Street, Southeast, Box 42 UMHC, Minneapolis, Minnesota 55455.

general and thoracic surgery, infections with sepsis, pancreatitis without infection, ruptured aneurysms, shock of any etiology, and prolonged periods of inadequate resuscitation from shock states. The clinical, physiologic, and metabolic responses that are observed represent one manifestation of what has been called the metabolic response to injury, since it affects end-organs such as heart, lung, kidney, liver, gut, muscle, and blood vessels. The metabolic response to injury also consists of the mediator systems that modulate the response organs. Some of these mediators are listed in **Table I**.

The effects of each of the potential mediators have been studied using the particular mediator of interest as an isolated effector either *in vitro* or *in vivo*. Several hormones, such as cortisol and catecholamine, have been found to be essential to both immediate and long-term survival. Others, such as eicosanoids, appear to be less essential to immediate survival and seem to serve as modulating agents of the metabolic response at a local level, as in the regulation of microcirculatory blood flow. The manifestations in the patient of this entire process represent the clinical, physiologic, and metabolic phenomena that have been described [*2,3*]. These manifestations are briefly summarized in **Table II**. It is important to remember that these characteristics were derived from observations taken during the phase of postresuscitative hypermetabolism. They, therefore, are not necessarily reflective of the injury, resuscitative, or organ failure phases of the overall response to injury. As such, however, these observations represent characteristics that can be used as monitored or outcome criteria in studies that are directed at either determining mechanisms of injury and response, or in studies that are evaluating a new therapeutic or support modality.

The general treatment program for this clinical problem consists of control or removal of the cause, circulatory resuscitation, and early nutrition/metabolic support (**Figure 1**) [*1,2*]. Overall reductions in morbidity and mortality were observed as well as improved outcomes reflective of the individual therapies [*4*]. These benefits appeared to reflect the treatment approach. The improved outcome following the institution of early fracture fixation and achievement of the upright chest immediately after polytrauma is one such example [*5*]. Early, invasive circulatory resuscitation using oxygen transport criteria has been associated with less organ failure and an improved outcome in general surgery patients [*6,7*]. Perioperative nutrition, primarily by the intravenous route, has been associated with a reduction in nutritionally related morbidity and mortality in those patients with moderate to severe starvation malnutrition who are undergoing surgical therapy [*8*].

Despite these results, however, the mortality risk for developing multiple organ failure syndrome remains high, as does the mortality risk from that disease. Single-agent attempts to modulate the response to injury, as in corticosteroids, inhibitors of eicosanoid production, and free radical scavengers, have not had substantial beneficial clinical effect [*9*]. In the case of nutrition/metabolic support, the beneficial effects derived from the treatment or prevention of starvation-induced single nutrient defi-

TABLE I
Potential Mediators of Metabolic Response to Injury

- Cytokines
  - Interleukin 1-8
  - Interferon γ
  - Platelet-activating factor
  - Tumor necrosis factor
- Eicosanoids
  - Prostaglandins (PGE_1, PGE_2, PGI_2)
  - Thromboxanes (TxA_2)
  - Leukotrienes (LTC_4, LTD_4)
- Mediator amines
  - Histamine
  - Serotonin
  - Epinephrine
  - Norepinephrine
  - Octopamine
- Opioids/other neurotransmitters
- Hormonal amines/peptides
  - Thyroxine
  - Growth hormone
  - Insulin
  - Glucagon
  - Catecholamine
  - Cortisol
  - Cortical releasing factors
- Complement
- Kinin
- Fibronectin
- Growth factors
- Enzymes
  - Proteases (acid and neutral)
  - Other lysosomal enzymes
- Nitric oxide (derived from L-arginine)
- Oxygen-derived intermediates

TABLE II
Characteristics of the Metabolic Response to Injury

- Clinical observations
  1. Persistent inflammatory response
     Fever, leukocytosis, physiology metabolism, activated macrophage
  2. Nosocomial infections
  3. Wound failures
  4. Malnutrition
     Altered body composition, single or generalized nutrient deficiencies
  5. Organ failures
     Lung, liver, kidney, gut
  6. Immune dysfunction
     Anergy, decreased B-cell function, decreased T-cell function
- Physiologic observations
  1. Increased oxygen consumption demand
  2. Increased cardiac output and oxygen delivery
  3. Increased demand for ventilation
  4. Decreased total systemic vascular resistance
  5. Decreased saturation of the central venous hemoglobin with a narrowed arteriovenous oxygen content difference
- Metabolic observations
  1. Increased gluconeogenesis and lipolysis
  2. Relative nonresponsiveness of the gluconeogenesis and lipolysis to downregulation by exogenous glucose
  3. Energy production by the process of aerobic glycolysis
  4. Utilization of a mixed carbon source for energy production: glucose, fatty acids of all chain length, and amino acids
  5. Net total body catabolism
  6. Change in body composition characterized by
     a. Reduction in lean body mass that is poorly responsive to exogenous amino acids and anabolic hormone modulation
     b. Redistribution of the body nitrogen to areas of active protein synthesis such as the viscera, wounds, and white cell mass

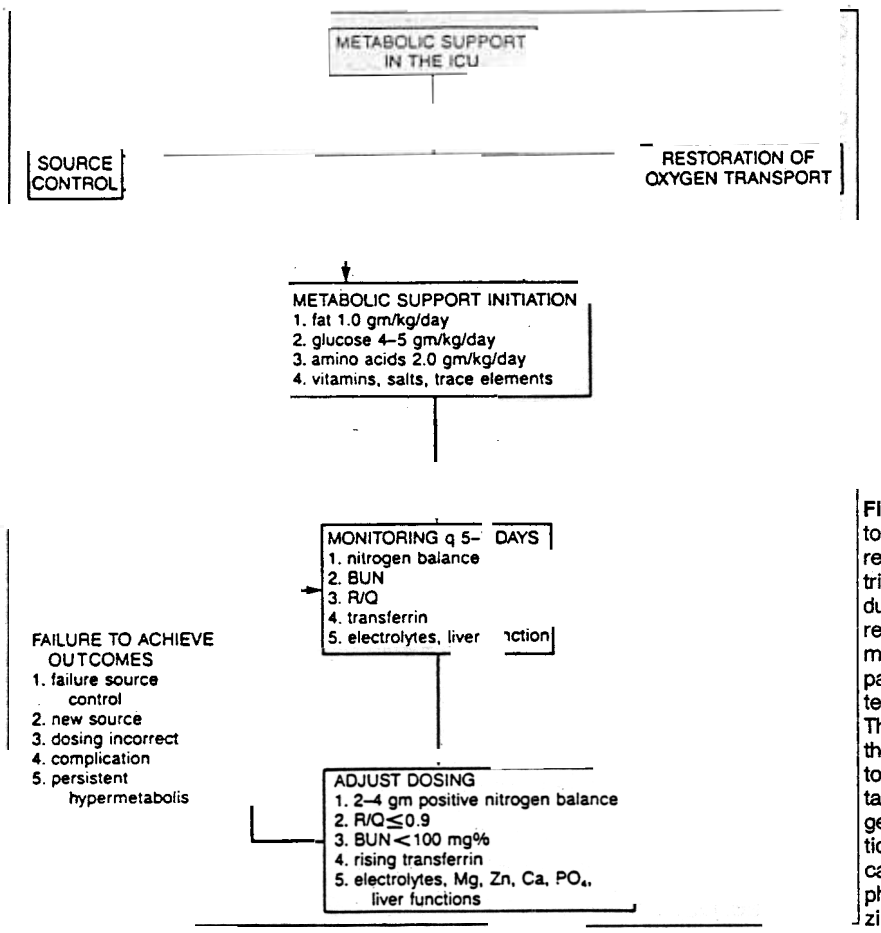

Figure 1. The general support regimen is to remove the cause, provide circulatory resuscitation, and then to administer nutrition/metabolic support. An overall reduction in morbidity and mortality has resulted from this approach. There remains, however, a significant group of patients who proceed to develop persistent hypermetabolism and organ failure. This persistent inflammatory response is the object of research that is attempting to modulate it and to improve host resistance. Several nutrients have been targeted as having activity for these functions. BUN = blood urea nitrogen; Ca = calcium; Mg = magnesium; PO_4 = phosphate; R/Q = respiratory quotient; Zn = zinc.

ciency or starvation-induced generalized nutrient deficiency [*10*]. Little direct effect on the disease process of hypermetabolism and organ failure has been observed. This was likewise so for the immune failure, wherein current nutrition support seems to have little effect on the disease-related dysfunctions of the specific or nonspecific immune defenses [*11,12*].

With respect to this latter problem, research focused on the route and timing of the nutrient administration. Enteral nutrition was observed to be safe and effective in surgical intensive care unit patients [*13*]. When nutrition is begun 3 to 4 days after the injury event, the incidence and mortality of multiple organ failure syndrome are not altered by the route of administration [*14*]. Very early enteral nutrition, however, has been associated with a lessening of the metabolic response to injury and fewer infections in animal models of burn injury and in a few, prospective clinical trials in trauma patients [*15,16*]. Further clinical trials are now in process.

The translocation hypothesis implicates the gut aerobic flora in the pathogenesis of nosocomial infections [*17*]. A number of approaches are underway to evaluate and treat the putative defect(s) in the gut mucosal barrier and to reduce the effects of the gut aerobic flora. These approaches range from mucosatrophic agents such as enteral nutrition and glutamine, to antibiotic regimens that are designed to selectively suppress the gut aerobic flora [*18,19*]. In the former case, clinical trials are inconclusive. In the latter case, clinical trials indicate that the incidence of nosocomial infections can be significantly reduced. This reduction in nosocomial infections, however, has not been accompanied by a reduction in either organ failure or mortality. In another study in trauma patients, sequential sampling of the portal vein blood after injury and resuscitation did not reveal the presence of either bacteria or endotoxin.

An overview of the data would support the position that after the cause has been controlled and appropriate circulatory resuscitation has occurred, a phase of systemic inflammation is entered that lasts for variable lengths of time and has variable outcomes. In some cases, such as those in which the cause is rapidly removed and resuscitation and supportive care rapidly begun, the hypermetabolism is short-lived and abates over several days. In others, the inflammation progresses to repair and fibrosis, and the surgical intensive care unit course is prolonged. An example of the latter would be the fibrosing phase of adult respiratory distress syndrome. In yet another scenario, the hypermetabolism persists and multiple organ failure ensues.

The research focus is shifting toward trying to understand the regulation of the processes of inflammation and repair and to find ways to modulate them to promote wound healing, decrease organ dysfunction and failure,

and return immune function to a homeostatic, responsive state. A number of approaches are being taken, ranging from antibodies against mediators such as endotoxin and tumor necrosis factor, to finding clinically useful ways to directly affect the cellular responses of inflammation and repair.

NUTRIENTS TARGETED TO MODULATE INFLAMMATION AND IMMUNE FUNCTION

Specialty nutrients are in this latter category. Because of the integral role of some nutrients in the regulatory process of inflammation, such as second-messenger generation, eicosanoid production and release, and lymphocyte proliferation in response to antigenic stimuli, nutrients are being investigated as a possible way to modulate inflammation. The nutrients being evaluated are those of a specialized nature and not necessarily part of a general nutrition regimen in either the dose or composition used. Nutrient deficiencies that could affect immune function, such as zinc deficiency, are well-handled with current nutrition regimens. The speciality nutrients that will be discussed are arginine, RNA, and n-3 PUFA.

Arginine: Arginine is a nitrogen-dense amino acid classified as semi-essential because it is required for growth and in post-traumatic states [*20*]. It is a potent endocrinologic secretagogue that can stimulate the release of growth hormone, prolactin, insulin, and glucagon. In cell culture systems, arginine is essential for the growth, but not the viability, of cells. *In vitro* cytokine production and release can occur effectively in the absence of arginine. The putative origin of these growth effects resides in the biochemistry of arginine since it is an essential component of polyamine and nucleic acid synthesis and would thus be necessary for mitotic responses.

Arginine is also a major source of nitrous and nitric oxide *in vitro* and *in vivo* [*21*]. The nitrous and nitric oxides are important mediators of vascular dilation, protein synthesis in hepatocytes, and electron transport in hepatocyte mitochondria.

Under experimental conditions, a number of *in vivo* immune effects have been observed with the administration of arginine. Some of these include increased survival in septic animals; increased survival of tumor-bearing animals, a phenomenon associated with reduced tumor size; an increase in the number of T cells and delayed-type hypersensitivity responses in athymic nude mice; increased thymic and peripheral blood lymphocyte responses in *in vitro* assays of mitogen-induced blastogenesis; and increased allograft rejection in rodents [*20*].

In humans, arginine has been associated with increased thymic and peripheral blood lymphocyte *in vitro* blastogenic responses to mitogens; and preservation of these same responses in surgical and in surgical intensive care unit patients. Recent data also associate arginine supplementation with reduced length of stay following major cancer surgery [*20,22*].

RNA: Purines and pyrimidines are precursors of DNA and RNA, which are necessary for protein synthesis and cell mitosis. Purines and pyrimidines have not been considered essential dietary nutrients. Excess dietary sources are excreted. The liver may be a major source of endogenous purines and pyrimidines for other tissues, but comparative contributions are not well documented. In addition to *de novo* synthesis from amino acids, bases or nucleosides from cell degradation can be reutilized through salvage pathways [*23*].

Restriction of dietary nucleotides results in suppression of cellular immune responses and prolongation of rodent allograft survival. The putative origin of these phenomena lies in an inability of these T cells to undergo blastogenesis in response to antigenic stimuli [*24*]. Uracil administration can restore delayed-type hypersensitivity responses to various foreign antigens in mice; can stimulate T-cell antigenic proliferative responses in T cells in mice; and can reduce abscess formation due to gram-positive organisms in mice. Dietary nucleotides may also be effective in macrophage activation of the T helper/inducer populations. Uracil has been reported to reverse the immunosuppression associated with blood transfusion in experimental settings [*24*].

Such observations would support the position of an additional dietary requirement for purines and pyrimidines, or at least uracil, under conditions of metabolic stress. Perhaps the salvage pathways become ineffective, synthesis is suppressed, or the requirements are greater than realized in these metabolic stress settings.

n-3 PUFA: A major component of the cell membrane is the polyunsaturated fatty acids (PUFA). These compounds are responsible for the structural and functional integrity of those membranes' eicosanoid production and release, and signal transduction through the phospholipid-dependent second-messenger pathways. The major PUFA constituent in the membrane is of the n-6 family derived from vegetable oils. There are very low levels of the n-3 family, major constituents of fish oils, primarily resulting from the low intake of fish high in these oils in the North American diet. The n-3 PUFA can preferentially replace the n-6 PUFA, altering the physiologic characteristics of the membrane to such stimuli as lipopolysaccharide (LPS) [*25*].

The incorporation of n-3 PUFA such as eicosapentanoic (EPA) acid (20:5n-3) into macrophages occurs within 3 to 6 hours in cell culture and is stabilized within a few days *in vivo* [*26*]. Once incorporated, the melting point decreases, fluidity decreases, and inositolphosphate production, dienoic eicosanoid release, and interleukin and tumor necrosis factor release in response to LPS are reduced *in vitro* and *in vivo* [*27*].

The release of dienoic eicosanoids and tumor necrosis factor and interleukin-1 release by the macrophage are directly related to the n-6/n-3 ratio, n-3 and n-6 total PUFA content, and relative PUFA composition in the cell membrane. In general, as the n-6 content increases, and the n-6/n-3 PUFA ratio increases over 1, the more dienoic eicosanoids are produced within and released from the cell membrane [*26*]. These two series of eicosanoids have different potency. The eicosapentanoic acid (20:5n-3) products are less inflammatory than those of linoleic acid (18:2n-6). A relative excess of linoleic acid substrate stimulates prostaglandin E_2 production, which

decreases the ability of cytokines to stimulate interleukin-2 synthesis by endothelial cells, and suppresses T-cell proliferative responses in response to lectin and specific antigen stimulation [25–28].

In animal models, n-3 PUFA incorporation into cell membranes of the hepatic macrophages and into the hepatocytes is stabilized within 3 to 5 days and is associated with a reduction in the n-6 PUFA content [26]. In rat models of bacterial peritonitis, n-3 PUFA in the diet was associated with a reduction in mortality [28]. In the mouse popliteal lymph node assay system, dietary n-3 PUFA as refined menhaden oil was associated with a return of the lymphocyte proliferative response to a magnitude equivalent to that with uracil administration.

COMBINED AGENT THERAPY

These three nutrients have been combined and administered as part of a nutritional support regimen. This approach was based on the rationale that the persistent inflammation observed in the clinical setting represented, in part, the presence of persistently activated macrophages and was associated with a depression of T-cell proliferative responses to specific antigenic stimulation, possibly related to excess prostaglandin E_2 production and excess cytokine release from the activated macrophages. In the clinical setting of source control, adequate circulatory resuscitation, and state-of-the-art nutrition/metabolic support, it has been hypothesized that n-3 PUFA would decrease macrophage prostaglandin E_2 and cytokine release and stimulate T-cell proliferative responses, and that arginine and RNA would act to directly stimulate T-cell proliferative responses.

The first studies from these human trials indicate that the additional selected nutrients are associated with a return to the *in vitro* T-cell proliferative responses to levels above those of normal, nonstressed man [29,30]. This result seems to occur independent of the nutritional outcomes of improved visceral protein synthesis and the achievement of nitrogen balance. The control group also achieved nitrogen balance and improved visceral protein synthesis, but with an associated continued depression of the *in vitro* immune function assays. Clinical outcome studies evaluating length of stay and the incidence of nosocomial infections are currently in progress.

REFERENCES

1. Cerra FB. The syndrome of multiple organ failure. In: Cerra FB, Bihari D, eds. New horizon series: cell injury and organ failure. Fullerton, CA: Society of Critical Care Medicine, 1988: 1–25.
2. Siegel JH, Cerra FB, Coleman B, *et al.* Physiologic and metabolic correlations in human sepsis. Surgery 1979; 86: 163–93.
3. Cerra FB. Hypermetabolism, organ failure and metabolic support. Surgery 1987; 101: 1–14.
4. Cerra FB. Multiple organ failure: clinical trade-offs. In: Lumb PD, Shoemaker WC, eds. Critical care: state of the art. Fullerton, CA: Society of Critical Care Medicine, 1990: 15–32.
5. Seibel R, LaDuca J, Hassett J, *et al.* Blunt multiple trauma, femur traction, and the pulmonary failure-septic state. Ann Surg 1985; 202: 283–95.
6. Shoemacher W. Hemodynamic and oxygen transport patterns in septic shock: physiologic mechanisms and therapeutic implications. In: Sibbald, Sprung, eds. Prospectives in sepsis and septic shock. Fullerton, CA: Society of Critical Care Medicine, 1985: 203–34.
7. Siegel JH. Through the glass darkly: the lung as a window to monitor oxygen consumption, energy metabolism and severity of critical illness. Clin Chem 1990; 36: 1585–93.
8. Buzby G. Perioperative nutrition support. JPEN J Parenter Enteral Nutr 1990; 14: 197S–9S.
9. Bone RC, Fisher CJ, Clemmer TP, *et al.* A controlled clinical trial of methylprednisolone in the treatment of severe sepsis and septic shock. N Engl J Med 1987; 317: 653–8.
10. Cerra FB. How nutrition intervention changes what getting sick means. JPEN J Parenter Enteral Nutr 1990; 14: 64–9.
11. Fry DE, Polk HC. Hose defense and MOF. In: Richardson JD, ed. Trauma: clinical care and pathophysiology. Chicago: Year Book Medical Publishers, 1986: 41.
12. Konstantinides FN, Kaproth PL, Cerra FB. Other aspects of metabolic monitoring in critically-ill patients. Clin Chem 1990; 36: 1594–1603.
13. Moore EE, Jones TN. Benefits of immediate jejunostomy feeding after major abdominal trauma: a prospective, randomized study. J Trauma 1986; 26: 874–81.
14. Cerra FB, McPherson J, Konstantinides N, *et al.* Enteral nutrition does not prevent MOFS after sepsis. Surgery 1988; 104: 727–32.
15. Jenkens M, Gottschlich M, Alexander JW, Warden GD. Effect of immediate enteral feeding on the metabolic response following severe burn injury. JPEN J Parenter Enteral Nutr 1989; 13 (suppl): 12S.
16. Moore FA, Moore EE, Jones TN, *et al.* TEN vs TPN following major abdominal trauma: reduced septic morbidity. J Trauma 1989; 29: 916–23.
17. Alexander JW. Nutrition and translocation. JPEN J Parenter Enteral Nutr 1990; 14: 154S–170S.
18. Souba WW, Herskowitz TR, Austgen MK, *et al.* Glutamine nutrition: theoretical considerations and therapeutic impact. JPEN J Parenter Enteral Nutr 1990; 14: 237S–44S.
19. Ramsey G, Ledingham I. Management of multiple organ failure: control of the microbial environment. In: Bihari DJ, Cerra FB, eds. New horizons: multiple organ failure syndrome. Fullerton, CA: Society of Critical Care Medicine, 1989: 327–37.
20. Barbul A. Arginine and immune function. Nutrition 1990; 6: 53–9.
21. Stuehr D, Gross S, Sakuma I, *et al.* Activated murine macrophages secrete a metabolite of arginine with the bioactivity of endothelium-derived relaxing factor and the chemical reactivity of nitric oxide. J Exp Med 1989; 169: 1011.
22. Reynolds JV, Thom AK, Zhang SM, *et al.* Arginine, protein calorie malnutrition and cancer. J Surg Res 1988; 45: 513–22.
23. Sonada T, Tatibana M. Metabolic fate of pyrimidines and purines in dietary nucleic acids ingested by mice. Biochem Biophys Acta 1978; 521: 55–66.
24. Kulkarni AD, Fanslow WC, Rudolph FB, *et al.* Effect of dietary nucleotides on response to bacterial infections. JPEN J Parenter Enteral Nutr 1986; 10: 169–71.
25. Kinsella J, Lokesh B, Boughton S, *et al.* Dietary PUFA and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. Nutrition 1990; 6: 24–45.
26. Cerra FB, Holman RT, Bankey P, *et al.* n-s PUFA as modulators of cell function in the ICU. Pharmacotherapy (in press).
27. Billiar TR, Bankey PE, Svingen BA, *et al.* Fatty acid intake and Kupffer cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation. Surgery 1988; 104: 343–8.
28. Cerra FB, Alden PA, Negro F, *et al.* Clinical sepsis, endogenous and exogenous lipid modulation. JPEN J Parenter Enteral Nutr 1988; 12: 63S–9S.
29. Cerra FB, Lehman S, Lonstantinides N, *et al.* Effect of enteral nutrient on *in vitro* tests of immune function in ICU patients: a preliminary report. Nutrition 1990; 6: 84–7.
30. Lieberman M, Shou J, Torres BS, *et al.* Effects of nutrient substrates on immune function. Nutrition 1990; 6: 88–92.