Herman Award Lecture, 1995: Infection-induced malnutrition—from cholera to cytokines\textsuperscript{1,2}

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ABSTRACT

Infection-induced malnutrition, the most common form of cytokine-induced malnutrition, results from the actions of proinflammatory cytokines, i.e., tumor necrosis factor (TNF) and interleukins 1, 6, and 8 (IL-1, IL-6, and IL-8). During acute generalized infections, these cytokines initiate the acute-phase reaction. This reaction is quite stereotyped, and includes fever, malaise, myalgia, headaches, cellular hypermetabolism, and multiple endocrine and enzyme responses. In addition, there is heightened catabolism of muscle proteins and many amino acids; flux of free amino acids into the liver; hepatic synthesis of acute-phase plasma proteins; sequestration of iron and zinc; gluconeogenesis; insulin resistance; impaired cellular uptake of fatty acids from plasma triglycerides; sizable losses of body nitrogen, potassium, magnesium, phosphate, and zinc; retention of body salt and water; heightened metabolic degradation and/or loss of vitamins; and an activation of the immune system. The pathogenesis of cytokine-induced malnutrition is thus vastly different from the malnutrition caused by uncomplicated starvation. Cytokine-induced malnutrition can have a devastating effect on the immune system and its functions. Although proinflammatory cytokines are found in mucosal fluids, where they contribute to the pathogenesis of inflammatory bowel diseases, it is not known whether cytokines play a role in toxigenic, secretory diarrheas such as cholera, which cause huge losses of body water, electrolytes, and bicarbonate while exhibiting no systemic manifestations of an acute-phase reaction. Am J Clin Nutr 1995;62:813–819

KEY WORDS

Infection, cytokines, interleukins, malnutrition, nitrogen, protein, lipids, amino acids, endocrinies, glucose, trace elements, electrolytes, vitamins, acute-phase reaction, cholera, diarrheas, immune system, nutritionally acquired immunodeficiency syndrome, NAIDS

INTRODUCTION

This Herman Award is very special to me, and I sincerely appreciate the honor that it brings. I cherish this award deeply because Bob Herman was one of my closest friends and colleagues for many years. His untimely death created a great loss for all of us who knew him (1), and for this Society as well. This Bob Herman Lecture now allows me to discuss a long-term interest, that is, infection-induced malnutrition, or, in more modern terminology, cytokine-induced malnutrition.

Acute febrile infections induce important losses of body nutrients. These losses can become quite large during severe, prolonged, or frequent infections. In infants and children, in elderly people, and in seriously ill patients, infection-induced malnutrition can become life-threatening.

The title of this lecture reflects the progression of my studies of infection-induced malnutrition over four decades, as shown in Figure 1. These studies began when I was Chief of the Department of Metabolism and the Metabolic Research Ward at Walter Reed Army Institute of Research. In 1959, I was asked to lead a small Army team to join Navy and Thailand investigators in studying the metabolic and physiologic aspects of cholera during an outbreak in Bangkok. Also, while at Walter Reed, Bob Herman was one of my resident physicians. That’s where we first began to collaborate on bench research projects. Our first collaborative paper was published in 1962 (2).

After completing the cholera studies, I was transferred to the Army Medical Laboratory at Fort Detrick, MD, subsequently named the US Army Medical Research Institute for Infectious Diseases. My mission there was to investigate the metabolic aspects of nondiarrheal infections. These studies continued for >20 y. Bob Herman and his wife Yaye would visit me there so that we could do bench research studies together. Together, they generated the major data for our last collaborative paper on vitamin metabolism and infection (3). For the past decade, I have been teaching about infection-induced malnutrition and nutritional immunology.

Several key milestones along the way included conferences and workshops sponsored by the National Academy of Sciences and the American Medical Association, meetings that I chaired or helped to organize. Each meeting led to important early publications (4–6). These included the first book on malnutrition and the immune response (4), edited by Suskind; the first comprehensive symposium on infection-induced malnutrition (5), which occupied two full issues when published in 1977 by The American Journal of Clinical Nutrition (AJCN); and the first comprehensive monograph on single nutrients and immunity (6), also published as a 1982 supplement to AJCN. Most recently, in 1992, the Journal of Nutritional Immunology was begun.

CHOLERA STUDIES

In the Bangkok studies (7–9), our group completely redefined the existing textbook concepts about the pathogenesis and

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therapy of cholera. We found that the intestinal mucosa did not slough off during cholera (7), as previously believed. Rather, the intestinal mucosa remained intact. Instead, toxin produced by the Cholera vibrio cells caused specific absorptive dysfunctions in mucosal cells. These toxic effects produced diarrheal losses of huge quantities of water, sodium, potassium, and bicarbonate (7, 9). Cholera stools were highly alkaline, and the enormous losses of body bicarbonate initiated a severe systemic acidosis (9).

Proper fluid and electrolyte-replacement therapy, with correction of the acidosis, proved life saving. These early studies in Bangkok brought cholera research into the modern era, and helped set the stage for the subsequent purification and characterization of the cholera toxin, discovery that cholera toxin stimulated adenylyl cyclase to cause a marked increase of cyclic AMP (adenosine-3':5'-cyclic monophosphate) in gut mucosal cells, discovery that intestinal absorption of glucose during cholera facilitated the absorption of salt and water, and formulation of the World Health Organization packets of sugar and electrolytes currently used for oral rehydration therapy in children with severe secretory diarrheas (10).

**FIGURE 1.** Temporal representation of the author's studies of infection-induced malnutrition. WRAIR, Walter Reed Army Institute of Research; USAMRIID, US Army Medical Research Institute for Infectious Diseases; IL-1, interleukin 1; NAS, National Academy of Sciences.

### METABOLIC BALANCE STUDIES

Because of the early successes with cholera, I was given the opportunity to initiate comprehensive studies of metabolic, endocrinologic, physiologic, and nutritional effects of nondiarrheal infections in research volunteers, as well as in laboratory animals. Initial data were obtained by using metabolic balance techniques (11) and by measuring adrenocortical hormones (12). According to the prevailing concepts in the early 1960s, responses to stress were largely initiated and controlled by the adrenal cortex.

For the balance studies (11), volunteers were hospitalized 2 wk before their purposeful exposure to an infectious microorganism. They were begun on a constant liquid diet that met all nutritional needs. Specimen collections were begun 9 d before exposure and were continued throughout the full course of illness.

Clinical illness was accompanied by anorexia and diminished food intake. Stool nitrogen losses did not increase, but urinary nitrogen excretion increased slightly. This combination of events produced sharply defined losses of body nitrogen soon after clinical illness began. Daily losses of nitrogen generated a large cumulative body deficit. When the patients were clinically ready for discharge, they were not yet beginning to reconstitute their large deficiency of body nitrogen (11).

Balance measurements of other major intracellular elements—potassium, magnesium, and phosphate—followed patterns of loss quite similar to those of body nitrogen (11). Zinc and sulfur losses also developed. On the other hand, sodium, chloride, and water were retained by the body during the acute illness, and then lost by diuresis during convalescence (11).

Paired-feeding studies in noninfected volunteers demonstrated only small transient losses of body nitrogen (11). These losses were rapidly regained. Other control studies showed that the hypermetabolism of artificially induced fevers caused similar large losses of body nitrogen and other elements (13). In contrast, nitrogen balance was not altered by the antibiotics used in therapy, or by hydrocortisone, when given in daily amounts that matched adrenal gland production during infection (11).

Once the metabolic balance studies were nicely underway, a continuing series of brilliant young physicians and newly graduated PhDs began to be assigned to my laboratories for their tour of Army service. They contributed much to subsequent endocrine, biochemical, and metabolic studies. Morton I Rapoport joined me in research on adrenal function and tyrosine metabolism (14–18). George E Shambaugh, III, investigated changes in thyroid function, glucose tolerance, insulin secretion, and tyrosine metabolism (19–23). George Lust performed biochemical studies documenting host enzyme responses (17, 24, 25).

Ralph D Feigen explored changes in plasma amino acids and the antidiuretic hormone (26–28); Albert S Kleiner inquired into changes in plasma proteins (26, 29, 30). Kenneth A Woeber measured growth hormone responses (31). Robert H Feiser (32–35), and later, Robert L Kaufmann (36, 37), investigated changes in lipid metabolism, whereas Elliot J Rayfield (38–41), David T George (38–41), and Randall T Curnow (38–40) performed additional studies on glucose metabolism and its related hormones.
### ENDOCRINOLOGIC STUDIES

#### Adrenal gland

When studied prospectively, we found that plasma cortisol lost its usual circadian periodicity during the acute phase of infectious diseases. Instead, plasma cortisol maintained its normally high morning concentrations throughout the entire day and night (12). Increases in urinary glucocorticoids and ketosteroids were actually quite small. These increases quickly reverted to normal when patients began to recover (12). Thus, the adrenal cortex clearly did not control the entirety of the host’s metabolic response to the stress of infectious diseases. Other investigators showed that adrenocorticoid excretion was subnormal during chronic infections (14). In contrast, plasma cortisol values could become very high during life-threatening illnesses. Such high values were caused by a failure of the liver to convert plasma cortisol to its water soluble metabolites. Unlike the glucocorticoids, aldosterone secretion patterns mimicked the febrile response pattern, and accounted for the acute renal retention of body sodium and chloride (12). As shown by others, adrenomedullary catecholamine values in plasma become markedly elevated during episodes of septic shock (41).

#### Thyroid gland

Shambaugh and I (20, 21) found a rapid decline of plasma protein-bound iodine, and a decreased binding of thyroxine to plasma proteins during acute infection. In laboratory rats, these changes were accompanied by a marked slow down of hormone production by the thyroid gland. This infection-induced evidence of hypothyroidism appeared to be caused by a decreased thyroidal responsiveness to thyroid stimulating hormone; (TSH) as well as by a diminished responsiveness of the anterior pituitary gland to declining concentrations of free thyroxine in plasma. Thyroid physiology returned to normal during convalescence. Later, others (42, 43) reported an increased peripheral conversion of thyroxine to reverse triiodothyronine rT3, rather than to T3, and also, a concomitant decline in production of thyrotropin releasing hormone from higher brain centers (43).

#### Pituitary gland

Other studies showed an increased pituitary secretion of adrenocorticotropin hormone (ACTH) (14) as well as growth hormone during acute infections (31). Large bursts of growth hormone secretion occurred during glucose infusions (39, 40). Water retention during acute febrile infections appeared to result from increased secretion of antidiuretic hormone by the posterior pituitary gland (27). Secretion of antidiuretic hormone could become inappropriately large during some infections.

#### Pancreas

Within hours after the initial onset of acute fever, fasting plasma concentrations of both insulin and glucagon were both abnormally elevated (19, 38). This finding was quite remarkable because insulin and glucagon production typically shows a reciprocal relation. Glucose tolerance tests, performed during the very early hours of acute fever, had already become abnormal, with patterns resembling those seen in adult-onset diabetes (19, 38). The administered glucose caused abnormally large insulin responses, giving evidence for the sudden development of cellular insulin resistance (19, 38–40). However, the high plasma glucagon values did decline appropriately, when the glucose was given (38–40).

### Evaluation of endocrine responses during infection

In evaluating our early endocrinologic findings during acute infectious illnesses, two important general conclusions could be drawn. First, endocrinologic responses to acute infection simply could not explain the numerous metabolic and biochemical events that characterized the totality of the body’s complex response to acute infectious disease. And second, the multiple endocrine responses seen during acute infection were similar to, and actually typified, the endocrine responses seen during other forms of stress.

### OTHER METABOLIC RESPONSES TO ACUTE INFECTION

Our combined metabolic and physiologic studies generated much fundamental information on a wide range of host responses to infection. Importantly, Robert W Wannamacher, Jr, (44–51) became the senior biochemist of our group and worked with Robert S Pekarek (45, 48, 50–54) and Michael C Powanda (47, 48, 50, 55, 56) on a wide variety of studies concerned with changes in trace element, amino acid, and protein metabolism, and the mediators that brought about these changes. Later, Philip Sobocinski and his team discovered the rapid, infection-induced synthesis of the hepatic metallothionein, which caused sequestration of zinc (57). Outside collaborators included David F Clyde, Herbert L DuPont, and Richard B Hornick of the University of Maryland (29, 51); Robert L Squibb at Rutgers (58), Buford L Nichols and his group at Baylor (59); and Robert S Lees (33) of the National Institutes of Health (NIH). These findings led to the development of new concepts about the comprehensive, cohesive, but complex nature of the body’s metabolic responses to an acute febrile infection.

Catabolic losses of weight and muscle mass were most apparent clinically (5). Free amino acids were being liberated from contractile muscle protein (5, 16, 26, 44–50). Many amino acids were then taken up by the liver, by phagocytes, by lymphocytes, and by other body cells (44–60). Some free amino acids were used to create new proteins, such as enzymes, acute-phase plasma glycoproteins, and metallothioneins (18, 22, 29, 30, 44–50, 55, 57). Many amino acids were used to create glucose, the principal fuel required for fever-induced hypermetabolism (44–50). The metabolic degradation or conversion of some free amino acids, such as tryptophan and phenylalanine, was accelerated (15, 16, 22, 55). In gram-negative infections an inhibition of lipoprotein lipase was found to cause large accumulations of triglycerides in plasma (36, 37). A catabolic destruction or urinary loss of body vitamins (3), best exemplified by vitamin A (60), once called the antinfection vitamin (61), appears to contribute importantly to the morbidity and mortality of some infectious illnesses (62, 63).

### THE ACUTE-PHASE RESPONSE

By the late 1960s, the body’s overall response to febrile infections was proving to represent a stereotyped admixture of
concomitant anabolic and catabolic events. When each individual response was plotted longitudinally, in comparison to the fever curve, a distinct pattern emerged. Some metabolic responses began in the incubation period, some at the onset of fever, some late in fever, and some during convalescence (5). No matter which microorganism was causing an acute, generalized, febrile infection, the onset of most metabolic, biochemical, or physiologic responses seemed to occur at relatively consistent, predictable times during the course of illness. These many responses could be categorized temporally by their relations to the time of onset of clinical symptoms and fever (5). In fact, virtually every metabolic or biochemical process under investigation was found to be influenced in some manner by the body’s response to an acute infection.

In today’s terminology, this overall response is termed an acute-phase response (64), an acute-phase reaction, or the systemic inflammatory response syndrome (65). This acute-phase response accompanies other severe medical and surgical problems, in addition to infection (64).

This complex response, which also activates complement and stimulates the immune system, appears to help defend the body (5, 64). However, on the darker side, acute-phase responses generate important nutritional costs (5), costs that can produce severe, life-threatening malnutrition. In addition, acute-phase responses which become excessive or overly prolonged can lead to hypotensive shock, multiple organ dysfunction, and death (64–66).

**DISCOVERY OF THE LEUKOCYTIC ENDOGENOUS MEDIAN ELE INTERLEUKIN 1**

As noted previously, endocrine responses could not explain or account for our emerging concepts about the acute-phase reaction and all its complexities.

Accordingly, we set out to see whether some unknown hormone-like substances were causing these acute-phase responses (54). Sure enough, when a milliliter of sterile plasma from an infected human being or laboratory animal was injected into a normal test rat, signs of an acute-phase response were initiated within minutes (50, 51, 54, 56, 57, 67). The response initiating, hormone-like substances we detected in plasma during acute infections were quite heat-labile (68). Therefore, they were not bacterial lipopolysaccharide endotoxins (67, 68). Rather, they proved to be small proteins. We could detect them in large quantities in chemically induced sterile exudates (54, 67, 68). Accordingly, we named these newly detected substances leukocytic endogenous mediators, or LEMs (54).

Although our crude LEMs met the technical definitions for hormones, LEMs were produced by diverse types of individual cells rather than by an anatomically defined glandular organ. For many years, before the advent of modern-day biotechnology, LEMs were difficult to isolate, purify, identify, or quantify. The precise amino acid sequence of LEMs remained undefined. But eventually, our metabolically important LEMs were equated with the endogenous pyrogens studied by fever physiologists, and with the immunologist’s lymphocyte activating factor, and all were renamed interleukin 1 (IL-1) (64, 66). A still-growing number of other interleukins were discovered, and in major breakthroughs, they were then produced in sizable quantities by biotechnologic methods. It now seems evident that our crude LEM preparations contained other proinflammatory cytokines in addition to IL-1 (66, 67, 69).

We thus were introduced into the world of cytokines, a world that keeps expanding, even though the individual actions of cytokines, and their interrelations are still incompletely understood. Acute-phase responses are initiated and controlled by the proinflammatory cytokines (64, 66, 67, 69–74), including IL-1, IL-6, IL-8, and tumor necrosis factor (TNF). The cytokine interferon-α can also contribute to the wasting syndrome of acute infections (75). Although numerous stimuli can initiate the cellular production of proinflammatory cytokines, bacterial endotoxins are somewhat unique because they primarily stimulate the release of TNF. As one of its actions, TNF inhibits lipoprotein lipase enzymes, thus accounting for the high plasma concentrations of triglycerides that develop during gram-negative sepsis (34, 36, 37).

**CURRENT CONCEPTS CONCERNING PROINFLAMMATORY CYTOKINES**

Because acute-phase reactions have such high nutritional costs (5, 76), new findings about the cytokines have potentially great nutritional importance.

**Control mechanisms for cytokine actions**

Like hormones, proinflammatory cytokine actions are regulated by many checks and balances, as shown in Figure 2. To act on a cell, a cytokine must first attach itself to a protein receptor on the cell surface. But cells produce many of these receptor proteins that are then released to float free in plasma (77, 78), where they can intercept and inactivate the matching cytokine. Other unique plasma protein molecules, receptor antagonists, can block cellular receptors for specific cytokines (77, 78). And other cytokines such as IL-4 and IL-10 can suppress the cellular production of proinflammatory cytokines (79–81).

**Cytokine assay in biological fluids**

Individual components of this complex system of checks and balances can now be measured in plasma, and their relations can be studied throughout the course of an acute-phase reaction (66, 69, 72, 78). These cytokines and their free receptors have also been found in mucosal fluids as well as in plasma (82–84). Raqib et al (84) recently reported longitudinal measurements of cytokines and receptor antagonists in the plasma and stools of patients with acute shigellosis. Concentrations of TNF-α, IL-1β, IL-6, IL-8, and IL-1 receptor antagonist in stools were quite high when shigellosis patients were first seen, and gradually returned to normal values during the next 2 wk (84). We still do not understand the pathogenic effects of these interacting molecules in either systemic or localized diseases. Findings like those of Raqib et al (84) raise yet unanswered questions about the potential role of cytokines, if any, in the pathogenesis of cholera and other secretory diarrheas, where nutrient losses are initiated by the actions of a specific intestinal toxin, rather than by a generalized acute-phase reaction.
INFECTION-INDUCED MALNUTRITION

In recent years, proinflammatory cytokines have been shown to influence another important area where nutrition and host defense seem to interact. These cytokines activate nitric oxide synthase in leukocytes (88–90). Nitric oxide synthase is an enzyme which oxygenates one of the guanido nitrogen groups of arginine to produce citrulline and nitric oxide (88). The December 18th issue of Science, in 1992, (91) named NO the “Molecule of the Year”, using the catch phrase “Just Say NO”.

Among its many important biological effects, NO has proven to be a highly potent microbiocidal and tumoricidal agent. The importance of NO in this regard may rival that of the more familiar free oxygen radicals in potency and effectiveness. Much more needs to be learned about the cellular production of NO in humans, and the varieties of microorganisms and parasites it may help to eliminate. Already, a complete enteral formula supplemented with arginine has shown amazing efficacy in shortening the duration of severe sepsis in intensive care patients (65).

CYTOKINE-INDUCED VERSUS STARVATION-INDUCED MALNUTRITION

The acute-phase reaction and its cytokine-driven hypermetabolism have high nutritional costs (5, 75, 76). One must be aware of these costs whenever the genesis of malnutrition is being considered.

The nutritional costs of cytokine-induced malnutrition during infection, as depicted in Table 1, differ in every way from those due to uncomplicated starvation. A large number of physiologic and metabolic processes react differently during uncomplicated starvation than during infection.

Cytokine-induced malnutrition is initiated by hypermetabolism (5, 76), with its high basal metabolic rates. Body nitrogen and other elements are lost quickly, while body water and sodium are being retained (5). Glucose and urea synthesis are both increased during cytokine-induced malnutrition, but ketone production is slowed (5). Oxidation of branched-chain amino acids is increased, and acute-phase plasma glycoproteins are created (5). And last, the immune system is activated. In each instance, an opposite response is typical of uncomplicated starvation.

Because starvation is rarely uncomplicated in infants and children, any resultant malnutrition is generally influenced importantly by cytokine-induced components. All of these many pathogenic differences must be taken into consideration whenever malnutrition is being studied, treated, or considered for public health interventions.

Time does not permit more than a passing, final mention of the other important aspect of the interaction between infection and malnutrition, ie, the devastating effects of cytokine-induced malnutrition on the immune system. Generalized and/or specific deficiencies of required nutrients (4, 6) can result in nutritionally acquired immunodeficiency syndromes, or NAIDS (61). The synergism between NAIDS and infectious diseases not only causes an estimated 30,000 childhood deaths a day worldwide, this synergism is also a major cause of deaths in seriously ill patients in our most modern hospitals (61).

TABLE 1

Differences in the pathogenesis of malnutrition

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<thead>
<tr>
<th></th>
<th>Starvation-induced</th>
<th>Cytokine-induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal metabolic rate</td>
<td>Decreased</td>
<td>Increased</td>
</tr>
<tr>
<td>Body nitrogen</td>
<td>Conserved</td>
<td>Lost quickly</td>
</tr>
<tr>
<td>Body water</td>
<td>Lost</td>
<td>Retained</td>
</tr>
<tr>
<td>Body sodium</td>
<td>Lost</td>
<td>Retained</td>
</tr>
<tr>
<td>Glucose synthesis</td>
<td>Inhibited</td>
<td>Stimulated</td>
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<tr>
<td>Ketone synthesis</td>
<td>Stimulated</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Urea synthesis</td>
<td>Reduced</td>
<td>Increased</td>
</tr>
<tr>
<td>Muscle BCAA oxidation</td>
<td>Minimized</td>
<td>Increased</td>
</tr>
<tr>
<td>Acute-phase plasma proteins</td>
<td>Unchanged</td>
<td>Produced rapidly</td>
</tr>
<tr>
<td>Antimicrobial defenses</td>
<td>Unchanged or weakened</td>
<td>Activated</td>
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BCAA, branched-chain amino acid.
other cause of human mortality can match that of NAIDS. But unlike our current epidemic of virally induced AIDS, NAIDS can be reversed by appropriate nutritional support.

I thank and acknowledge the important contributions of my numerous colleagues and collaborators throughout the years, and acknowledge the courage and dedication of the many Seventh-day Adventist and other research volunteers who participated in these studies.

REFERENCES