WORLD HEALTH ORGANIZATION
MONOGRAPH SERIES
No. 57

INTERACTIONS OF NUTRITION AND INFECTION

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WORLD HEALTH ORGANIZATION

GENEVA

1968

PRINTED IN BELGIUM

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That malnutrition increases susceptibility to infectious disease seems a reasonable assumption, and clinical observation in areas where malnutrition is common has generally lent support to this belief. Equally reasonable is the supposition that infectious diseases have an adverse effect on the nutritional state. It is only comparatively recently, however, that systematic studies have been undertaken to explore in detail the complex interactions between nutrition and infection.

WHO began to take an interest in this problem early in its history. In 1950 a Joint FAO/WHO Expert Committee on Nutrition recommended that studies should be made on the relation of nutritional status to resistance to intestinal parasites. During the next few years, evidence gradually accumulated that protein-calorie malnutrition in infants and young children was the most serious nutritional problem in developing countries and that acute communicable diseases of viral and bacterial origin were among the main contributory factors. Furthermore, a series of investigations by the Institute of Nutrition of Central America and Panama (INCAP), with WHO support, demonstrated the interaction between nutritional deficiencies and many of these diseases, especially acute diarrheal disease. Meanwhile, experience gained in various WHO-assisted programmes for the prevention and control of communicable diseases suggested that many were associated with malnutrition.

The time therefore seemed ripe for a broad examination of the interrelationships between malnutrition and diseases caused by a variety of pathogenic organisms, including bacteria, rickettsiae, viruses, protozoa, and helminths. In 1965, a WHO Expert Committee on Nutrition and Infection reviewed the whole question and put forward suggestions for a comprehensive programme of research. This was designed to determine the effects of infection on nutritional status, the effects of malnutrition on resistance to infection, and the mechanisms of interaction between nutrition and infection. Such a programme demanded, in the first instance, a careful sifting of the vast amount of epidemiological, clinical and experimental evidence already available. The present monograph is intended to meet this requirement.

The three authors have been collaborating closely for many years. Dr Gordon and Dr Taylor first came together in 1953, when they pooled their

ideas concerning synergism and antagonism in associated diseases and set out to explore these relationships, both in the field and in the laboratory. Meanwhile, Dr Scrimshaw was collecting data in Panama and in Guatemala on the association of malnutrition and acute infection among the local populations. In 1958, he met Gordon and Taylor and the three decided to undertake a detailed review of the published evidence and of their own experiences concerning synergism and antagonism in relation to nutrition and infection. This review, published in 1959 in the American Journal of the Medical Sciences, *provided a basis for the first four chapters of the present monograph, but much new material has been added, and the evidence has been re-evaluated.*

Chapter 5 is an expanded version of a paper published in 1963 which analyses and illustrates the epidemiological principles that have to be observed in carrying out field studies on problems of the kind discussed in the monograph. Chapter 6 is devoted to a particularly striking and important example of a synergism between infection and malnutrition—that of weanling diarrhea. This brings together a vast store of information from a number of articles already published by the authors elsewhere and some more recent findings of studies carried out by INCAP. The last chapter presents a short summary of the present state of knowledge regarding interactions of nutrition and infection, and discusses the public health implications.

The manuscript of the monograph was circulated in draft form to seventeen experts in twelve countries for their comments and suggestions, which have been taken into account in preparing the final text. The names of these reviewers are listed on page 8. Their collaboration, and that of the many persons who have supplied unpublished information, is greatly appreciated, both by the authors and by WHO.

Dr Scrimshaw, Dr Taylor and Dr Gordon have performed a great service in awakening clinical interest in the interrelation of infectious disease and malnutrition and drawing attention to its public health importance. The World Health Organization is deeply indebted to them for the thoroughness with which they have undertaken this comprehensive review of the subject and the immense amount of time they have devoted to the project.

CHAPTER 1

BASIC PRINCIPLES AND CONSIDERATIONS

Introduction

Throughout history, man has recognized an association between famine and pestilence. A relation becomes obvious when a natural or man-made disaster drastically curtails the production and distribution of food and simultaneously causes a breakdown in personal hygiene, environmental sanitation, and the provision of medical care. The resultant crowding of people and the social disorganization favor the spread of infectious disease. Far more relevant scientifically than these secondary associations are field and clinical observations, as well as many experimental studies, proving that commonly occurring interrelationships between infection and malnutrition are direct and causal. These direct interactions fall into two basic patterns: malnutrition generally alters resistance of the host to infection, and infectious disease exaggerates existing malnutrition.

Multifactorial Causality of Disease

Progress in medicine and public health depends primarily on understanding the causes of disease. In modern academic medicine, however, and even in public health practice, causation is too often equated with discovering the agent of disease. From an epidemiologic point of view, the activity of the agent is merely one of a triad in the complex of causality, the other two elements being host characteristics and environmental factors. Neither the presence of an infectious agent nor the deficiency of an essential nutrient will alone determine whether disease occurs.

Host factors interact with infectious agents to produce clinical disease. Environmental factors influence both agent and host; in fact, they may determine the nature and outcome of interactions between the two (Gordon, 1958). For example, if tuberculosis were caused merely by infection with the tubercle bacillus, the clinical disease would be highly prevalent. The

fact is that an exposure that is readily resisted by most persons causes disease in a selected few. Furthermore, an attack is more likely to be severe, and even to prove fatal, in a malnourished or otherwise susceptible person.

Similarly, when the cause of a nutritional disease is identified as a deficiency of a specific nutrient, this is often still insufficient to explain why the disease occurs. For example, hypochromic, microcytic anemia is not necessarily due to a dietary deficiency of iron. It may occur in persons whose iron intake would have been completely adequate were it not for interference with iron absorption by excessive dietary phosphates or for abnormal iron loss resulting from severe hookworm disease, menorrhagia, a duodenal ulcer, or even excessive sweating. Environmental and host factors may render a given intake of a specific nutrient either wholly adequate or grossly deficient.

Too often the clinician and the public health worker are encouraged to identify the agent of disease and then, in attempts at prevention or control, to concentrate on measures directed against this agent. Because modern clinical medicine has developed effective treatment for many common diseases, this approach appears to work well in hospitals. Such a limited attack may fail miserably, however, in preventing a relapse once the patient returns to his home environment. For prevention of disease in the general community, measures based on a narrow etiological approach are also frequently ineffective. Preoccupation of public health authorities with the causative agents of disease is likely to result in control measures that are uneconomical and unsuccessful because they do not attack the most vulnerable links in the multiple chains of causation (Gordon, 1965a, b).

Weanling diarrhea is a dramatic example. This newly designated syndrome, discussed in detail in Chapter 6, results from the combined effects and interaction of malnutrition and infection. During the weaning and immediate post-weaning periods, major adjustments of the host to new influences combine to cause maximum simultaneous predisposition to risk from malnutrition and repeated enteric infections.

Kinds of Interaction

Effect of infection on nutrition

A convincing accumulation of scientific evidence is presented in the next chapter to show that all infectious diseases have direct adverse metabolic effects and that frequently they also influence the amount and kind of food consumed. When individuals or populations are suffering from malnutrition, even though minimal or borderline, these effects of infectious disease on nutritional status are likely to have significant clinical and public health importance.

It should not be forgotten that a high prevalence of debilitating infectious disease may also affect the nutritional status of a population by reducing the ability of many of its members to produce or earn their food supply. Populations with a great deal of endemic malaria, severe hookworm disease, schistosomiasis, and other infections common to tropical, underdeveloped areas are likely to lack the physical stamina to be efficient in agricultural and industrial labor. Heavily parasitized farm animals give a poor return for the food they consume and for the effort in caring for them. These direct effects on economic development provide major justification for expanded health programs.

Effect of nutrition on infection

The data presented in Chapter 3 clearly demonstrate that nutritional deficiencies generally reduce the capacity of the host to resist the consequences of infection. An aggravation of disease, or synergism, is the expected result in man whenever nutritional deficiency is sufficiently severe. Such effects are also observed in most studies with laboratory animals, particularly when infection is due to bacteria, rickettsia, intestinal protozoa, or intestinal helminths.

In some studies with laboratory animals involving viral, protozoal, or helminthic infections of systemic origin, but rarely with other infectious agents, specific vitamin and mineral deficiencies have been found to inhibit the activity of the agent more than they interfere with host resistance. When other than the three highly host-dependent types of organisms just mentioned are involved, antagonism between infectious agents and malnutrition has only rarely been demonstrated in laboratory animals. Moreover, there is no verified instance in which a natural or experimental nutritional deficiency has been shown to be antagonistic to any infection in man.

Nutritionally induced determinants of synergism may include: (*a*) reduced capacity of the host to form specific antibodies; (*b*) decrease in phagocytic activity of microphages and macrophages; (*c*) interference with production of non-specific protective substances; (*d*) reduced non-specific resistance to bacterial toxins; (*e*) alterations in tissue integrity; (*f*) diminished inflammatory response and alterations in wound healing and collagen formation; (*g*) effects originating in alterations of intestinal flora; and (*h*) variations in endocrine activity. Evidence is presented to show that all may be involved in varying degrees in the synergism between nutrition and infection.

The principal determinant of antagonism is that the metabolic requirements of the infectious agent are not met because of a nutritional deficiency of the host. The factor that limits development or activity of the agent may be an essential nutrient whose deficiency is less critical for the host than the agent, or it may be a dietary constituent, such as para-aminobenzoic acid, that has little or no nutritional significance for man.

Nutrient excesses may have either an adverse or a favorable effect on reproduction or pathogenicity of infectious agents.

In succeeding chapters the effect of many different nutritional deficiencies inducing these interactions is described for a wide variety of infectious agents and host species. The principles that emerge from the resulting analysis have direct clinical and public health application.

Ecologic Determination of Disease

Both infectious and nutritional diseases are dynamic, changing in character and frequency as the conditions causing them change. Host and environmental changes more often control these shifts than do variations in agent virulence, which so often attract greater scientific attention. The disease patterns of a given area or population vary with time. At any one period, wide differences are seen from one geographic area or population to another. The way in which prevalence and nature of disease differ with time, place, and person can be illustrated by specific examples among both nutritional and infectious diseases.

Variations with time

In New York City in 1900, the death rate from diarrheal disease among children less than one year old was 4496 per 100 000 of the population of that age. In 1920 it had dropped to 1796; in 1961 it was only 45. For children one to four years of age, the rates changed from 470 per 100 000 in 1901 to 120 in 1920 and to only 2.4 in 1961. Obviously, many environmental and host factors were involved in this dramatic decline, but the rates in New York City as recently as the early 1900's were worse than in many technically underdeveloped areas today.

Similar phenomena explain the virtual disappearance of *Mehlnährschaden*, or "starch dystrophy", a disease exceedingly common in Europe at the beginning of the century. It was caused by infants being fed too much carbohydrate and too little protein, and is now recognized to have been a form of the severe protein malnutrition known as kwashiorkor. Pellagra provides the best example of an altered frequency of a nutritional disease in the USA; in the early 1930's, it claimed thousands of victims in the southern states; today, it has almost disappeared.

Unfortunately, the trend is not always so favorable. Kwashiorkor and marasmus are increasing in many of the burgeoning cities of Latin America, Asia, and Africa. One reason is a general extension of the middle- and upper-class pattern of early weaning, an almost inevitable situation as more young women are employed in industry and domestic service. Because they are unable to keep their babies with them as they did in the villages,

these working mothers feed them starchy, low-protein gruels, a poor substitute for mother's milk or the cow's milk formulas used by the economically privileged.

Another aspect of time distributions of disease is the periodicity some of them manifest. In Chapter 2 it is shown that kwashiorkor often has a distinct wave of increased incidence a few weeks after an outbreak of acute diarrheal disease. Epidemics of measles and other common communicable diseases of childhood also frequently precipitate a rise in kwashiorkor.

Variations with place

The distributions of ascariasis and hookworm disease depend upon a climate and soil favorable to the embryonation of eggs and larvae (Babbott et al., 1961). Where the soil is frozen hard or is non-existent, as in parts of the Arctic, or is dry sand, as in the desert, the propagation of these diseases is manifestly impossible.

Today, most of the classical deficiency diseases are sharply localized according to the extent of social, economic, and technical development. Little is contributed to an understanding of their distribution by the simple knowledge that goiter is due to iodine deficiency, beriberi to a lack of thiamine, pellagra to a niacin-tryptophan deficiency, or xerophthalmia to inadequate intake of vitamin A. For example, it is necessary to know that occurrence of goiter is influenced by differences in iodine content of soil and water, goitrogenic substances in the diet, hardness of water, water pollution, and other environmental factors, and that today it is being reduced by less monotonous diets, importation of food from non-goitrous areas, and the iodization of salt. Environmental and host factors are the real determinants of prevalence of goiter.

Variations with person

According to statistics published by WHO (1964) the mortality from measles in Mexico (1961) was 83 times as high as in the USA; in El Salvador (1962), 128 times as high; in Guatemala (1962), 268 times as high; and in Ecuador (1960), 274 times as high. Measles is a highly communicable disease acquired by nearly all children early in life, often earlier in developing regions than in more advanced areas (Gordon et al., 1965). Death from measles is rare among well-nourished children, even without treatment with sulfonamides and antibiotics. There is no evidence that virulence of the measles virus is any different in Latin American countries from what it is generally.

The main reason for the dramatic differences in mortality rates is that young children in Mexican, Guatemalan, and Ecuadorian villages are more susceptible hosts. In Africa, measles is frequently a still more fulminating

disease. The malnutrition during the critical period between weaning and school age that is responsible for the readily apparent retardation of growth and development also reduces resistance to infectious diseases. Many factors such as housing, local medical practices, social customs, and physical status combine to influence the spread of measles among susceptible young children, as well as the incidence of secondary infections. An improvement in nutrition by itself has, however, been observed to lower measles mortality.

The preceding examples illustrate the general observation that factors responsible for differences in disease distribution are by no means static. Many once-serious diseases have ceased in time to be important in highly industrialized countries. This process is at work today in the less developed countries. Health workers have the obligation to understand the environmental and host factors involved in causality as well as those that relate to specific agents of diseases, and to use this knowledge to accelerate favorable time trends. It follows that the geographic distributions of disease and the related socio-economic distribution of people within an area are subject to similar dynamic interpretations. Particularly in less developed areas, the interaction of malnutrition and infection is a major ecologic determinant of existing patterns of disease.

Synergism and Antagonism

The combined effect of malnutrition and infection cannot be predicted from the occurrence and characteristics of either alone. Infectious disease nearly always makes co-existing malnutrition worse. Furthermore, the consequences of infection are likely to be more serious in a malnourished host than in a well-nourished one.

When infection aggravates malnutrition or malnutrition lowers resistance to infection, the relationship between the two can be classified as *synergistic*, i.e., the simultaneous presence of malnutrition and infection results in an interaction that is more serious for the host than would be expected from the combined effect of the two working independently. An infection, through precipitating clinical malnutrition, can result in further synergism as the infection, in turn, becomes more severe in the malnourished host. Thus it is possible for the mutual interaction of an infectious disease and a state of malnutrition to create a vicious circle, which often results in a fatal outcome.

In some special circumstances, which will be discussed, malnutrition is more likely to discourage multiplication of the agent than to affect the resistance mechanisms of the host. In this event, the interaction between malnutrition and infection can be identified as *antagonistic*, the combined effect being less than would have been expected.

Numerous examples of both synergism and antagonism are given in succeeding chapters.

Definitions

Epidemiology

Schneider (1946a) has attempted to distinguish between "susceptibility factors" and "resistance factors" in the diet. The former decrease the effect or extent of infections when withheld (antagonism), whereas the latter increase the severity of infections when they are deficient (a part of the total concept of synergism). We find it less confusing to consider increased susceptibility and decreased resistance as synonymous. Throughout, we have adhered to the following definitions, which are taken, with minor re-wording, from the tenth edition of the handbook *Control of communicable diseases in man*, published by the American Public Health Association (Gordon, 1965a).

1. *Fatality*—An expression of the severity of disease as judged by the frequency of deaths among the patients or sick persons in which those deaths occur. It may express the general characteristic in relation to an area, a disease, or a class of diseases. It is commonly employed quantitatively as a ratio of the number of fatal cases to total cases in a specific clinical or epidemiological experience where all cases have been followed to completion (acute disease) or for a stated period of time (chronic disease). Common usage of the ratio is disease-specific; thus, the case fatality of diphtheria is 4%. (*Compare* 7. Mortality.)

2. *Incidence* — A general term used to characterize the frequency of occurrence of a disease, an infection, or other event over a period of time and in relation to the population in which it occurs. Incidence is expressed more specifically as a rate, commonly the number of new cases during a prescribed time in the unit of population in which they occur; thus, cases of tuberculosis per 100 000 population per year. (*Compare* 8. Prevalence.)

3. *Infection*—Invasion of a living host (man, animal, or plant) by an organism; its development or multiplication there; and a reaction of tissues to its presence or to toxins it generates. Infection is not synonymous with infectious disease; the result may be inapparent or manifest (see 5. Infectious Disease). The presence of living infectious agents on exterior surfaces of the body or upon articles of apparel or soiled articles is not infection but contamination. The term "infection" should not be used to describe conditions of inanimate matter, such as soil, water, sewage, milk, or food; the term "contamination" applies to these conditions.

4. *Infectious Agent*—An organism capable of producing infection or infectious disease. Most infectious agents are micro-organisms (e.g.,

bacteria, protozoa, spirochetes, viruses, rickettsiae, bedsoniae), but some are helminths.

5. *Infectious Disease*—A disease of man, animal, or plant resulting from an infection.

6. *Morbidity* — A general and variously used term expressing the number of sick persons or cases of disease in relation to the population in which they occur. Quantitative expression of morbidity is best attained by incidence rates (see 2. Incidence), occasionally by prevalence ratios (see 8. Prevalence). Disease-specific incidence rates are common usage in expressing morbidity, sometimes further qualified for age, sex or other attribute, and usually representing cases per 100 000 population per year. *Attack rate* is an incidence rate often used for particular populations, observed for limited periods and under special circumstances, as in an epidemic. The *secondary attack rate*, in communicable disease practice, expresses the number of cases among familial or institutional contacts occurring within the accepted incubation period directly following exposure to a primary case, in relation to the total of such contacts; may be restricted to susceptible contacts when determinable. *Case rate* expresses the incidence of clinically recognized cases; *infection rate*, the sum of infection and infectious disease. *Admission rate* (USA) is the usual term for morbidity in military populations, an incidence rate that includes both patients admitted to hospital and those confined to quarters.

7. *Mortality* — A general term characterizing the frequency of deaths over a period of time in the total population (the sick and the well) in which those deaths occur (*compare* 1. Fatality). Commonly expressed quantitatively as a mortality rate (death rate), the number of deaths in a unit of population occurring within a prescribed time. Crude mortality rates, deaths from all causes, are usually stated as the number of deaths per 1000 population per year. Disease-specific mortality rates are usually expressed as the number of deaths per 100 000 population per year.

8. *Prevalence* — A general term used to characterize the frequency of a disease or other event at a particular time and in relation to the population from which drawn. Prevalence is expressed more specifically as a ratio, prevalence ratio, the number of cases of disease present in a specified population unit at a particular instant of time. Thus, the prevalence ratio of tuberculosis is the number of active cases (all forms, old and new) existing at a designated time per 100 000 persons. (*Compare* 2. Incidence.)

The World Health Organization (Bulletin, 1966) has published a note amplifying the concepts of incidence and prevalence, and giving suggestions for their use and application.

Nutrition

No corresponding set of definitions is available for the various nutritional states. The word "malnutrition" itself is used for a variety of conditions. The following definitions are proposed:

1. *Malnutrition* — A pathologic state resulting from a relative or absolute deficiency or excess of one or more essential nutrients sufficient to produce disease. Disease may be clinically manifest or it may be detectable only by biochemical or physiological tests. Four forms are distinguished:

(*a*) *Starvation* implies the almost total elimination of food, and hence the rapid development of severe undernutrition, marasmus, or inanition.

(*b*) *Undernutrition* is the pathologic state resulting from the consumption of an inadequate quantity of food over an extended period of time. *Marasmus* and *inanition* are synonymous with severe undernutrition.

(*c*) *Specific deficiency* refers to the pathologic state resulting from relative or absolute lack of an individual nutrient.

(*d*) *Imbalance* refers to a disproportion among essential nutrients that has pathologic consequences, whether or not there is an absolute deficiency of any nutrient, as determined by the requirements of a balanced diet.

(*e*) *Overnutrition* is the pathologic state resulting from consumption of an excessive quantity of food, and hence a caloric excess, over an extended period of time.

2. *Toxicity* — The pathologic consequences of excessive intake of certain vitamins, minerals, or amino acids.

Microbiology

The nomenclature of pathogenic agents as used throughout the text is not necessarily that of the original authors, but represents the best judgment of current taxonomic usage.

Interpretation of Experimental Results

It should be emphasized that, with simple undernutrition and starvation, no characteristic clinical or biochemical signs of specific nutrient deficiencies occur, except for a progressive loss of body mass. Only when diets are deficient in one or several essential nutrients relative to caloric intake and to other nutrients are specific signs and symptoms likely to develop. The problem of interpretation is complicated by the fact that under field conditions several specific nutrient deficiencies are likely to exist simultaneously, along with some caloric restriction.

In animal feeding experiments to test the effects of deficient diets, two types of controls are required: *ad libitum controls*—animals that are allowed to eat whatever quantity of the complete or control diet they desire, even though they may consume more food than the animals fed the deficient or experimental diet; and *pair-fed controls*—animals that are allowed to consume only as much of the control diet by weight as the test animals eat of the experimental diet. Sometimes paired-feeding is accomplished by forced-feeding the test animals so that they eat as much as the *ad libitum* controls.

Under experimental conditions, animals fed a deficient diet *ad libitum* usually decrease their food intake. When this occurs, only pair-fed control animals will show whether the observed results are the direct effect of the nutrient deficiency, the indirect effect of decreased food intake, or both. Unfortunately, until recently most investigators failed to take into account the reduced food intake of animals fed deficient diets. When nutritional alterations of resistance to infection are studied in experiments with both pair-fed and *ad libitum* controls, the results show that the commonly observed reduced intake of the deficient diet may account for all, none, or a specific and measurable part of the total effect on resistance.

In some experiments, excessively high dietary protein or calorie levels have been fed to control animals. There is increasing experimental evidence that the imbalance produced when intakes are too high may significantly lower resistance to disease.

Trends in Medical and Public Health Emphasis

Soon after the First World War, the separate currents of research on infectious disease and research on nutritional deficiencies began to come together. This historical circumstance largely accounts for the appearance at that time of a large number of published reports of synergistic interaction.

Examples of synergism accumulated rapidly as microbiological research continued to identify new infectious agents. With growing appreciation of the influence of constitutional factors on host resistance, new skills were developed to study specific immunity, cellular metabolism, and phagocytic action as they affect the reactions of hosts to invasion.

Concurrently, increasing numbers of nutrients were recognized; and the scientific basis of nutrition was established by clinical, biochemical, and field studies. Laboratory studies of specific nutritional deficiencies among animals were frequently complicated by serious epidemics of infectious disease, the symptoms of which were sometimes erroneously attributed to the deficiencies since the studies did not include well-nourished controls.

When specific nutrients were given to persons with nutritional deficiencies, the results of this replacement therapy were so dramatic that susceptibility to infection was identified with malnutrition, and it was easy to conclude

that these same nutrients would be protective to normal human populations. This notion was furthered by the type of oversimplification implicit in the use of such terms as "anti-infective vitamin" for vitamin A, and by the growing commercial effort to sell vitamins to the public. As a result, medical practice and public gullibility in the more developed countries carried the use of vitamins far beyond physiologic needs and scientific evidence of benefit. To some extent this situation still prevails.

About two decades ago, a sharp reaction set in against the use of vitamins and against the view that nutrition in general is a major factor in resistance to infection. This reaction had its origin in the excessive claims made for the value of vitamins in preventing and treating infections, the abuses in vitamin administration, and the lack of result when these nutrients were added to an already adequate diet. It was reinforced by a number of experiments in which a nutrient deficiency resulted in antagonism rather than synergism. In the enthusiasm for "debunking" vitamins, little attention was given to certain facts: first, that the experimental demonstrations of antagonism were limited to systemic viral and protozoan infections and, second, that human populations were not likely to experience the type and severity of malnutrition induced in the animal experiments.

The greater severity of poliomyelitis in the privileged populations of highly developed countries was also responsible for a major misconception. Some authorities suggested that malnutrition was protecting the children of less developed countries against paralysis and other serious consequences of poliomyelitis. This appeared reasonable when antagonism between several specific deficiencies and poliovirus in animals was demonstrated. It is now known that children of the lower socio-economic groups in developing regions are exposed to poliovirus infection at an earlier age than that at which paralysis is likely to occur. A high-level herd immunity is thus maintained by frequent, inapparent infections. Despite this fact, the behavior of poliomyelitis is still often erroneously cited as evidence that malnutrition may increase resistance to infection rather than lower it.

The data in succeeding chapters indicate that, under field conditions, malnutrition in man, if severe enough to influence a given infection, is regularly synergistic.

As the growing numbers of observations on an interrelation between nutrition and infection were brought together, a general opinion was formed that results were too variable to demonstrate any general principles or patterns. This mistaken impression that the interactions are unpredictable still exists. Confusion also followed because of failure to understand that the demonstration of lack of ability of malnutrition to influence antibody formation in a given experiment is no proof that it does not influence other protective mechanisms, such as phagocytic activity or tissue integrity.

It was reported at conferences held in 1949 (Clark et al.) and 1955 (Miner) that the relationship of each nutrient to each infectious agent in

each host would need to be worked out individually. This was the position taken in most reviews of the subject (Ackert, 1942; Schneider, 1946a; Chandler, 1951; Smith, 1955; Platt, 1958a; Geiman, 1958). Too little attention was paid to two useful summaries in the older literature that had arrived at more positive conclusions. Clausen (1934) suggested that although nutrition does not, as a rule, influence frequency of infection, an inadequate diet may greatly enhance its severity. This generalization is still valid. In reviewing over three hundred publications concerned only with the effect of vitamins, Robertson (1934) stressed the frequency with which experimental deficiencies of vitamins A and C lowered resistance to bacterial infection. This conclusion is strongly supported by the evidence presented in succeeding chapters.

Our review (Scrimshaw, Taylor & Gordon, 1959) came at a time of renewed interest in this subject. Since then, reviews by Hodges (1964) and Delaunay (1964) have appeared, and the World Health Organization has convened an expert committee (World Health Organization, 1965) on this aspect of nutrition. The conclusion is that it is not necessary to consider each combination of nutrient deficiency, host, and infectious agent separately. Instead, principles emerge of such reliability that they serve as a practical guide for both clinical and public health workers.

Nature of the Evidence

A common criticism of conclusions on the nature and importance of interactions of nutrition and infection is that modern standards of scientific investigation have not been met in the evidence published. The specified reasons usually include a lack of sufficient information on the composition of the diet, the absence of adequate controls, inadequate numbers of animals, and other technical considerations. The criticism is entirely valid, but should not obscure the fact that there are enough well-planned and carefully conducted studies to establish beyond reasonable doubt the existence of basic patterns of interaction.

It has been difficult to decide which experiments and which reports to omit from consideration in this monograph on grounds of unreliability. We have followed the rule of discarding an observation only where there was good reason to doubt its validity. Obviously, the reports cited vary widely in quality and credibility. However, the same criteria have been applied to all types of observation, whether they indicate no effect, antagonism, or synergism. More stringent standards and elimination of experimental studies lacking pair-fed controls would not alter the primary conclusions.

Most of the recent experiments do meet present standards of scientific validity. Knowledge of nutrient requirements has improved, there is a better understanding of the biological activity of infectious agents, and

modern principles of experimental design and analysis are generally applied. A number of the more recent studies permit clarification and expansion of conclusions drawn from earlier and less decisive studies.

In summary, it should be realized that there is no contradiction in the fact that interaction of nutrition and infection may result in synergism, antagonism, or no effect, depending upon the combination of host, agent, and environment. Characteristic interaction patterns have been defined. The conclusion of greatest clinical and public health significance to be drawn from the material presented is that synergism of malnutrition and infection is particularly common in populations of less developed areas.

CHAPTER 2

EFFECT OF INFECTION ON NUTRITIONAL STATUS

Introduction

The ways in which infectious disease influences the nutritional state of the poorly nourished received much attention in the early decades of the present century. Communicable diseases were then highly prevalent; knowledge of nutritional deficiencies was advancing rapidly; and connection between the two was increasingly evident. Eventually interest declined and was superseded by a concern for the reverse relation, that of malnutrition to resistance. A three-day conference on "Nutrition in Infections", held in New York in 1955, dealt solely with the effect of nutrition on infection, and made essentially no reference to the effect of infection on nutritional status (Miner, 1955). This may be partly explained by the fact that populations of North America and Europe are now so well nourished that the stress of acute infection on nutrition is rarely of practical significance.

Interest in this relationship has recently revived, largely because of recognition that kwashiorkor is an important disease in many less developed areas and of the demonstration that infection precipitates this syndrome in children suffering from subclinical, chronic, protein malnutrition.

Infections have a deleterious effect on the nutritional status of the host through physiologic and anatomic changes. These changes become evident in such systemic reactions as fever, leukocytosis, and stimulation of adrenal cortical activity. Local reactions include diarrhea, tissue inflammation and necrosis, increased mucus secretion, fatty liver, and changes in skin and hair. The investigations of earlier years, together with recent studies, provide conclusive evidence that almost all infections produce changes capable of influencing nutritional status.

The main research effort in this field has utilized laboratory animals because they can be subjected to rigidly controlled diets, induced infections,

and complete examination of tissues. For ethical or practical reasons, some studies can be done only with laboratory animals. Others can be conducted more profitably in man. Metabolic effects are best measured in hospitalized patients. The deleterious nutritional results of reduced food intake due to loss of appetite, of "therapeutic" diets less adequate in protein than normal diets, and of purgatives and other medicines are best observed in patients under clinic or field conditions. Information on mortality, morbidity, and general community health can be obtained only by investigating whole populations. The numerous clinical studies dealing with biochemical or metabolic consequences of infection are valuable in helping to understand the sequence of events, but they do not take the place of epidemiologic investigations in assessing the public health significance of a situation.

The observed effect of infection on nutritional status varies with time, place, and person. Less developed areas of the world are today experiencing many of the problems that confronted physicians and public health workers in Europe and the USA fifty years ago. The age or the physiologic state of the host often determines whether nutritional deficiency will be manifest or clinically inapparent under a given circumstance. For example, growing children and pregnant and lactating women are particularly vulnerable. An added stress such as infection, often relatively innocuous of itself, may be sufficient to precipitate acute malnutrition.

Many physicians and public health workers from economically favored countries are now called upon to advise or actually work in less privileged areas. What is still more important, many of the eventual health leaders of the latter countries are being trained under conditions far different from those in which they will practice. Both groups of workers need to be familiar with the seriousness of the synergism between infection and malnutrition when both conditions are present simultaneously in appreciable numbers of the population.

This chapter provides a summary of present knowledge and suggests further research. The number of studies dealing with the effect of bacterial and helminthic infections on nutrition is relatively large. Fewer reports are available on viral and rickettsial diseases, principally because techniques for studying these infectious agents have developed more recently. Little information is at hand on fungus infections or on the injuries and infections arising from arthropods, although in tropical areas these agents are responsible for a significant amount of disability.

Evaluation of the effect of infectious disease on nutritional status was a direct objective of most of the reports now to be discussed. Sufficient investigations are cited to document the important impact of infection on nutrition, particularly when a borderline deficiency already exists. Systematic review of all articles on the various infectious diseases would yield many additional pertinent references, but is deemed unnecessary.

Infection and Protein Nutritional Status

Intestinal infection of bacterial origin

Bacterial infections of the intestinal tract have an adverse influence on protein nutrition that is of major public health importance. In less developed countries this is most evident among young children during the weaning period, when inadequate diets lead to malnutrition. Some of the earliest observations were on the effects of typhoid fever and other enteric infections on nitrogen excretion because these diseases were prevalent in the more developed countries at the time when human requirements for protein were first being recognized. Furthermore, their relation to the intake, digestion, and absorption of food was obvious.

In his Harvey lecture of 1908, MacCallum (1910) drew attention to the findings of Vogel (1854) and Traube (1855) that typhoid fever causes a striking increase in urinary output of nitrogen. He referred to a patient described by Leyden & Klemperer (1841) who experienced a loss of 100 g of nitrogen, equivalent to 3.2 kg of muscle tissue, in only 12 days. A patient described by Müller (1884) lost nitrogen corresponding to 2.5 kg of muscle in eight days. Two important papers by Coleman & Gephart and by Coleman & DuBois in 1915 similarly reported large nitrogen losses due to typhoid fever, even when adequate calories were fed. As a result, therapeutic diets for patients with typhoid fever were changed from a low to a high protein content.

In typhoid fever, Krauss (1926) found a two- to three-fold increase in nitrogen excretion together with a decrease in urinary creatinine due to loss of muscle mass. Shaffer & Coleman (1909) were unable to maintain nitrogen balance in typhoid fever patients even with 90 calories and 1.6 g of protein per kilogram of body-weight. One patient with paratyphoid fever excreted 14.5 to 16.0 g of urinary nitrogen daily at a time when the intake was 2.2 g of nitrogen and 56 calories per kilogram of body-weight. All of the nitrogen loss was through the urine; fecal nitrogen was within normal limits. Although diarrhea was a regular feature, nitrogen absorption was little affected. Modern chloramphenicol treatment of typhoid fever has reduced the duration of the febrile period, but a strongly negative nitrogen balance is still a prominent feature of the disease (Woodward & Smadel, 1964).

Close (1953) described a child recovering from kwashiorkor whose serum albumin rose within seven weeks of therapy from 1.05 to 3.58 g per 100 ml, only to fall to 1.59 g per 100 ml five days after the onset of typhoid fever. A decrease in serum albumin is characteristic of acute infections. In an authoritative review of the characteristic pattern of plasma proteins in acute infectious disease, Belfrage (1963) described a more or less regular alteration for the various clinical types. Acute bacterial infections such as

pneumonia produce marked changes in all blood serum components, especially a decrease in albumin and an increase in alpha and beta globulins. An increase in the gamma globulin fraction is associated with lymphoid activity and prolonged antigenic influence. Experimental fevers induced by bacterial endotoxins produced essentially the same changes as bacterial infections of short duration. Most recently, Crawley and co-workers (1966) have described an early increase in blood urea nitrogen and a slow decrease in serum proteins as a result of the intravenous administration of a purified enterotoxin, type B.

Blood amino acid changes may prove to be one of the earliest detectable metabolic responses to infection. An early increase in whole blood amino acids was observed in young men with experimentally induced typhoid fever who subsequently developed symptoms, but not in those who had no clinical illness (Feigin et al., 1968). A significant decrease in blood amino acid concentration followed the development of the disease in subjects who became ill. In experimental tularemia in man, the amino acid decrease occurred 12 to 60 hours after exposure and prior to clinical symptomatology (Feigin & Dangerfield, 1967). In Venezuelan equine encephalomyelitis, blood amino acid response tended to occur at least a day earlier in subjects inoculated at 8 a.m. than in those infected at 8 p.m. (Feigin et al., 1967).

An outstanding epidemiologic feature of kwashiorkor is the frequency with which it is precipitated by an attack of acute diarrheal disease. Many observers have emphasized this sequence, among them Brock & Autret (1952), in Africa; Autret & Béhar (1954), in Central America; Waterlow & Vergara (1956), in Brazil; Jelliffe and co-workers (1954), in Jamaica; Van Der Sar (1951), in Curaçao; Gerbasi (1956, 1957), in Sicily; Pretorius and associates (1956), in South Africa; Cravioto (1958), in Mexico; Gupta (1958), Rao and co-workers (1959), and Bhattacharyya (1961), in India; and Ryan & Murrell (1964), in New Guinea. Gopalan (1955), in India, and Jelliffe and associates (1960), in Trinidad, noted that infectious diarrhea reached its peak at the beginning of the dry season, when flies were prevalent, and was, in turn, followed by outbreaks of kwashiorkor three to four weeks later. A survey of severely dehydrated children with gastroenteritis showed that over 60% had severe hypoalbuminemia (Truswell et al., 1963).

Smythe (1958), in Cape Town, observed a regular overlapping of the seasonal prevalence of gastroenteritis and kwashiorkor, which he believed to be due to metabolic effects of the infection along with a marked disturbance of the intestinal flora. In studying the absorption of amino acids from an isolated Thiry loop in an adult man, Orten and co-workers (1962) found that spontaneous infection in the loop retarded total absorption of an 18-amino-acid mixture by 14%. Administration of neomycin markedly altered the intestinal flora and decreased absorption by 30%. In general, the rapidly absorbed amino acids in the mixture were less inhibited than those more slowly absorbed.

In acute gastroenteritis, as in typhoid fever, only a small part of the adverse nitrogen balance is apparently attributable to decreased absorption of nitrogen. As early as 1915, Holt and associates noted that even with severe diarrhea nitrogen absorption rarely fell below 75%. Chung (1948) and Chung & Viščorová (1948) found that the loss of nitrogen in diarrhea was much less than that of fat, and that the feeding of protein consistently increased the net absorption of nitrogen. In diarrhea, however, nitrogen absorption was reduced from 90% to approximately 75% and, in one instance, to as low as 27%.

It is relevant that the administration of antibiotics to patients on restricted vegetarian diets resulted in reduction of fecal nitrogen, along with an increase in urinary nitrogen, urea, and ammonia comparable to levels seen in patients on an iso-nitrogenous diet supplying protein of animal origin (Deosthale et al., 1964). The effects of the antibiotic and the animal protein diet given together were not additive. Obviously, the type of intestinal flora associated with the vegetable protein diet was less favorable for nitrogen absorption than that present when animal protein was in the diet.

Chickens infected with *Salmonella pullorum* (Ross et al., 1956) showed a seven- to ten-fold increase in blood urea, even though the formation of blood urea through the ornithine-citrulline cycle is normally insignificant in chicks and other animals that excrete nitrogen as uric acid. Administration of arginine apparently prolonged survival by activating this cycle. Measurements of urinary nitrogen were not made, but a significant amount of urea nitrogen in the urine was the probable reason for the nitrogen imbalance. A similar nitrogen loss occurred in dogs with sterile abscesses and minimal febrile reaction (Cook & Whipple, 1918; Daft et al., 1937; Yuile et al., 1953).

The general deduction is that, although intestinal infections of bacterial origin may cause some decrease in absorption of nitrogen from the gastrointestinal tract, the more important and constant effect is increased urinary nitrogen and a secondary anorexia. In a poorly nourished child, diarrhea may begin as an acute infection and end as chronic diarrhea, perpetuated by protein deficiency.

Tuberculosis

The nutritional effects induced by tuberculosis were among the first to be studied. The interest still continues because tuberculosis is an important cause of death everywhere, and especially in less developed regions.

As early as 1922, McCann showed that urinary nitrogen excretion was markedly increased in patients with tuberculosis. Krauss (1926) described a two- to three-fold increase in febrile tuberculosis and a change in urinary ratio of sulfur to nitrogen from 1:7 to 10:7. In afebrile patients the effect was small. More recently, Johnston (1953), Co Tui and co-workers (1954),

and Rao & Gopalan (1958) have confirmed these results in active, febrile tuberculosis.

Getz and associates (1951) have emphasized the relatively low levels of serum albumin in patients with tuberculosis, compared with other illnesses. Excessive sputum conceivably has nutritional significance, since normal loss of nitrogen in sputum averages 0.68 g per day, and may be as high as 1.7 g even for healthy persons (Kocher, 1914).

Reports from West and Central Africa (Morley, 1959; De Maeyer, 1957), from South Africa (Pretorius et al., 1956), and from India (Jayalakshmi & Gopalan, 1958; Bhattacharyya, 1961) all suggest that tuberculosis can precipitate kwashiorkor in children already suffering from chronic malnutrition. This would be expected in view of the strongly negative effect on nitrogen balance.

Guinea-pigs with tuberculosis showed a negative nitrogen balance except when fed a high-protein diet (Rao & Gopalan, 1958). Tuberculosis produced low serum albumin levels in chicks (Wogan et al., 1961), although increases were observed in alpha-3, beta, and gamma globulin fractions. The amounts of protein and of lysine in the liver were also depressed (Squibb et al., 1965).

In summary, the increased excretion of nitrogen and the decreased intake of food associated with active tuberculosis not only complicate clinical management, but may be of considerable public health importance in regions where protein malnutrition is common.

Other acute bacterial infections

Other infections also affect nitrogen balance and, as recently demonstrated, bring changes in concentrations of amino acids in blood and urine. In calves infected with *Bacillus abortus*, there is an increase in serum globulin and a tendency for a decrease in albumin fractions (Howe & Sanderson, 1924).

Rats injected with *Pasteurella tularensis* showed a disappearance of serum cystine, arginine, and phenylalanine within 72 hours, as measured by paper chromatography, along with a marked lowering of other free amino acids in the blood (Woodward et al., 1954). These changes did not occur with either killed or avirulent bacilli. After the rats recovered, plasma amino acid levels returned to normal. Excretion of free amino acids in the urine was unchanged.

In extensive and well-controlled studies in man (Beisel, 1966; Beisel et al., 1967), healthy young adults experimentally inoculated with *P. tularensis* experienced a marked increase in urinary nitrogen excretion coincident with onset of fever.

Kocher reported in 1914 that 2.5 to 3 g of protein per kilogram of body-weight were required daily for nitrogen equilibrium in patients with erysipelas,

pneumonia, and pyelonephritis. In erysipelas (Coleman et al., 1922) and in arthritis (Cecil et al., 1922), nitrogen equilibrium, even with adequate calories, may require up to 15 g of nitrogen daily.

Johnston & Maroney (1938) demonstrated that even chronically infected tonsils exert a marked catabolic effect. Isonitrogenous diets were fed, urine samples collected daily, and feces pooled for three-day periods in a group of children. In nine children with enlarged tonsils, a history of recurrent attacks, but no acute infection at the time, the average nitrogen balance was −0.06 g of nitrogen per kilogram of body-weight per day. After tonsillectomy the balance rose in all patients to an average of +1.52 g.

Nitrogen balance studies at the Institute of Nutrition of Central America and Panama (INCAP), originally designed to determine the quality of dietary protein, were complicated by a variety of intercurrent infections, including asthmatic bronchitis, bronchopneumonia, tonsillitis, sinusitis, and staphylococcal abscesses (Scrimshaw et al., 1960). Such infections were regularly followed by a decided drop in nitrogen retention, which persisted for one to two weeks after recovery.

Almost all bacterial infections produce an increased urinary excretion of nitrogen. Generally they also result in some decrease in protein intake. Both effects depend on the severity of the disease and, with respect to urinary nitrogen, on the nutritional state of the host as well. The return to a normal nitrogen balance is frequently delayed beyond clinical recovery from the acute episode.

Viral infections

Specific studies of viral infections and the nutritional status of the host are relatively few, and most of them are recent.

Field observations suggest that measles, of all the common communicable diseases of childhood, imposes an unusually severe nutritional stress. Morley believes that measles precipitates kwashiorkor in malnourished children of West Africa more frequently than any other infectious disease (Morley & MacWilliam, 1961; Morley, 1962; Morley et al., 1963; Morley, 1967). Purcell (1939) and Sai (1965), in Ghana; De Maeyer (1957), in the Congo; Gans (1961), in Lagos; Avery (1963), in Sierra Leone; Bezon (1959), in Algeria; Restrepo Molina (1955), in El Salvador; and Netrasiri & Netrasiri (1955), in Thailand all emphasize the importance of this disease as a contributory cause of kwashiorkor. A fall in serum albumin in measles has been observed by Mansharamani (1961) and Giuliani (1963). Diarrhea is a frequent accompaniment of measles in malnourished children (Morley, 1962; Scrimshaw et al., 1966*a*,*b*) and must impair intestinal absorption to some degree. Patients studied by INCAP showed a negative nitrogen balance during the febrile stage, due primarily to increased urinary excretion of

nitrogen. Salomón and associates (1968) have cited examples of kwashiorkor precipitated by measles, German measles, chickenpox, and whooping cough.

Kearney and associates (1948a) showed that Theiler virus encephalomyelitis in mice precipitated a tryptophan deficiency in animals on a diet low in that nutrient. Symptoms were identical with those caused by tryptophan deficiency alone, but death occurred at about 18 days instead of 34. These investigators mentioned similar results with diets deficient in either methionine or valine, but gave no details. One possible mechanism is suggested by the finding that cellular protein synthesis has been found to be markedly depressed after infection of mouse L cells in tissue culture with Venezuelan encephalomyelitis virus (Lust, 1966).

Sanslone & Squibb demonstrated that nitrogen retention in chicks infected with Newcastle virus increased during the incubation period and decreased during the active stage of the disease. The increase seemed to be due to the infection *per se*, whereas paired-feeding studies indicated that the decrease was due to a reduction in food intake. The loss in body-weight and increase in liver size in chicks with this disease were accompanied by a lower level of free amino acids in the blood, similar to that in starved birds (Squibb, 1964a).

Whedon & Shorr (1957) attributed the negative nitrogen balance in children with paralytic poliomyelitis to atrophy of muscle, because of a parallel decrease in body-weight and a drop in creatinine excretion in the urine. Studies of chickenpox by Wilson and co-workers (1961) suggest, however, that viral infections not associated with muscular paralysis also have a significant depressing effect on nitrogen balance. A markedly increased nitrogen retention for at least two weeks after recovery from the acute disease was evident in young children fed a daily ration of 1.5 to 2.0 g of protein (unpublished INCAP data, 1966). Chickenpox is also known to precipitate kwashiorkor in poorly nourished children (Salomón et al., 1968). Even as mild a viral infection as that induced by 17-D yellow fever vaccine (Gandra & Scrimshaw, 1961) or smallpox vaccine (unpublished INCAP data) produced a detectable effect. Increased nitrogen losses in children with influenza have been demonstrated by Górnicki and co-workers (1967). Beisel et al. (1967) have reported that the onset of negative nitrogen balance in sandfly fever coincided with the onset of clinical symptoms in six subjects.

The apparent effect of stress on nitrogen balance is less marked in laboratory animals already severely depleted of protein (Cuthbertson, 1954, 1961). This is consistent with the findings of Davies and associates (1959) in a patient with chronic hepatitis and an initial serum albumin level of 1.4 g per 100 ml. The disappearance rate of intravenously administered ^{131}I-labeled serum albumin and the total excretion of nitrogen in the urine were only about half the normal amounts. Unfortunately, details of dietary intake were not given, and protein intake may have been reduced.

Thus, all viral diseases, even the mildest, exert a detectable adverse effect on nitrogen balance. Epidemiologic evidence indicates that viral diseases precipitate kwashiorkor in a substantial number of underprivileged children.

Rickettsial infections

The nutritional consequences of rickettsial infections have not had much study. The frequency of edema and low serum protein levels in Rocky Mountain spotted fever led Harrell and associates (1946) to carry out nitrogen balance studies in a group of adult patients. Over 4.0 g of protein per kilogram of body-weight were required to maintain balance while fever was present. Beisel et al. (1967) have shown that Q fever also markedly increases urinary excretion of nitrogen in young men, even in one asymptomatic case displaying only rickettsemia.

Rickettsial diseases do not seem to differ from other infections in producing deleterious effects on the protein nutritional status of poorly nourished persons.

Protozoal infections

Many investigations, in man and in animals, have been concerned with the nutritional consequences of acute and chronic malaria. Except for two studies of trypanosomiasis in monkeys, no systemic protozoal disease other than malaria seems to have been investigated for its effect on nitrogen metabolism.

The protein metabolism of rats infected with *Plasmodium berghei* malaria is particularly instructive (Dema et al., 1959). Infected animals have been shown to be less able to absorb protein nitrogen, partly because of reduced intestinal proteolytic activity. Increased nitrogen excretion in the urine and a lower ratio of urea to total urinary nitrogen in the infected animals were even more important. Other statistically significant changes included increased liver fat, more total body water, and lower values for hematocrit, hemoglobin, and serum protein. Uninfected pair-fed controls showed no significant change in protein metabolism.

MacDonald (1960) has shown that the combination of experimental malnutrition and *P. berghei* infection in mice resulted in more hepatic fibrosis than in similarly infected well-fed animals. These results confirmed the hypothesis of Walters & Waterlow (1954) that malaria and malnutrition are jointly responsible for the frequent hepatic fibrosis of children in Gambia. Zuckerman & MacDonald (1964) have subsequently shown that hepatic fibrinogenesis in mice infected with *Schistosoma mansoni* is increased in animals consuming a high-carbohydrate, low-protein diet compared with control animals with the same infective dose.

A steady increase in concentration of all amino acids in erythrocytes was observed as parasitemia progressed in chicks infected with *P. gallinaceum* (Rama Rao & Sirsi, 1958). Aspartic acid, valine, glycine, threonine, and phenylalanine reached three to six times their original values. However, the plasma concentration of most amino acids fell during parasitemia, after a slight initial increase during the incubation period. Liver and brain tissues showed a steady decrease of almost all amino acids. Because of a probable close relation to plasmodial metabolism, the investigators making this study directed particular attention to the observed increase of amino acids in the erythrocytes.

In 1918, Barr & DuBois observed that, without exception, a negative nitrogen balance occurred during the acute, febrile period of malaria. In a study of 12 young men with experimentally induced *P. vivax* malaria, Taylor and co-workers (1949) found that levels of serum albumin were 14.5% lower when patients were experiencing five to eight paroxysms and an average of 193 hours of fever at 101 °F (38.3 °C) or more than during control periods. Oomen (1957) noted that Indonesian children between one and 12 years of age who were suffering from both malnutrition and chronic malaria had larger livers than those suffering from one of these conditions alone. Malaria prevalence was estimated by splenic enlargement.

Chicks with acute coccidiosis produced by injection of *Eimeria acervulina* or *Eimeria necatrix* in the fourth week of life gained only 63 g during the fifth week, as compared with 247 g for controls (Panda, 1963). During the sixth week, however, both groups had the same rate of gain, and during the seventh to fourteenth weeks the rate was slightly greater in the previously infected birds (Panda et al., 1962a). Nitrogen retention during the fourteenth week was also slightly greater in the infected chicks (Panda et al., 1964). The adrenal cortex was larger and cells of the islets of Langerhans were hypertrophied and more numerous (Panda & Combs, 1964). These late findings were presumably compensatory.

Acute trypanosomiasis produces a greatly decreased serum albumin and increased serum gamma globulin levels in monkeys (Woodruff, 1959; Smithers & Terry, 1959). Mice injected with a "non-pathogenic" strain of *Trypanosoma duttoni* and then starved, died significantly earlier than controls (Sheppe & Adams, 1957).

Janz & Pinto (1963) were unable to establish that rats infected with *T. rhodesiense* experienced any significant change in nitrogen metabolism directly attributable to the infection. With lower food intake and a continuing fever, changes in nitrogen metabolism were demonstrable.

Giardia lamblia may attach itself to the intestinal mucosa in man in sufficient numbers to impair nutrient absorption (Cortner, 1959). Undoubtedly, nitrogen absorption is affected, although no direct studies have been made.

Both trypanosomiasis and malaria tend to produce, in their febrile stages, a negative nitrogen balance, but only when the concentration of

parasites is relatively great. Severe infections with pathogenic intestinal protozoa also influence nitrogen balance adversely, although few have been studied.

Helminthic infections

Most of the infections thus far discussed cause an increased excretion of nitrogen in the urine, with little or no interference with nitrogen absorption from the gastrointestinal tract. Investigators of the nutrition of domestic animals have long recognized that helminthic infections interfere directly with protein utilization. Many years ago, the University of Cambridge Institute of Animal Pathology carried out nitrogen balance studies in sheep infected with a variety of nematodes. Fluctuations in protein absorption paralleled changes in the worm burden (Stewart, 1932-33). Even when many worms were present, the digestion of other components of the ration was unaffected, with the possible exception of crude fiber.

It is conceivable that in some helminthic infections anti-enzymes directly inhibit pepsin and trypsin in the intestinal lumen. For example, Sang (1938) assigned the name "ascarase" to an enzyme isolated from *Ascaris lumbricoides* that *in vitro* has an inhibitory effect on pepsin and trypsin and *in vivo* a significant capacity to decrease food absorption. Since absorption of nutrients other than proteins may be affected at the same time, mechanical damage to the mucosa may also be a factor.

Franklin and associates (1946) found that *Trichostrongylus colubriformis* infection in sheep depressed not only protein digestibility, but also calcium and phosphorus utilization. Sheep on a hay diet lost weight and died when infected with *Trichostrongylus axei* whereas control animals kept worm-free remained unaffected (Gibson, 1954).

Working with rats experimentally infected with *Trichinella spiralis*, Rogers (1942) observed a marked lowering of protein digestion in the immediate 4 to 12 days after infection. A second and less marked response occurred after 30 days, presumably due to the departure of adult female worms from the intestinal wall. A decrease in nitrogen excretion in the urine also suggested that the acute infection affected protein absorption. By the twenty-fourth day, urinary nitrogen had increased to three times the normal amount. Rogers attributed this to tissue inflammation and destruction.

Heavy infection of rats with the hookworm *Nippostrongylus muris* markedly reduced net protein utilization, mostly by decreasing the amount of absorbed dietary nitrogen (Platt et al., 1961). This was also true of puppies infected with *Toxocara canis* (Platt & Heard, 1965).

Human adults with heavy hookworm infection had an average nitrogen absorption of 62.5%, compared with 73.3% in worm-free subjects on the

same diet (Darke, 1959). Holmes & Darke (1959) emphasized the greater importance of hookworm disease when food supplies were limited. Puerto Rican army recruits with severe hookworm disease had decreased intestinal absorption of nutrients (Sheehy et al., 1962). In severe infections more albumin was lost into the gut than would be expected from loss of red cells alone, since the hookworms appeared to ingest tissue fluid as well as capillary blood (Gilles et al., 1964).

In careful studies of nine children with heavy *Ascaris lumbricoides* infection, Venkatachalam & Patwardhan (1953) observed a decrease in fecal nitrogen per 24 hours from 1.3 to 0.7 g after de-worming. They attributed the original loss to an antiproteolytic substance secreted by the parasite. Darke's (1959) findings in nine patients in Buganda, Africa, were similar.

Schistosomiasis due to *Schistosoma haematobium* may produce a loss of albumin in the urine sufficient to result in lowered serum albumin and severe edema. Losses of 8 to 9 g of albumin within 24 hours were observed in two patients with severe disease (Farid, personal communication, 1965).

Loughlin & Mullin (1955) suggest that infectious diseases due to intestinal parasites and a variety of other causes have the capacity to shorten the time that food remains in the gastrointestinal tract of children ("intestinal hurry"). The result is a diminished opportunity for digestion and absorption. The anorexia, indigestion, colitis, and bloody stools in severe cases of trichuriasis fall into this category (Jung & Beaver, 1951). Venkatachalam & Patwardhan (1953) list "intestinal hurry", mechanical blockage, and mucosal damage as factors in the effect of ascariasis on the nutritional status of children.

Many investigators mention the frequent association of intestinal helminths and kwashiorkor. Among those expressing a belief that helminths are often a factor in precipitating the nutritional disorder are Peña Chavarría and co-workers (1948), in Costa Rica; Gillman & Gillman (1951), in South Africa; Jelliffe (1953a), in Western Nigeria; Williams (1953), in Ghana; Thomson (1954), in Malaya; De Silva (1954), in Ceylon; Netrasiri & Netrasiri (1955), in Thailand; Stransky & Reyes (1955), in the Philippines; Platt (1958b), in Africa; Pohowalla & Singh (1959), in India; and Fraga (1965), in Brazil. In a child with prior growth failure, there was a gain in body-weight with no change in diet following treatment of a heavy ascaris infection (Jelliffe, 1953a).

It should be noted, however, that Bray (1953) found no difference in fecal nitrogen in West African children before and after treatment for ascariasis and hookworm disease. On the basis of measurements of fecal fat and the urinary excretion of a test oral dose of D-xylose, Kotcher and co-workers (1966) found no evidence of intestinal malabsorption in children with strongyloidiasis or hookworm infection, but they did not measure nitrogen absorption. On the other hand, De Lima et al. (1966) reported D-xylose almost uniformly lower in 20 children with various combinations of

hookworm, *Trichuris*, *Strongyloides*, *Ascaris*, and *Giardia* than in 12 children without intestinal parasites. However, primary differences in nutritional status as well as the presence or absence of parasitism may have contributed to the results. Unpublished INCAP observations indicate that these diseases produce a detectable effect on nitrogen balance, as determined by examination of children on a constant intake of protein before and after treatment, only when they occur in a severe form. In India, the frequency with which intestinal parasites and ova were found in the intestines of children admitted to the Pediatric Hospital was essentially the same, whether or not they were suffering from kwashiorkor. (India, Council of Medical Research, Nutrition Research Laboratories, 1965).

By interfering with intake, absorption, and retention of protein nitrogen, almost any unusually heavy infection by an intestinal helminth can probably induce protein malnutrition in persons whose diet is otherwise adequate. Severe intestinal helminthic infections are common in many populations. The mere presence of a helminth does not, however, justify an assumption that it has clinical or metabolic significance. The relative importance of these infections in contributing to protein malnutrition is often overemphasized, for the simple reason that intestinal helminths are visible or are readily demonstrated microscopically, whereas other infectious agents or the action of nutrients can be recognized only by complicated technical procedures. Unfortunately, few specific epidemiologic data are available regarding schistosomiasis, filariasis, onchocerciasis, and other helminthic infections that might well be expected to exert a systemic nutritional effect.

Excess of protein or amino acids

Increasing attention is at present being given to possible imbalances of nutrients, particularly the amino acids. Proof has accumulated that excess consumption of some nutrients markedly alters the requirements for certain others (Harper, 1957-58; Hsu, 1963). For example, excess methionine increases the requirement for tryptophan to the point of creating a relative deficiency. An instance of such antagonism was reported by Gershoff and associates (1952), who found that excess methionine interfered with type 2 poliovirus in mice; as they had previously demonstrated an antagonism between the virus and tryptophan deficiency, they concluded that the methionine acted by creating a relative deficiency of tryptophan. This methionine-induced tryptophan deficiency was further accentuated by giving the analogue 6-methyltryptophan.

A second instance, reported by Squibb (1964b), is that Newcastle disease, a viral infection of chicks, is made worse by either a deficiency or a surfeit of protein. The surfeit was induced by a diet containing 41% of casein. Two possible explanations were offered: first, that the raised energy requirements resulting from the infection caused an increase in the metabolic

use of protein, thus producing an amino acid imbalance; second, that the extra protein caused an increased multiplication of the virus.

Infection and recovery

If the increased urinary nitrogen excretion associated with trauma and acute infection arises from so-called "toxic destruction" of protein, as was formerly believed (DuBois, 1927; Peters & Van Slyke, 1946), it then becomes necessary to explain why the "destruction" is much less in malnourished persons than in those with adequate protein stores (Fleck & Munro, 1963). Increased loss of urinary nitrogen with no apparent tissue destruction also occurs through stress of psychological origin (Scrimshaw, 1963; Ohlson, 1958).

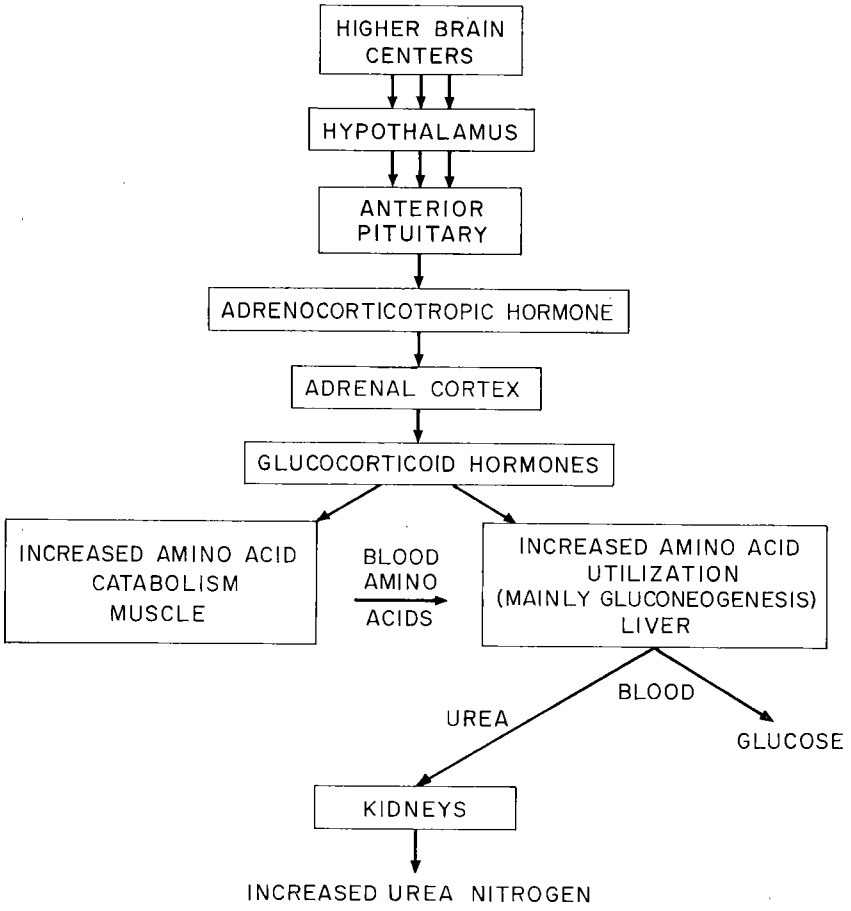
Much of the excess nitrogen in the urine of individuals with infectious disease probably comes from skeletal muscle, mobilized as a result of the adrenal cortical response to additional adrenocorticotropic hormone (ACTH) produced by the anterior pituitary during stress from infection. The increased 17-hydroxysteroid hormones exert a catabolic influence on muscle, so that amino acids are released into the blood-stream. At the same time, these hormones are anabolic for the liver and stimulate gluconeogenesis from amino acids.

The endocrine relationships are shown schematically in Fig. 1. It cannot be assumed, however, that they comprise the whole mechanism. Adrenalectomized animals on a maintenance dose of cortisone are capable of the stress response, although it has been postulated that this arises from adrenal tissue outside the adrenal glands.

During recovery from stress of infectious origin, additional nitrogen must be retained to replenish that lost from muscle and other tissues. The so-called "anabolic phase" of recovery from infection or trauma was measured quantitatively in patients with typhoid fever and pneumonia as early as 1875 (Svenson, 1901), and is now so well known as to require no further documentation.

Waife and associates (1950) studied 12 patients with protein depletion and a complicating infection. Six patients given relatively large protein supplements in addition to an already adequate diet retained an amount of nitrogen directly proportional to that provided in the supplement. Four patients retained little or no additional nitrogen. Another patient, receiving a somewhat smaller protein supplement of about 4 g daily, utilized this nitrogen with increasing efficiency so that, toward the end of 38 days, she was storing more and more of the total protein intake. The twelfth patient, whose diet was unsupplemented and regarded as the control, apparently began to reach nitrogen equilibrium after 50 days of a mildly positive balance; a nitrogen balance of +3.54 g in the first ten days dropped to +0.51 g for the last five days. These investigators, in an effort to interpret

FIG. 1. SIMPLIFIED SCHEME SHOWING EFFECT OF STRESS OF INFECTIOUS, TRAUMATIC, OR PSYCHOLOGICAL ORIGIN ON PROTEIN METABOLISM

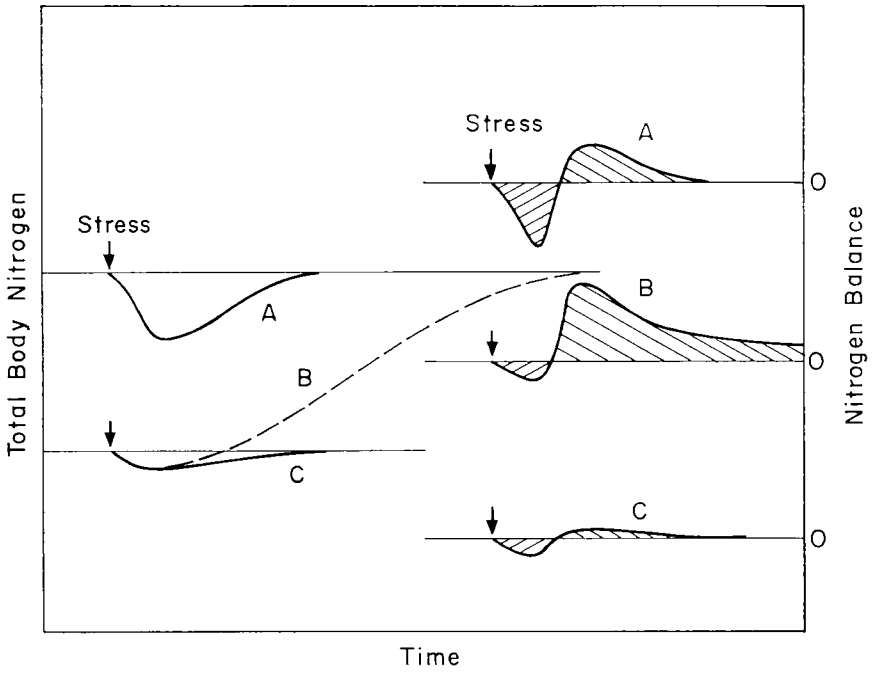


the variable results, proposed the scheme from which Fig. 2 has been derived.

The magnitude of the initial catabolic response to stress, as measured by the nitrogen balance technique, appears experimentally to be inversely proportional to the degree of nitrogen depletion in the animal. The magnitude of the stress itself may or may not be reflected in the nitrogen balance, depending upon the initial state of nutrition of the host. Fig. 2 shows diagrammatically the expected changes in nitrogen balance under various conditions.

Whatever the initial response, an anabolic phase of nitrogen balance would be expected after the catabolic phase if the dietary protein intake were sufficient. In a repleted animal subjected to a short period of acute

FIG. 2. EXPECTED CHANGES IN NITROGEN BALANCE UNDER VARIOUS CONDITIONS



A = well nourished
B = poorly nourished before stress, well nourished after
C = poorly nourished throughout

stress, equilibrium might be regained quickly, whereas a depleted animal would require a long time regardless of the smaller initial response.

The previously described effects of infection on nitrogen metabolism are consistent with the interpretation just given. Since psychological stress is also followed by an increase in urinary nitrogen, the action of the endocrine mechanism is probably much more important in stress from infection than is the direct "toxic" destruction of protein by the infectious agent. This conclusion receives support from a recent report by Rapoport et al. (1968) describing endocrine and liver enzymes in the course of experimental pneumococcal infection in mice.

Another way in which infectious disease may increase nitrogen loss is through the sweating associated with fever. Under minimum sweating conditions (27-28 °C, 43-45% humidity), the nitrogen loss in perspiration has been assessed at 0.36 g per day for an adult male. Exposure to moist heat (37-39 °C, 64-73% humidity) can result in nitrogen losses equivalent to 20 g or more of protein (Mitchell & Hamilton, 1949).

These observations were recently confirmed by Consolazio and associates (1963) by intermittently exposing human volunteers to different temperatures

and humidities for periods of 8 to 24 hours over several days. A compensatory reduction in urinary nitrogen output was not observed. Clearly, the increased loss of amino acids and other nitrogenous compounds during fever deserves more attention than it has thus far had.

Infection and Nutritional Status, Other Nutrients

Vitamin A

Because low intakes of vitamin A are common in human diets, the direct effect of infection on vitamin A metabolism is of practical importance.

Chicks infected with *Eimeria tenella* had decreased liver stores of vitamin A, even when large amounts of the vitamin were fed to them (Erasmus et al., 1960). Although the enhanced vitamin A in the diet did not alter the severity of the coccidiosis, it resulted in better appetites and faster growth in the surviving chicks. Coryza of chickens also lowered vitamin A levels in the tissues, but paired-feeding studies indicated this was due to a drastic decrease in food intake (Squibb et al., 1955). Newcastle disease had no effect on vitamin A reserves of chicks nor on absorption of this nutrient from the gut. Furthermore, vitamin A therapy of chicks with adequate nutritional reserves prior to infection was without benefit (Squibb, 1961a).

Because turpentine-induced abscesses lower vitamin A concentrations in the blood of rats by as much as 50% and also decrease the concentration of vitamin A in the liver, it is often presumed that septic abscesses have the same effect. When excess vitamin A was supplied in the diet both to rats with turpentine-induced abscesses and to control rats, concentrations in the liver were almost identical, suggesting that absorption and storage proceeded normally despite the simulated infection (Kagan, 1955).

Soliman (1953) found low liver reserves of vitamin A in cattle with lungworms and in guinea-pigs with livers infected with the lungworms *Dictyocaulus viviparus* and *D. filaria*. The reserves averaged 2.5 IU per gram, compared with 23 IU in healthy animals.

Thirty days after administration of 100, 500, or 1000 *Ascaridia galli*, chickens maintained on a constant diet had vitamin A levels in the liver of 122, 104 and 88.5 μg per 100 g, respectively, compared with 177 μg in control birds (Šihobalova & Kustova, 1950; Šihobalova et al., 1951). The process of formation of antibodies to *Ascaridia* in the chicken not only lowered the vitamin A concentration in the liver but also increased vitamin A in the mitochondria (Leutskaya, 1963). The changes were more marked with purified antigen than with broth cultures (Leutskaya, 1964a,b).

Rats infected with scabies developed signs of vitamin A deficiency on a deficient diet much more rapidly than those free of infection (LeGallic, 1955).

In children, concentrations of vitamin A in the blood are appreciably reduced in pneumonia, rheumatoid arthritis, acute tonsillitis, and rheumatic fever (Shank et al., 1944; Jacobs et al., 1954). Intestinal absorption of vitamin A may also be impaired in the presence of *Giardia lamblia* (Chesney & McCoord, 1934; Katsampes et al., 1944). Normally, vitamin A is not excreted in the urine, but does appear in such pathologic states as obstructive jaundice, chronic nephritis, and pneumonia (Moore, 1957; Goldsmith, 1959).

As early as 1892, Spicer, in England, noted that children with meningitis, infantile diarrhea, chronic tuberculosis, measles, whooping cough, and severe chickenpox frequently developed xerophthalmia. Subsequently, a number of investigators noted that infection precipitates acute clinical avitaminosis A in persons with a latent deficiency (Oomen, 1958). This nutritional disease is still an important cause of blindness in Indonesia and other countries where vitamin A deficiency is endemic among young children (Oomen, 1959). Although no longer a problem in the USA and Western Europe, xerophthalmia and keratomalacia still occur sporadically, after infections, among malnourished infants in nearly all developing countries, even those in which vitamin A deficiency is not common among older children and adults.

In reviewing the frequency of night-blindness in the tropics, Rodger and associates (1960) have emphasized the lower serum carotene and vitamin A values in persons with hookworm disease compared with persons in the same communities without the infection. Steatorrhea and decreased absorption of vitamin A and xylose were found to be common in Puerto Rican army recruits with severe hookworm disease (Sheehy et al., 1962); and, in Colombia, malabsorption has been reported in patients with anemia and multiple intestinal parasites, including hookworms (Vélez & Orrego, 1963).

Onchocerciasis reportedly causes less blindness in endemic areas where agricultural development is good than it does where farming conditions are poor (Rodger, 1960, 1962). There is evidence that vitamin A is the important variable, but other factors may well be responsible. Rodger (1957) described high infection rates with the parasite in eastern Nigeria; but blindness was less frequent where red-palm oil, which is high in vitamin A, was used extensively. However, supporting data have not been published.

In summary, infections exert a sufficiently adverse effect on vitamin A nutrition to have practical significance in animal husbandry and in public health practice. This has been proved by both experimental studies in animals and clinical and field observations in man.

Thiamine

A variety of studies on laboratory animals and man support the conclusion that infection affects thiamine metabolism.

Although mice infected with Western equine encephalitis virus and fed a diet severely deficient in thiamine showed no clinical signs of infection, they developed thiamine deficiency earlier than did uninfected thiamine-deficient animals (Kearney et al., 1948b). In rats infected with the tapeworm *Hymenolepis diminuta* and injected with ^{35}S-labeled thiamine, Chandler and co-workers (1950) found that the worms obtained thiamine from the blood and tissues rather than directly from the gastrointestinal contents of the animal host. Rama Rao & Sirsi (1956) demonstrated that three- to four-week-old chicks infected with *Plasmodium gallinaceum* had a markedly lower level of thiamine in the blood during the acute infection.

Although quantitative data are few, many qualified observers, themselves interned in Japanese prisoner-of-war camps in the Second World War, remain convinced that bacillary dysentery precipitates acute beriberi in persons on thiamine-deficient diets for long periods (Smith & Woodruff, 1951). Suzman (1955) cites examples of beriberi following pneumonia and malaria. In persons with borderline thiamine deficiency, even bed-bugs have been listed among stress factors capable of producing acute beriberi (Platt, 1958b).

Najjar & Holt (1943) demonstrated that man utilizes thiamine and other B-complex vitamins that are the result of synthesis by intestinal bacteria. When the intestinal flora of nine adolescents on diets marginally deficient in thiamine was suppressed by succinylsulfathiazole, thiamine promptly disappeared from the urine, but reappeared when the sulfonamide was discontinued. Obviously, any pathologic process interfering with the normal distribution and function of intestinal bacteria can affect thiamine nutrition adversely in the presence of insufficient vitamins in the diet (Ellinger et al., 1947).

In summary, infectious disease can precipitate clinical beriberi in persons maintained on a diet inadequate in thiamine. In some regions, many people consume such diets; and the number is often greatly augmented in times of natural disaster or civil disturbance. Under such circumstances the action of infection on thiamine nutrition has major public health importance.

Other B-complex vitamins

Experimental studies in man have proved that infections have an effect on other B-complex vitamins such as niacin, pyridoxine, folic acid, and vitamin B_{12}.

Naturally occurring respiratory infections have precipitated megaloblastic anemia in monkeys maintained on a diet low in folic acid, as have subcutaneous abscesses induced by turpentine (May et al., 1952). Luhby (1959) concluded that 9 of 27 children with megaloblastic anemia would not have developed that condition from diet alone; a superimposed infection was the deciding factor.

Campbell & Pruitt (1952) claimed that, in patients with acute infectious hepatitis, supplementary vitamin B_{12} brought about an earlier return of appetite and normal liver size than did a high-protein, high-carbohydrate diet, even when this was supplemented with brewers' yeast as a source of B-complex vitamins. Although the study included 100 patients, the implication that viral hepatitis puts a special stress on vitamin B_{12} metabolism cannot be accepted without better evidence, because the chronic nature of the disease and the tendency toward remissions make therapeutic measures difficult to evaluate.

The classic studies of Von Bonsdorff in Finland (1948, 1952, 1956, 1964) have demonstrated that the fish tapeworm *Diphyllobothrium latum* has such a voracious appetite for vitamin B_{12} that it often causes megaloblastic anemia in affected persons, a finding substantiated by the use of ^{60}Co-labeled vitamin B_{12} (Scudamore et al., 1961) and the determination of vitamin B_{12} blood serum levels (Nyberg et al., 1961). Recovery from the anemia follows expulsion of the worms (Palva, 1962). This subject has been reviewed by Nyberg and co-workers (1961). Even optic atrophy has been attributed to vitamin B_{12} deficiency in fish tapeworm carriers (Björkenheim, 1966).

In the experience of Layrisse and associates (1959), patients with anemia due to hookworm infection had a low absorption of folic acid and low levels of serum vitamin B_{12}.

Khalil (1924) reported that pellagra in Egypt was six times more frequent in persons with hookworm disease than in those without that infection.

The observations of Jiménez Díaz and co-workers (1952) that the feces of children suffering from acute enteritis were unusually low in thiamine and pyridoxine but unchanged in riboflavin content are significant. Had this been a non-specific effect from reduced food intake, the riboflavin content of the feces would also have been affected. As Frazer (1949) has suggested for sprue, the effect may be due partly to interference with bacterial synthesis in the small intestine, after replacement of the normal duodenal flora by a microbiota characteristic of the lower intestinal tract.

Ellinger & Abdel-Kader (1947) showed that the intestinal flora contains niacinamide-synthesizing *Escherichia coli* as well as niacinamide-consuming bacteria such as *Shigella sonnei*, *Shigella dysenteriae*, *Shigella flexneri*, *Streptococcus faecalis*, and *Proteus vulgaris*. A predominance of one group over the other may affect the niacinamide nutritional state. The ingestion of *p*-aminomethylbenzene sulfonamide increased the niacinamide content of the fecal flora and urinary output of *N*-methylnicotinamide by stimulating the niacinamide-synthesizing coliform bacteria and inhibiting the growth of non-coliform micro-organisms. The predominance of dysentery bacilli in the intestine would conceivably lead to consumption of niacinamide and might aggravate a deficiency in the pellagra-preventive factor.

Trager (1947b, 1954) has found that *Plasmodium lophurae* malaria in chickens lowered by half the levels of biotin and co-enzyme A in the liver

after only five days. He believes the pathology of the infection to be influenced by this action.

Although infection often causes a deficiency of an essential nutrient, micro-organisms occasionally increase the supply. Levels of niacinamide tend to be high in the tissues of tuberculous patients (Abdel-Kader et al., 1951; El-Ridi et al., 1958), which may explain the rarity of coexistent pellagra and pulmonary tuberculosis. *Mycobacterium tuberculosis* is a niacin-synthesizing micro-organism (Bird, 1947). Tryptophan, a precursor of niacin elaborated by coliform bacilli (Ellinger et al., 1947; Ellinger & Abdel-Kader, 1949), has been shown to have an inhibitory action on human strains of *Mycobacterium tuberculosis*, both *in vitro* and *in vivo*, in the guinea-pig (Abdel-Kader & Zaki, 1958), the indole ring of tryptophan being mainly responsible for the tuberculostatic effect (Abdel-Kader & Zaki, 1958, 1960a,b). In this instance, the body apparently utilizes a metabolic product as a defense against an invading pathogenic micro-organism. From tissue culture studies it is known that the filterable agent of psittacosis is capable of synthesizing folic acid (Bader & Morgan, 1961).

The important role of the intestinal flora in synthesizing vitamins has long been recognized (Nightingale et al., 1947; Elvehjem, 1948; Johansson & Sarles, 1949). The synthesis of the B vitamins (Barnes et al., 1960; Wostmann et al., 1962) and of vitamin K (Gustafsson, 1959; Wostmann et al., 1963) is particularly important. Some of these vitamins are absorbed directly from the gut; others are available only to animals that practice coprophagy (Levenson & Tennant, 1963a; Barnes et al., 1963; Daft et al., 1963).

In summary, systemic infections are able to induce anemia when folic acid is deficient. Fish tapeworm may cause anemia because of its high requirement for vitamin B_{12}. Although pellagra is sometimes associated with infection in persons deficient in niacin, proof is lacking that infection is a major factor. There is no reason to believe that the demonstrated effect of infection on pyridoxine metabolism has clinical significance. Various mechanisms are involved in the deleterious effect of infections on the various other B vitamins, but more work is required to establish their clinical role.

Ascorbic acid

Interest in infection and ascorbic acid dates back to the early years of the present century, when scurvy was a common disease among children of North America and Europe.

In 1924, Nassau & Scherzer reported an earlier onset of scurvy in guinea-pigs on a deficient diet when an experimental trypanosomiasis was also present. Guinea-pigs on scorbutic diets died of scurvy much sooner in the presence of chronic "healed" tuberculosis (Bieling, 1927). Rinehart and

co-workers (1934) reported that scorbutic guinea-pigs developed mild lesions suggestive of rheumatic fever, which were considerably worsened by superimposed infection with beta hemolytic streptococcus. Well-nourished animals exposed to the infection did not develop appreciable lesions.

Even in the rat, which does not normally require dietary ascorbic acid, Nyden (1948) was able to demonstrate interference with the reduction of ascorbic acid in the spleen and adrenals of animals infected with *Trypanosoma hippicum*. The reduced form of ascorbic acid was present in a lower concentration, despite a normal level of the oxidized vitamin. The effect was less noticeable in skeletal muscle and was absent in liver and blood plasma. Similarly, McKee & Geiman (1946) described significantly low ascorbic acid levels in the plasma and whole blood of monkeys with *Plasmodium knowlesi* parasitemia.

According to Kroshman (1940), children infected with malaria showed a marked decrease in blood concentrations of vitamin C and increased excretion in the urine when compared with non-infected children living under comparable conditions. Lotze (1938) and Mohr (1941) described similar findings in adults. Typhoid fever markedly decreased blood serum levels of ascorbic acid, and dehydro-ascorbic acid accumulated in the blood (Banerjee & Belavady, 1953). Vaishwanar and associates (1959) reported low levels of blood ascorbic acid among schoolchildren in India after an outbreak of influenza. Wirth (1952) believed that, in two cases of hemorrhagic measles in patients whose diets were deficient in vitamin C, the added stress of the viral infection was sufficient to precipitate the clinical picture of vitamin C deficiency.

As early as 1917, Hess called attention to the frequency with which children from low-income families in New York City developed florid scurvy after contracting a febrile illness such as otitis media, pneumonia, or nephritis. Although Hess was not aware that scurvy was a vitamin-deficiency disease, he concluded that it was "precipitated in infants with 'latent' scurvy when an infectious disease such as 'grippe' was superadded". Even vaccination against smallpox has been reported to have had this effect in malnourished German children (Stern, 1923).

Sweany and associates (1941) noted that ascorbic acid saturation decreased with an increasing severity of tuberculosis. In 32 fatal cases the vitamin-C content of the tissues was low relative to the intake of the vitamin. From these data, they estimated that extra amounts of vitamin C up to 150 mg per day disappeared from the body of patients with severe tuberculosis. Getz and co-workers (1951) also found serum ascorbic acid values especially low in tuberculous patients.

Recent INCAP studies have demonstrated an increased loss of vitamin C in the urine during the height of the primary reaction to vaccination against smallpox and to vaccination with attenuated measles virus, as well as during

the acute clinical stages of measles and chickenpox (INCAP unpublished data, 1962).

Clinical manifestations of ascorbic acid deficiency and low urinary excretion of this vitamin were reported for schoolchildren of Madagascar Island having severe ascariasis or other intestinal parasitic disease (Dodin, 1955). No proof of a causative relationship was given.

Among some less privileged populations, the ability of infectious disease to worsen the ascorbic acid nutritional status of man continues to have public health significance.

Vitamin D

In early pneumonia superimposed on rickets, Perevoščikova and co-workers (1956) noted a pronounced decrease in levels of phosphorus and, occasionally, of calcium in the blood, the reaction being in direct proportion to the severity of the infection. Even large doses of vitamin D were unable to bring calcium and phosphorus levels to normal in the blood. The conclusion is that pneumonia may aggravate rickets. Rickets has also been shown to be more severe in chicks suffering from coccidiosis (Stafseth, 1931).

Vitamin K

Increased prothrombin times, indicative of a greater requirement for vitamin K, were observed by Squibb (1964c) in chicks with Newcastle disease. This happened only in the early stages of disease in chicks fed on diets causing low vitamin K reserves. A commercial diet or a single massive dose of menadione sodium bisulfite, injected 24 hours prior to blood sampling on the fifth day after inoculation, provided sufficient vitamin K to maintain normal prothrombin times.

Iron

The effects of acute infection on iron metabolism in man are well documented, although some of the mechanisms are only partly understood. These effects have considerable clinical and public health importance.

Infections influence iron metabolism most directly through loss of blood and a resulting anemia. The figure of 0.67 ml of blood lost per day per hookworm, frequently cited in the older literature (Faust & Russell, 1957), is now known to be an overestimate. By measuring the excretion of ^{51}Cr-labeled hemoglobin, Roche and co-workers (1957a,b) showed that each *Necator americanus* causes daily losses of 0.031 ± 0.017 ml of blood or 2.74 ± 1.50 ml per million eggs. Gilles and associates (1964) obtained a figure of 0.05 ml of blood lost per worm per day for this species. Using the same techniques, Farid and co-workers (1965a) found that the blood

loss for *Ancylostoma duodenale* was five to ten times greater than that cited for *Necator*. They reported a loss per worm per day of 0.26 ± 0.045 ml. In heavily infected patients, the loss ranged from 14 to 45 ml of blood per day, with a daily iron loss of 3.56 to 9.94 mg. Mean values were 26.4 ml of blood and 6.06 mg of iron. These investigators concluded that the mean blood loss per 1000 ova per gram of feces was 4.47 ± 1.6 ml.

^{51}Cr escapes from the blood only through the worm, and is not absorbed from the gastrointestinal tract. By studying hemoglobin labeled simultaneously with ^{51}Cr and with ^{59}Fe, Roche & Pérez-Giménez (1959) demonstrated that an average of 44.1% of the iron lost through hookworms was reabsorbed; individual figures ranged from 13.1% to 76.4%. Two patients studied by Gilles and associates (1964) reabsorbed 47% and 60%, respectively, of the iron from hemoglobin entering the gut. No alteration in red cell survival was found.

In patients with hemoglobin values less than 6.5 g per 100 ml as a result of *Ancylostoma duodenale* infection, Farid and co-workers (1965b) found, by ^{51}Cr determination, a shortened red cell half-life; this returned to normal when the anemia was corrected. They believed that iron-depleted red cells were sequestered by an enlarged spleen. Layrisse and associates (1961, 1964, 1965) reported similar findings, although they attributed the reduced red blood cell survival to lack of an intrinsic factor within the cell.

An adequate dietary intake of iron often compensates for a mild to moderate hookworm infection, so that iron-deficiency anemia does not develop (Walker, 1955). Even with light infections (fewer than 100 worms), the loss may be 1 to 1.5 mg per day (Tasker, 1961). The data of Vinke & Jansen (1962) from Curaçao suggest that hookworms lower hemoglobin only when the infection is heavy and the diet deficient. In mildly endemic hookworm areas, the prevalence of iron-deficiency anemia may not correlate with the frequency of hookworm infection (Scrimshaw et al., 1953; Mayet & Powell, 1966).

In hyperendemic areas, however, 10% to 20% of hookworm patients may have more than 2000 eggs per gram of feces (Roche et al., 1957a), equivalent to infection with at least 100 worms; uncommonly, a single human host may have 500 worms or more (Faust & Russell, 1957). Anemia is usual with such severe infections.

In Georgia, USA, Hill & Andrews (1942) observed a progressive decline in hemoglobin with hookworm infection only when counts were in excess of 2500 ova per gram of feces. Dealing with infections in most instances in excess of 2000 ova per gram of feces and several with more than 10 000, White and co-workers (1957), in Peru, found a reasonably good inverse correlation between numbers of *Necator americanus* eggs and hemoglobin values, as did Farid & Miale (1962) for *Ancylostoma duodenale* in Egypt. Among Nigerians, iron-deficiency anemia was found only in persons with more than 20 000 ova per gram of stool, or about 800 worms (Gilles et al.,

1964). Obviously, a synergistic combination of a diet low in iron and a heavy hookworm infection has major health significance for some populations.

Studies by Cruz (1934), in Cuba, and by Rhoads and associates (1934a) and Payne & Payne (1940), in Puerto Rico, indicate that anemia resulting from hookworm disease responds promptly to the addition of iron to the diet, or more slowly to anthelminthic treatment. White and co-workers (1957) demonstrated good hemoglobin response to as little as 15 mg of iron daily. Needless to say, the most rapid and lasting improvement occurs when the two procedures are combined (Rhoads et al., 1934a,b; Cruz & de Mello, 1948).

Pérez & Comparini (1960), in Guatemala, found hookworm infection in 61% of general hospital patients with hemoglobin levels below 6 g and in only 5.5% with normal levels. Chiriboga and associates (1950) reported a frequency of 42% microcytic and 9.5% macrocytic anemia among 21 patients having more than 500 hookworms, as judged by fecal egg counts. With lighter infections, anemia was much less frequent. As suggested by Foy & Kondi (1960, 1961), a direct relationship almost certainly exists between number of bleeding points, extent of mucosal damage due to hookworms, and nutritional status.

An outstanding feature of chronic malaria is the anemia it produces. This is apparently due to the fact that the malaria parasites meet their high protein requirements by splitting hemoglobin, leaving large amounts of unused heme as malaria pigment (Ball et al., 1948; Deegan & Maegraith, 1958). The pigment is picked up by the reticuloendothelial system and only slowly reoxidized to hemosiderin to be re-used (Rigdon, 1945). Iron may also be lost in malaria through hemoglobinuria (especially in "blackwater fever") and, to a small extent, through the gastrointestinal tract (Deegan & Maegraith, 1958).

In a study by Chiriboga and associates (1950), 20 patients with malaria who also had more than 500 hookworms each were found to have an average of 23% macrocytic and only 15% microcytic anemia, whereas 9 of 19 patients with malaria alone had macrocytic anemia but no instance of the microcytic anemia classically associated with iron deficiency.

From studies in East Africa in areas where malaria was uncontrolled and in others where control measures were successful, Draper (1960) cited evidence that the rise and fall of hemoglobin levels directly paralleled the prevalence of malaria. Unfortunately, types of anemia and of malaria were not specified.

In well-fed mice with experimental hepatosplenic schistosomiasis, DeWitt & Warren (1959) observed development of a severe anemia (hemoglobin 4-6 g per 100 ml) in some of the animals in the tenth week after infection. Although the type of anemia was not recorded, it was probably the anemia of chronic infection.

Some evidence exists that *Schistosoma mansoni* causes iron-deficiency anemia (Jamra et al., 1964), although blood loss is irregular and may be negligible (Walters & Waterlow, 1954). Serum bilirubin, fecal urobilinogen, reticulocyte counts, and red cell half-life (determined by labeling with ^{51}Cr) were normal in patients investigated by Farid and co-workers (1964). Such anemias as occurred were apparently due to inadequate iron intake and responded readily to ferrous sulfate. In *Schistosoma haematobium* infection, the blood loss, estimated through uptake of ^{59}Fe, averaged 4 to 10 ml of blood per day (Farid, personal communication, 1965). Ten patients had an average hemoglobin of 4.3 g per 100 ml.

Even extensive bedbug bites have been suggested as a factor in the development of iron deficiency in children, through direct loss of hemoglobin (Venkatachalam & Belavady, 1962). *Trichuris* infection has been reported to produce a fecal blood loss of about 0.005 ml per worm per day, sufficient to cause anemia in children with heavy infections of over 800 parasites (Layrisse et al., 1967).

The hypochromic, microcytic anemia characteristic of iron deficiency also occurs in populations in which neither hookworm nor malaria is endemic. Of 91 English women in the thirty-second to thirty-fourth week of pregnancy and having a urinary infection, 56 were described as having iron-deficiency anemia; in 30, the hemoglobin rose significantly when the infection was treated (Giles & Brown, 1962).

The anemia associated with chronic infectious disease varies greatly with the nature and severity of the infection. "Anemia of infection" was first explored systematically in a series of papers from the University of Utah. Cartwright and co-workers (1946) showed that both staphylococcal and sterile turpentine abscesses produced hypoferremia and anemia in dogs. Wintrobe and associates (1947) obtained the same results with pigs and also showed that the hemopoietic response of iron- or pyridoxine-deficient animals to the missing nutrient was markedly impaired in the presence of infection.

The anemia of infection is not the result of blood loss, overt hemolysis, or iron deficiency, and does not respond to administration of folic acid or vitamin B_{12}. The bone marrow has a decreased proportion of red cell precursors, some increase in proportion of immature cells, and poor hemoglobin production in normoblasts (Harris, 1963). Administration of radioactive iron revealed reduced iron-binding capacity and thereby decreased iron retention (Bush et al., 1956; Princiotto et al., 1964). Cartwright and associates (1946) state that the effect can be simulated in dogs by injection of ACTH.

The life span of erythrocytes is shortened by a variety of chronic infectious diseases (Ebaugh et al., 1954; Freireich et al., 1954; Bush et al., 1956) and by acute rheumatic fever (Reinhold, 1954). The bone marrow in these situations is unable to increase red cell production by the 50% needed to

compensate for a shortened life cycle, although normal bone marrow can increase erythrocyte output six- to eight-fold (Brown, 1950). Apparently, patients with chronic infection exhibit marked inhibition of erythropoiesis. A defect in the release of iron from the reticuloendothelial cells to transferrin for hemoglobin synthesis has been interpreted as at least one responsible factor (Gubler et al., 1950; Freireich et al., 1957a,b). This, together with an elevated erythrocyte protoporphyrin concentration, has been viewed as a quantitative defect in the rate of conversion of protoporphyrin to hemoglobin (Vaughan, 1948; Cartwright & Wintrobe, 1952).

Among 28 patients with a chronic infection accompanied by weight loss, Clark and co-workers (1947) found total hemoglobin reduced, on an average, to 58% of values calculated according to normal weight. Comparing 15 patients with infectious and "toxic" diseases with 17 controls, Jarnum & Lassen (1961) found a lower serum concentration of transferrin that closely paralleled a fall in albumin. They attributed both findings to a temporary decrease in the rates of synthesis to values less than those of breakdown.

Hemolytic anemia may develop in association with some acute infections to such an extent as to dominate the clinical findings. Jandl and co-workers (1961) pointed out that the following conditions may also cause anemia: an acute, often lethal, intravascular hemolysis during invasion of the bloodstream by such organisms as *Clostridium perfringens*; a direct infection of red cells by *Bartonella bacilliformis*, causing splenic trapping and hemolysis; the acute or chronic hemolysis associated with cold agglutinins or hemolysins; and increased splenic sequestration.

Dogs with experimentally induced staphylococcal abscesses developed anemia within two weeks (Matsumoto et al., 1962). Another group of dogs whose reticuloendothelial system was blocked by intravenous injection of India ink also developed anemia. With both infection and blockage of the reticuloendothelial system, the severity of anemia increased. A frequent association of anemia with amebic abscess of the liver has been noted by Mayet & Powell (1964).

To summarize the studies cited, hookworm disease is apparently responsible for much iron-deficiency anemia, due to blood passing through the worms into the intestine. Malaria, especially chronic malaria, often results in significant iron loss. Chronic infections of bacterial or viral origin produce the so-called "anemia of infection" by interfering with iron-binding capacity and erythrocyte life span. Some acute infections induce hemolytic anemia.

Other minerals

Numerous systemic infectious diseases, as well as local enteric infectious processes, cause diarrhea and, with it, a frequent profound disturbance of electrolyte balance. There is limited evidence that infections also cause

significant alterations of calcium and phosphorus metabolism in domestic and laboratory animals.

Nematode infections of sheep contribute to less-effective utilization of both calcium and phosphorus (Shearer & Stewart, 1932-33; Franklin et al., 1946), but the metabolism of sodium and potassium does not appear to be affected. In studies of lambs experimentally infected with *Cooperia curticei* or *Trichostrongylus clubriformis*, Andrews (1938) found no effect on absorption of calcium and phosphorus, although weight gain per unit of food consumed was less.

It has been observed that *Trichinella spiralis* in rats interferes more with assimilation of calcium than with that of protein (Rogers, 1942). In the same study, urinary phosphorus excretion fell notably during the acute infection, indicating that absorption of this mineral from the gastrointestinal tract was reduced. Rao & Gopalan (1958) found a marked calcium and phosphorus loss in tuberculous guinea-pigs compared with non-infected animals on a comparable diet.

In an extended study of nearly 1000 adolescent girls (Johnston, 1953), 39 with active tuberculosis had a negative calcium balance that was corrected only by increasing the calcium intake during several months.

Large increases in serum copper values have been reported for patients with tuberculosis (Panvalkar et al., 1961), but the significance of this finding is unknown.

Any infection causing diarrhea results regularly in a disturbed mineral balance, since the loss in feces is usually sufficiently large to deplete the body of sodium chloride, potassium, and phosphorus, as well as other nutrients (Rapoport et al., 1947; Achor & Smith, 1955). The potassium loss occasioned by kwashiorkor is particularly significant (Hansen & Jenkinson, 1956). Diarrhea often appears early in kwashiorkor as the result of an accompanying infection. It then continues after the infection has run its course because of the changes in intestinal physiology induced by the protein deficiency. By the time kwashiorkor has fully developed, a profound hypokalemia is usually present (Hansen & Jenkinson, 1956; Kahn, 1959; Smith & Waterlow, 1960), as well as serious serum and tissue changes relating to other electrolytes.

One of the most dramatic metabolic effects in any infection is the fulminating electrolyte imbalance in cholera, so commonly followed by circulatory collapse. Although the end results have long been known, recent studies with modern biochemical methods have greatly clarified understanding of this sequence of events. Years ago, O'Shaughnessy (1832) pointed out that, in addition to the obvious dehydration, there occurred a massive salt and bicarbonate loss from the blood, these elements being "present in large quantities in the peculiar white ejected matters". Studies in Bangkok, Dacca, and Calcutta (Phillips, 1963) have shown that the electrolyte loss is primarily in potassium, bicarbonate, and, to a lesser degree, sodium and chloride. The potassium loss can result in a 15% to 30%

deficit of body stores. It appears to be responsible for the kidney damage that so commonly results in uremia. Other evidence suggests that a toxin paralyses the "sodium pump" in the intestinal mucosa, preventing reabsorption of electrolytes from the upper bowel, where they are normally present during digestion (Huber & Phillips, 1960; Phillips, 1963). Major therapeutic advances from this work have reached the point where prompt treatment should make cholera a far less terrifying disease.

In young men with acute tularemia, Beisel & Sawyer (1964) observed an increased excretion of potassium. A sudden renal retention of chloride was characteristic of recovery. Negative calcium balance followed the peak of fever (Joy et al., 1964; Beisel et al., 1964, 1967).

The extensive literature on malaria and electrolyte balance has been reviewed by McKee (1951). Much discussion has centered on the suggestion that the typical paroxysms of chills and fever are due to the massive release of potassium from erythrocytes at the completion of the sporulation cycle (Zwemer et al., 1940). Although this explanation suffices with respect to laboratory animals, the process appears somewhat more complicated in humans (Flosi, 1944; Overman et al., 1949a). A part of the electrolyte effect has been attributed to exhaustion of the adrenal cortex (Overman et al., 1949b). By contrast, sodium and phosphorus values of plasma are decreased. It is believed that the sodium effect is due to such factors as altered cell permeability and excessive sweating (Overman et al., 1949a). The phosphorus decrease may be due to the demonstrated high requirement of the malaria parasite for this electrolyte as part of its dependence on phosphorylative glycolysis for energy requirements (Speck & Evans, 1945a,b; Speck et al., 1946; McKee, 1951).

Eimeria necatrix infection in chicks has been shown to interfere with the absorption of orally administered zinc-65 (Turk & Stephens, 1966), a finding consistent with the interference of this organism with absorption of vitamin A (Erasmus et al., 1960), previously referred to.

Infectious disease thus appears to interfere with calcium and phosphorus metabolism in both animals and man. Furthermore, diarrhea, a symptom of most enteric and some systemic infections, causes a potassium and chloride loss that requires prompt corrective measures. For these reasons, a major part of the public health effort to reduce infant and child mortality from diarrheal disease is directed toward early rehydration and restoration of electrolyte balance.

Lipids

Surprisingly little is known about the action of infection on fat metabolism. Infections such as influenza and pneumonia commonly result in increased liver and fecal fat (Boyd, 1961). This also occurs after infection with *Giardia lamblia* (Cortner, 1959) and perhaps other intestinal parasites.

Von Brand & Mercado (1958) infected rats with *Plasmodium berghei* and found that liver lipids were increased in direct proportion to the parasitemia.

Some intestinal infections clearly decrease absorption of fat, with the result that steatorrhea follows, provided the diet has a continuing normal fat content. Steatorrhea, however, is not harmful; and absorption of fat remains relatively high. Since the anorexia of infection makes the maintenance of an adequate caloric intake difficult, fat should be continued in the usual amounts as a much-needed source of calories (Holt et al., 1915; Chung & Viščorová, 1948; Chung, 1948).

The conclusion follows that, although infection often increases the amount of fat in the liver, any clinical significance is doubtful. The appearance of steatorrhea during enteric infection indicates a somewhat less efficient utilization of this important source of energy, but is no reason to reduce dietary intake of fat. Unless intestinal helminthic infections are very severe, fat absorption is not impaired (Abdalla et al., 1963; Kotcher et al., 1966; Layrisse et al., 1964). The same appears to be true for giardiasis (Palumbo et al., 1962).

Carbohydrates

Markedly low levels of blood glucose are common in infectious disease, probably secondary to reduced caloric intake. The action of systemic protozoa on glucose and glycogen metabolism has special interest. A decrease in blood glucose levels in experimental trypanosomiasis has been shown by Schern (1925), Regendanz & Tropp (1927), von Brand (1961), and others. Regendanz (1929) described the same phenomenon for *Haemobartonella muris* in the duck.

During parasitemia of rats infected with *Plasmodium berghei*, Mercado (1952) found that whole-blood glucose levels fell to 68 mg per 100 ml from the normal level of 96 mg per 100 ml and that serum levels showed a similar decrease from 115 mg per 100 ml in normal serum to 55 mg in parasitized animals. Subsequently, Mercado & von Brand (1954) showed that liver glycogen decreased by about 90% in rats heavily infected with *P. berghei*, and that carcass glycogen was only 40% less. Simultaneous administration of fructose and meticortelone stimulated glycogenesis (Mercado & von Brand, 1957).

Malaria also causes a drop in whole-blood glucose levels in ducks (Marvin & Rigdon, 1945) and monkeys (Christophers & Fulton, 1938). Devakul & Maegraith (1958) reported well-maintained blood-sugar levels in rhesus monkeys until the terminal stages of *P. knowlesi* infection. Devakul (1959) has reported a blood sugar level of 4 mg per 100 ml in a patient with *P. falciparum* malaria. The liver glycogen of animals dying from malaria is low, but glyconeogenesis is possible even in terminal stages of the disease, as indicated by increased glycogen in liver and skeletal muscle after intra-

venous administration of glucose or adrenal cortical hormones. The investigators believe that direct competition for glucose between parasite and host is exaggerated when glucose supplies are limited.

Malaria parasites of several species satisfy their energy requirements *in vitro* by oxidizing glucose, mannose, fructose, or glycerol (Maier & Coggeshall, 1941). *In vivo*, however, they depend almost wholly on glucose. The parasitized erythrocyte utilizes about 25 to 100 times the amount of glucose consumed by a normal red cell. Under anaerobic conditions most of this glucose is converted to lactic acid, demonstrated in monkey malaria to cause a distinct fall in pH of the blood (McKee, 1951). *P. vivax* uses *in vitro* three or more times the glucose consumed by other malarial species (Geiman, 1948). The phosphorylative anaerobic glycolysis from which the malaria parasite derives its energy utilizes only 10% of the total energy available from the complete oxidation of glucose (McKee, 1951). Whatever the mechanism, malaria is characterized by low levels of blood glucose and liver glycogen.

Staphylococcal enterotoxin poisoning in monkeys has also been shown to produce early changes in glucose metabolism, possibly related initially to catecholamine release and, later, to increased utilization of glucose and to metabolic acidosis (Crawley et al., 1966). Such changes were seen during clinical illness from tularemia in seven human subjects (Shambaugh & Beisel, 1967), and probably occur with most other infections.

Enzymes

The general opinion derived from an extensive literature is that infections commonly alter the activity of essential enzymes. For example, *Pasteurella tularensis* reduces aconitase and furmarase activity in liver tissue of rats by more than a third (Woodward & Miraglia, 1961). Recent studies with bacteriophage-infected *Escherichia coli* (Flaks et al., 1959) make it clear that introduction of viral deoxyribonucleic acid (DNA) in the host cell alters the pattern of enzyme activity. A possible relation to the nutritional status of higher organisms has not been explored.

In the late stages of *Plasmodium knowlesi* infection in monkeys and *P. berghei* infection in mice (Riley & Maegraith, 1961), blood serum contains a factor that inhibits the normal oxidative phosphorylation of liver mitochondria *in vitro*. Although not specific, since it occurs with any prolonged anemia or with starvation, this may be an important factor in pathogenesis. Other enzyme systems of monkeys (Maegraith et al., 1962) and mice (Riley & Maegraith, 1962) also depressed under these conditions include succinic, glutamic, and beta-hydroxybutyric dehydrogenases, and dinitrophenol-stimulated adenosine triphosphate (ATP) activity. Both latent and magnesium-stimulated ATP activity are increased.

In observations on 32 children with scarlet fever (Véghelyi, 1949), all except 5 had a marked decline in duodenal amylase, trypsin, and lipase activity, and all had lowered values for blood amylase. Presumably, the effect was primary, not secondary to protein or other nutritional deficiency, since the children appeared well nourished. There is evidence, from the USSR, of decreased duodenal activity in opisthorchiasis (Sokolova, 1948); and Plotnikov (1953) has reported the same effect in pancreatitis associated with *Schistosoma japonicum*. Decreased activity of intestinal enzymes in infectious disease could conceivably lead to poorer digestion and absorption of nutrients, as it does in kwashiorkor, thus exacerbating the nutritional difficulties of children already suffering from protein deficiency.

The ability of various infections to increase alkaline phosphatase and other blood and tissue enzymes is outside the scope of this presentation. The marked effect of infections on neutrophil alkaline phosphatase of man has been reviewed by Beisel (1967).

Unknown factors

A number of studies suggest a probable nutritional disturbance after an infection, without giving any indication which nutrients were involved. An example is the report of Gibson (1954) on sheep infected with *Trichostrongylus axei* and fed a diet of hay. Infected animals developed severe loss of weight, severe oligocythemia, and a tendency toward neutrophilia just preceding death. Control animals fed the same diet had no symptoms.

Effect of Infection on Growth and Development

In addition to altering absorption, metabolism, and the excretion of specific nutrients, as this chapter has described, infections reduce food intake by an action on appetite. Also, as a part of therapy, a frequent custom is to withdraw solid food or otherwise change the diet during an illness, thereby reducing nutrient intake. Purgatives and other medicines likewise interfere with absorption and utilization of nutrients. Consequently it is not surprising that severe or prolonged illness has an adverse effect on growth and maturation, especially when a child is already malnourished.

Several investigators have failed to find any deleterious effect of the usual infectious diseases on the growth of well-nourished children (Martens & Meredith, 1942; Sontag & Lipford, 1943; Cahn & Roche, 1961). Other long-term studies of well-nourished children have identified instances in which growth appears to have been depressed (Maresh & Washburn, 1940; Valadian et al., 1959). The Oxford Child Health Survey of 650 children observed from birth to five years of age (Hewett et al., 1955) ended with the demonstration that children escaping all illness would be one inch taller than those who had experienced "severe" illness, and 0.4 inches taller than those

suffering an "average" amount of sickness. No corresponding effect on bone maturation was observed.

Most investigators in developing countries have been content to recognize a retardation of growth and development in malnourished children who experience a heavy and continued burden of infection. Usually no attempt has been made to distinguish the relative importance of infectious disease and malnutrition. Worse still, some observers have arbitrarily assigned major importance to one or the other according to personal bias, with no facts to support their contention.

Bruce-Chwatt (1952), in Southern Nigeria, described exaggerated flattening of growth curves for children who acquired malaria after five months of age. The eradication programs against malaria in some hyperendemic areas offer a rare opportunity to compare nutritional status before and after a prevailing high incidence of the disease. However, Draper & Draper (1960) failed to find an increased growth in children following area elimination of malaria.

The experience at INCAP indicates that most infectious diseases inhibit growth and development in poorly nourished children, although the relative importance of infection and other factors is often difficult to determine. During an acute disease such as measles, malnourished children lose considerable weight (Morley et al., 1963, 1968; Scrimshaw et al., 1966). Salomón et al. (1968) have reported significant loss of weight in such children after whooping cough, mumps, rubella, and chickenpox, as well as measles. Growth is generally inhibited during both illness and convalescence.

Among children participating in a comparative study of diets of animal and vegetable protein-based mixtures, Truswell and co-workers (1959) noted a sharp drop in weight in those who contracted chickenpox; nitrogen balance was not measured.

Experimental feeding programs among pre-school children in rural Guatemala improved growth and development, but the children were still markedly retarded for their age, compared with well-nourished Guatemalan children (Scrimshaw et al., 1965). The depressing effect of chronic diarrheal disease on the growth and development of children in Chile has been emphasized by Martinez & Weidenslaufer (1963).

Crowley and associates (1956) found that loss of weight in Puerto Rican army recruits was directly related to hookworm infection and was lessened by appropriate therapy. Further investigations are badly needed.

Studies with rats make it clear that pneumococcal septicemia and chronic otitis media have the same effect as acute starvation, in that they cause immediate slowing of chondroplasia followed by a reduction of osteoblastic activity (Acheson & MacIntyre, 1958; Acheson, 1959). Lines of arrested growth appear, similar to those often visible in the roentgenograms of the metaphyses of young children subject to malnutrition. It is clear that

relatively short, acute infectious diseases are able to cause permanent stunting of the skeleton in the rat.

Depression of body-weight of cockerel chicks by Newcastle disease was found to be directly proportional to severity of infection (Squibb, 1961a, b). In subsequent work, alterations in the amino acid pool of infected animals were demonstrated, and it was suggested that this was the mechanism responsible for growth depression (Squibb, 1966). In the experience of Lev & Forbes (1959), germ-free chicks grew faster than animals reared under natural circumstances and having the usual intestinal flora. A main inhibitory effect was from a strain of *Clostridium perfringens* normally present in the conventional chick intestine; it could be reduced to inconsequential levels by an appropriate antibiotic.

The known ability of partially germ-free NCS mice to grow well on a deficient diet has been related to their simple intestinal flora. These mice, however, develop more exacting nutritional requirements when treated with endotoxin. Dubos and associates (1965), in studying the influence of infection on nutritional requirements of germ-free mice, reported an increased need for some essential amino acids, apparently because of an infectious process. Their work confirmed an effect of the indigenous bacterial flora on nutritional state.

The widespread use of antibiotics in commercial feeds for poultry and swine is based on extensive experimental evidence of their ability to increase rates of gain in weight of growing animals (Schendel & Johnson, 1954). Antibiotics presumably act by suppressing unfavorable intestinal micro-organisms (François, 1959; François & Michel, 1964; Jukes, 1955). Holtzman & Visek (1966) present evidence that the mechanism of improved growth in rats fed chlortetracycline may be the reduction in the number of micro-organisms releasing ammonia in the intestinal tract (indicated by a slower turnover in urea hydrolysis in animals given the antibiotic). They suggest that the ammonia released by bacteria in the intestinal tract may cause a thickening reaction in the mucosal wall that might impose a greater demand for nutrients or perhaps cause a loss of necessary substances in the feces.

Daily administration of antibiotics to malnourished children living in an insanitary environment has given equivocal results. Scrimshaw and co-workers (1954) demonstrated a significant increase in height and weight of Guatemalan schoolchildren fed 50 mg of aureomycin daily for six months, compared with those given placebos. At the end of 12, 18, and 24 months, however, there was no difference between experimental and control groups. Penicillin under similar circumstances had no effect at all on growth. MacDougall (1957) found a highly significant increase in the average weekly weight gain of severely undernourished hospitalized African children given aureomycin over periods of two to seven weeks; no increase was observed in a control group given a placebo.

In spite of their effectiveness in the treatment of acute infectious disease, there is no evidence that the prolonged administration of antibiotics to children under natural conditions, even in developing countries, is of any value in increasing their growth and development or in decreasing the frequency of diarrheal disease.

An increased rate of growth in mice infected with *Trypanosoma duttoni* (Lincicome & Shepperson, 1963) is an exception to the usual depressant effect of infection on growth. Mice were observed for a month in two experiments, and for 58 days in a third. Enhancement of growth was apparent at the close of all three studies, despite a three-week period of parasitemia.

Although *Schistosoma mansoni* infection in rats had only a slight effect on food intake and growth, a reduction in maze learning was observed by Kershaw and associates (1959). The results were shown not to be due to decreased food intake *per se*.

Work with animals shows that factors interfering with early growth also influence motor development (McCance, 1962; Lát et al., 1960), the number of neurons in the brain (Lowry et al., 1962; Heard et al., 1961), and behavior (Cowley & Griesel, 1963; McCance, 1962; Nováková, 1966). There is some evidence that malnourished children with arrested somatic growth and biochemical maturation have a coincident retardation of mental development (Stoch & Smythe, 1963; Geber & Dean, 1957; Robles et al., 1959; Wug et al., 1964; Ramos-Galván et al., 1960, 1965; Coursin, 1965).

Summary

The effect of many specific infectious diseases on the nutritional state of the invaded host needs further study. Some nutrients have had little or no attention. Nevertheless, the available facts are sufficient to establish conclusively that most infections have some adverse effect on nutritional status. This has public health significance wherever substantial numbers of persons have nutritionally inadequate diets.

Even trivial infections result in increased loss of nitrogen in the urine. Infections also contribute to protein and other nutrient deficiencies by decreasing appetite and diminishing tolerance to food. In areas where diets are already quantitatively or qualitatively inadequate in protein, the diet given to persons with an infectious disease is usually even more deficient in protein. Treatment of infection often includes administration of purgatives and other medicines with adverse effects on nitrogen absorption or nitrogen retention. Finally, the acute diarrhea so characteristic of most intestinal and some systemic infections has the particular characteristic of decreasing nitrogen absorption. Severe helminthic disease also reduces nitrogen absorption, even in the absence of diarrhea.

It is generally accepted that kwashiorkor is precipitated by acute diarrheal disease, measles, or some other infection superimposed on a diet already dangerously low in usable protein or calories. Evidence also exists that keratomalacia, scurvy, and beriberi are frequent aftermaths of an infectious process in persons subsisting, respectively, on diets deficient in vitamin A, ascorbic acid, and thiamine. Experimental studies in laboratory and domestic animals and in man confirm the adverse effect of infection on the metabolism of these vitamins. Although the evidence is less convincing, infection may precipitate pellagra in niacin-deficient persons.

Infections interfere with the metabolism of calcium and phosphorus. The electrolyte imbalance commonly associated with diarrhea is of major clinical and public health significance in many regions. Chronic infections alter iron metabolism and erythrocyte production to such an extent that the so-called "anemia of infection" develops. Microcytic anemia also results from intestinal bleeding occasioned by severe hookworm disease, and occasionally from urinary blood loss, as with *Schistosoma haematobium*. Infection with the fish tapeworm *Diphyllobothrium latum* frequently leads to macrocytic anemia through avidity of the parasite for vitamin B_{12}.

Frequently, infection leads to an accumulation of fat in the liver and some degree of steatorrhea, although usually neither finding is of clinical importance. Many infections lower blood glucose and limit deposition of glycogen in the liver. Specific infectious diseases also influence various types of enzyme activity.

To an important extent, infectious disease, in conjunction with reduced food intake and an altered metabolism of protein and other specific nutrients, is associated with retardation of growth and maturation of young children. Endocrine factors are directly involved. The need for more protein during convalescence from an infectious disease is a further limiting factor in the growth of those children whose usual diet is of borderline protein adequacy.

Infections so consistently worsen nutritional status that they must be taken into account in all clinical problems and public health programs that involve persons whose diet is inadequate or whose nutritional status is suboptimal.

CHAPTER 3

EFFECT OF MALNUTRITION ON RESISTANCE TO INFECTION

Introduction

This chapter offers a systematic review of published evidence on the extent to which nutritional status conditions the host to infectious disease. Each cited interaction of malnutrition and an infection is presented according to five characteristics: the nutritional deficiency, the species of the host, the type of infection, the results observed, and the significance of the contribution. The aim is to identify patterns of response and possible determinants of the observed results.

Not all the studies cited have equal merit. Design of experiment and completeness of data are often disappointing. Nevertheless, the observations fall into patterns sufficiently distinct to be useful in devising public health measures for prevention and control. They also permit better understanding of the clinical behavior of infectious disease in malnourished persons.

An effort has been made to include all useful references, whether they reveal synergism, antagonism, or absence of effect. Many studies have been omitted because of insufficient numbers of animals or subjects, inadequate controls, or uncertain evidence of an altered nutritional status.

Tables 1 to 18 present a concise summary and classification of reported interactions between nutrition and infection. The text provides additional information and a narrative account. No attempt is made in this chapter to explain the effects observed; Chapter 4 deals with mechanisms.

Multiple Nutritional Deficiencies

Many observations on nutritional deficiencies of man deal with situations in which poor diets are improved by concurrent provision of several nutrients. Because of the multiple factors involved, the results cannot appropriately be tabulated according to specific nutrient deficiencies. In Table 1, therefore, they have been grouped together as "effects of multiple deficiencies".

TABLE 1. EFFECTS OF MULTIPLE NUTRITIONAL DEFICIENCIES ON INFECTIOUS DISEASE

Infectious agent or disease	Host	Response	Reference	Remarks
Bacteria				
Mycobacterium tuberculosis	Man	Synergistic	Leitch (1945)	Qualitative improvement of the diet with no change in total calories drastically reduced a high tuberculosis rate in a Norwegian naval training school after improved housing had failed to do so
M. tuberculosis	Man	Synergistic	Downes (1950)	Familial contacts of 194 families with tuberculosis were divided into two matched groups; the group given multivitamin and mineral supplements had significantly fewer cases than the control group
M. tuberculosis	Man	Synergistic	Faber (1938)	During First World War, tuberculosis mortality in Denmark rose 30% when the diet lacked meat and fish (1916-17); it decreased markedly when German blockade increased local consumption of meat (1917-18)
M. tuberculosis	Man	Synergistic	Cochrane (1945)	Among prisoners of war in Germany, tuberculosis prevalence was high among Russians; much less among Frenchmen with Red Cross food parcels; and zero among British prisoners who had the best food (other conditions were not uniform among the three groups)
M. tuberculosis	Man	Synergistic	Palmer, Jablon & Edwards (1957)	Of 70 000 white men recruited for US Navy (1949-51) 4 times as much tuberculosis among those 15% or more underweight for height
M. tuberculosis	Man	Synergistic	Grafe (1950)	Wartime tuberculosis rates doubled in Germany by 1946 and dropped to prewar level in 1948 when food supply (and other factors) improved
M. tuberculosis	Man	Synergistic	Siebert (1946)	During widespread malnutrition in Germany in 1946, tuberculosis more malignant, more lesion breakdowns, higher mortality than before or since
M. tuberculosis	Mouse	Synergistic	Sengupta & Howie (1948-49)	Mice fed a complete diet had greater resistance than those on milk and whole ground wheat
M. tuberculosis	Guinea-pig	No effect	Mouriquand et al. (1923)	No higher mortality in animals fed a generally deficient diet
Salmonella enteritidis	Mouse	Synergistic	Webster (1930)	Animals receiving bread and milk diet more susceptible than those receiving complete diet
" Diarrhea "	Man (children)	Synergistic	García-Erazo (1960)	Longitudinal study of 100 children: diarrhea more frequent in malnourished children

TABLE 1 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Acute diarrhea	Man (children)	Synergistic	Oropeza & Torres (1963)	Morbidity in malnourished children 3 times that in the satisfactorily nourished, and mortality 7 times
Acute diarrheal disease	Man (children)	Synergistic	Gordon et al. (1964b)	Diarrheal disease more severe in pre-school children judged malnourished by their degree of retardation in weight for age
Acute respiratory disease	Man (children)	Synergistic	García-Erazo (1960)	Longitudinal study of 100 children; acute respiratory disease more frequent in malnourished
Natural infection	Man (children)	Synergistic	Schilling & Naquira (1962)	More bronchopneumonia and other secondary infections complicating measles in young children with marasmus or pluricarencial syndrome than in those of good nutritional state
" Chronic diarrhea "	Monkey	Synergistic	Radhakrishna Rao (1942)	Animals on diets deficient in protein, vitamins, and calcium developed chronic diarrhea that could be reversed by improved diet; diarrhea in controls mild and of short duration
Pasteurella pseudotuberculosis	Guinea-pig	Synergistic	Zachorowski (1952)	Spontaneous outbreak associated with " diet deficient in greens "
Rickettsia				
Rickettsia prowazekii	Man	Synergistic	Gordon (1948)	Typhus more severe among malnourished Russian civilians in Europe at end of Second World War, than among better nourished Allied troops
Virus				
Rous sarcoma virus	Chicken	Antagonistic	Rous (1911)	Thin birds more resistant than well-nourished ones; nature of deficient diet not stated (included because of historical significance)
Infectious hepatitis	Man	Synergistic	Hahn & Bugher (1954)	Disease much more severe among undernourished African tribesmen than in the better-fed
Yellow fever virus	Mouse	Synergistic	Kuczynski (1937)	High fatality in poorly nourished animals compared with pair-fed controls
Poliomyelitis	Rabbit	Antagonistic	Rivers (1941)	Infected " malnourished " animals had fewer, smaller lesions than controls
Protozoa				
Plasmodium gallinaceum	*Aedes aegypti*	Synergistic	Terzian et al. (1953)	On sugar diet alone, high susceptibility judged by oocyst density in stomach after infection; decreased density when niacin, thiamine, biotin, calcium pantothenate, para-aminobenzoic acid, folic acid, pyridoxine, or vitamin C added to diet

TABLE 1 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Eimeria tenella	Chicken	Synergistic	Allen (1932)	Diet deficient in protein, vitamin A, and possibly B-complex caused more hemorrhage, and 23% fatality compared with 5% in well-fed animals
Entamoeba histolytica	Man	Synergistic	Alexander & Meleney (1935)	In two rural communities in Tennessee, white community with food supplements and green vegetables had 40% incidence of amebiasis, but dysentery was rare; Negro community with monotonous and deficient diet had much acute dysentery
Helminths				
Hymenolepis fraterna	Rat	Synergistic	Shorb (1933)	Rats fed diet of white bread and water, deficient in vitamins, minerals, protein, and fat had more worms than controls
H. fraterna	Mouse	Antagonistic	Shorb (1933)	Mice on deficient diet of bread and water had smaller worms and lost them rapidly
H. diminuta	Rat	Synergistic	Vavilova (1946)	After ingesting 1500-2000 eggs, 92% of animals on a vitamin-free diet infected, 80% when only thiamine added, 23% when only vitamins A and D added, compared with 46% on a stock control diet; largest number of worms in animals consuming vitamin-free diet
H. diminuta	Rat	Antagonistic	Addis & Chandler (1944)	Average number of worms reduced by a diet deficient in either fat-soluble vitamins or B-complex vitamins; reduced more when both missing (*see* text)

Tuberculosis

Most studies of the effect of diet on resistance to tuberculosis fall into this category. For example, Leitch (1945) reported a high incidence of tuberculosis among cadets in the Trondheim Naval Training School in Norway prior to improvement of their diet in 1925. Net caloric intake remained unchanged, but the diet was fortified with margarine, cod liver oil, whole wheat bread, fresh fruit, vegetables, and milk. Tuberculosis morbidity promptly dropped to the usual level for young adults of that area. This observation has particular significance since previous attempts at control through improved housing and general hygiene had been unsuccessful.

In Harlem, New York City, 194 families exposed to reinfection with tuberculosis were divided into two groups, matched for family size and related factors, and studied for five years. One group was given supple-

mentary vitamins and minerals; the other served as a control. Despite difficulties in follow-up, recurrence of tuberculosis was shown to be significantly lower among those receiving vitamin therapy (Downes, 1950).

Numerous experiences in time of war support these observations. Mortality from tuberculosis increased sharply in Paris, France, during the War of 1870-71, as well as during both World Wars (Marche & Gounelle, 1950). In 1916 and 1917, when diets lacked meat and fish, the mortality from tuberculosis increased by 30% in Denmark (Faber, 1938), and to an appreciable extent throughout Europe (Rich, 1951). When the German blockade of Denmark later forced up the local consumption of dairy products previously exported, the rate decreased (Faber, 1938).

During the Second World War, death rates from tuberculosis in Germany doubled (Cochrane, 1945). In 1946, when malnutrition was widespread, tuberculosis was especially malignant, with more breakdowns of quiescent lesions and a higher mortality than before or since (Siebert, 1946; Grafe, 1950). By 1948, rates had returned to pre-war levels, in conjunction with an improved food supply (Grafe, 1950). The increase in tuberculosis in the Warsaw Ghetto early in the Second World War was attributed in part to deterioration of nutritional status (Schechter, 1953).

It is pertinent that among 70 000 white men recruited by the US Navy from 1949 to 1951, morbidity rates for tuberculosis among persons who were 15% or more underweight in relation to their height were four times as high as among persons of normal or excess weight. No relationship between body build and infection with tubercle bacilli could be determined on the basis of reaction to the tuberculin skin test (Palmer et al., 1957).

Orr and associates (1931) reported a 6% prevalence of tuberculosis in a predominantly cereal-eating tribe in East Africa, compared with 1% in a tribe with considerable meat and blood in the diet; the two environments were otherwise ecologically similar. Pneumonia and tropical ulcers were also more frequent in the cereal-eating tribe. When the diet of laborers from this tribe was supplemented with milk, meat, and greens, their death rate dropped from 35.4% in 1923 to 10.8% in 1924.

Of related interest is the observation that mice infected with *Mycobacterium tuberculosis* had a lower survival rate on diets limited to wheat and milk than did controls fed complete diets (Sengupta & Howie, 1948-49).

Diarrheas and dysenteries

Numerous comprehensive studies in developing countries have shown that acute diarrheal disease and acute upper respiratory illnesses occur more frequently and last longer among malnourished than among well-nourished children (García-Erazo, 1960; Bevan, 1962; Sabin, 1963; Gordon et al., 1963; Gordon et al., 1964a,b,c). Severe, chronic diarrhea was reported to be common among laborers in the present Tanzania until

their poor diets were improved (McKenzie, 1940). The interrelationship of malnutrition, especially protein deficiency, and diarrheal disease is presented in detail in Chapter 6.

Sharp contrasts in fatality from bacillary dysentery were observed among different population groups in Malaya (Fletcher & Jepps, 1924). Deaths per 100 cases were negligible among Europeans, whereas rates ranged between 2% and 3% for Eurasians, well-to-do Asiatics, and prisoners. They were 25% to 36% among coolie laborers. Nutritional differences were extreme. The Indian coolies had much overt beriberi, inanition, and xerosis, a disease sequence clearly synergistic with dysentery. Differences in death rates did not appear to be due to lack of medical care, since treatment was provided to poorer patients. Among the lower social classes and the heavily exposed laborers, an immunity conceivably acquired during childhood could be expected to limit attack rates. It would not normally account for the high fatality; indeed, the reverse would be more reasonable.

In laboratory investigations, Radhakrishna Rao (1942) observed that monkeys fed diets deficient in protein, calcium, and several vitamins developed chronic diarrheal disease. This condition could be reversed by improving the diet. In well-nourished monkeys, diarrheal disease was ordinarily mild and short-lived.

Respiratory diseases

General nutritional deficiency has also been reported to be synergistic with acute respiratory disease in guinea-pigs (Zachorowski, 1952) and with salmonellosis in mice (Webster, 1930). Robertson & Tisdall (1939) attempted to determine more specifically the factors responsible for the lowered resistance to *Salmonella muriotitis* in rats fed a deficient diet. Animals were observed for 28 days after the infective dose, and the degree of resistance was measured by the percentage survival. In order of decreasing effect were deficiencies of B-complex vitamins, vitamin D, vitamin A, minerals, and proteins.

Other infectious diseases

Warner & Winterton (1935) noted a lower incidence of rheumatic fever among children in an institution after their diet, formerly seriously inadequate, was improved. Other studies on diet and rheumatic fever (Hess, 1920, 1932; Jacobs et al., 1954) gave demonstrable results only when diets were seriously inadequate before supplementary feeding began.

Other investigations concern the feeding to laboratory animals of diets generally deficient and yet normal for local human populations. For example, Passmore & Sommerville (1940) fed one group of monkeys a rice diet similar to that widely consumed by low-income families in southern

India. Another group of animals received a satisfactory lacto-vegetarian diet of milk, legumes, whole wheat, and fruit. Animals on the rice diet developed diarrhea, conjunctivitis, xerosis, and mild scurvy. Inoculation with malaria produced similar degrees of parasitemia and duration of infection in both groups.

The uncertain relationship between frequency of trachoma and presence of malnutrition has been summarized by McLaren (1963). In Tanzanian villages of Africa with adequate rainfall and a good food supply, the prevalence of trachoma among 800 schoolchildren was 10%; in a dry, famine area, it was 60% for 450 schoolchildren. Xerophthalmia was also common. Dust, flies, and personal hygiene were environmental factors influencing the results.

A number of authors have called attention to the high mortality from measles in the developing countries. This is attributable in large part to the poor nutritional state of the people (Morley & MacWilliam, 1961; Ristori et al., 1962; Taneja et al., 1962; Morley, 1962). There is evidence from field studies in Guatemala that measles fatalities among young children can be reduced by supplementary feeding alone (Scrimshaw et al., 1966). More complications were observed in malnourished than in well-nourished children in India (Ghosh & Dhatt, 1961), in Venezuela (Flores Chacín et al., 1962), and in Guatemala (Vega et al., 1964).

Differences in frequency of amebic dysentery in two rural communities in Tennessee, USA (Alexander & Meleney, 1935), are pertinent. Although *E. histolytica* was present in 38% of residents of a hill town having a predominantly white population and consuming a variety of foods, amebic dysentery was rare. A second community, a river town, predominantly Negro, had better sanitation and a better water supply. The population had half as many carriers of amebae but a great deal of clinical dysentery. Similar amounts of protein, fat, and carbohydrates were available to both groups, although the second town had fewer fresh vegetables and other accessory foods.

The first report on the viral etiology of a tumor (Rous, 1911) also presented the first evidence for a nutritional effect on a viral infection. Thin chickens were more resistant to the infectious agent than healthy, well-nourished birds. Kuczynski (1937) found that poorly nourished mice infected with yellow fever virus experienced a higher fatality than did pair-fed controls. Malnutrition reduced the resistance of guinea-pigs to the virus of foot and mouth disease (Olitsky et al., 1928). On the other hand, the frequency of occurrence and the severity of poliomyelitis in rabbits (Rivers, 1941) were less in malnourished than in well-nourished animals.

During an outbreak of infectious hepatitis in Nigeria, the disease was more common among well-fed persons, but deaths were negligible. Undernourished tribes had a greater severity and a higher case fatality (Hahn & Bugher, 1954).

The observations by Rajasuriya & Nagaratnam (1962) in a general hospital in Colombo, Ceylon, on amebiasis of the liver suggested nutrition as one factor in pathogenesis. Deficient diets, excessive use of alcohol, and preceding acute infectious disease were considered the major determinants of incidence and severity.

Among American personnel living in the Sudan, four well-nourished persons developed cutaneous leishmaniasis, but two elderly persons in poor nutritional state had full-blown kala-azar (Cahill, 1964). We are unable to document Strong's statement (1942) that epidemics of kala-azar occasionally follow famines.

DeWitt and associates (1964) attempted to determine the effect of an improved diet on young men with heavy *Schistosoma mansoni* infections who had been living on a low-protein diet (mainly rice and beans). Abundant meat, milk, butter, cheese, and eggs were provided to 21 subjects for 15 months, with the result that "over-all benefits were derived". Quantitative evidence was limited mainly to the occurrence of fewer side effects after treatment with stibophen than in controls consuming the low-protein diet. Egg counts were inconclusive, and all patients had high titers to serologic tests for schistosomiasis.

During an epidemic of typhus fever in Europe at the close of the Second World War the disease was reported to be much more common and more severe among Russian prisoners of war and displaced persons than among other prisoners of war and soldiers in active service, who were better fed. Supporting evidence is provided by one of the present authors (Gordon, 1948) who worked in that epidemic.

A 23% fatality rate was reported in chickens given diets deficient in protein, vitamin A, and possibly B-complex, and infected with *Eimeria tenella*. Only 5% of well-fed birds succumbed (Allen, 1932).

Aedes aegypti mosquitos were more susceptible to *Plasmodium gallinaceum* when fed only sugar (Terzian et al., 1953). Oocyst density in the stomach decreased when the diet was supplemented with any one of several vitamins: niacin, thiamine, biotin, calcium pantothenate, para-aminobenzoic acid, pyridoxine, or vitamin C. It increased with folic acid. Where appropriate, results of this study are included in the tables dealing with specific nutrient deficiencies. It is included in this section because a simple sugar diet is deficient in all essential nutrients except calories.

Rats maintained on white bread and water—a diet deficient in thiamine, minerals, and riboflavin—were highly susceptible to *Hymenolepis nana* (Shorb, 1933). The same situation in mice had the opposite result. When the diet of control mice was restricted to the same low intake as that of alcohol-fed mice, both groups had more cysticercoids following infection with *H. nana* than did control animals fed *ad libitum* (Larsh, 1950).

Addis & Chandler (1944) reported reduced numbers of *Hymenolepis diminuta* per rat in animals given diets deficient in fat-soluble or B-complex

vitamins. The numbers were least when both nutrients were omitted. The mechanism is uncertain, since remaining worms grew normally in the absence of the vitamins. Vavilova (1946) reported the opposite results with the same agent; rats consuming vitamin-free diets had more worms than animals receiving stock diets. Furthermore, after ingesting 1500 to 2000 eggs, 92% of animals on the vitamin-free diet became infected; 80% became infected when thiamine was added, and the infection rate dropped to 22% with supplementary vitamins A and D. Under the same circumstances, 46% of rats on a stock diet became infected.

Infections with organisms of generally low pathogenicity often complicate the treatment of persons debilitated by malignant lymphomas. Severe enterocolitis has been described in which *Strongyloides stercoralis* was the immediate cause of death in a man with Hodgkin's disease and in another with lymphocytic leukemia. In both cases the patients, already debilitated by malignant disease, were overwhelmed by strongyloidiasis (Rogers & Nelson, 1966).

To summarize, diets deficient in multiple nutrients frequently lead to a lower resistance to infection. Studies of general malnutrition without reference to specific nutrients are of public health importance because of the many world populations that now exist on deficient diets. However, multiple nutritional deficiencies in man are so frequently synergistic with infection as to support the need for systematic and precise search for the specific deficiencies primarily responsible.

Protein and Amino Acid Deficiencies

A variety of published evidence demonstrates conclusively that protein malnutrition, when sufficiently severe or prolonged, has a profound effect on resistance to infection. Table 2 condenses the results of studies in which protein was clearly the major limiting dietary factor. Results of studies concerned with specific amino acid deficiencies are shown in Table 3, and Table 4 lists additional reports in which inadequate supplies of several nutrients existed, although protein deficiency was apparently the principal one. In protein malnutrition, synergism with infection is usual; rarely does deficiency of this nutrient interfere with multiplication of the agent rather than resistance of the host.[1]

The frequent occurrence and severity of intercurrent infections in kwashiorkor are widely recognized (Trowell, 1949; Frontali, 1953; Jelliffe, 1953a; Williams, 1953; Trowell et al., 1954; Béhar et al., 1958b; Wills & Waterlow, 1958; Scrimshaw et al., 1960; Waterlow et al., 1960; Bhattacharyya, 1961; Phillips & Wharton, 1968). Lack of febrile and leukocytic response to infection is characteristic of children with kwashiorkor.

TABLE 2. EFFECTS OF PROTEIN DEFICIENCY ON INFECTIOUS DISEASE

Infectious agent or disease	Host	Response	Reference	Remarks
Bacteria				
Mycobacterium tuberculosis	Mouse	Synergistic	Hedgecock (1955, 1958)	Greatest protection at 20% dietary protein; decreased with less or more protein; protective effect abolished by lard or methyl linoleate
M. tuberculosis	Mouse	Synergistic	Dubos & Pierce (1948)	Higher fatality with corn meal and gelatin than with bread and milk diet
M. tuberculosis	Mouse	Synergistic	Dubos et al. (1955)	Survival time of infected animals decreased by fasting 36-48 hours after infection; corrected by complete diet but not when protein omitted
M. tuberculosis	Mouse	Synergistic	Schaedler & Dubos (1956)	Higher fatality and more tissue invasion with 7.5% gelatin diet than with 7.5% gelatin plus 18% casein
M. tuberculosis	Mouse	Synergistic	Dubos & Schaedler (1958)	Highest fatality on 8% casein; less with 8% casein plus 12% amino-acids or 20% casein
M. fortuitum	Mouse	Synergistic	Schaedler & Dubos (1959)	Fatality rate higher with 5-8% casein than with 15-20%
M. tuberculosis	Rat	No effect	Metcoff et al. (1949); Metcoff (1949)	Relatively mild deficiency (8% compared to 18% casein) and 58 days maximum duration
M. tuberculosis	Rat	Synergistic	Koerner et al. (1949)	With 15% or 25% protein, all animals died within 150 days; with 40% protein, little or no progression
M. tuberculosis	Rat	Antagonistic	Ratcliffe (1951) (abstract)	With 40% casein, slightly larger tubercles than with 6-8%, but only after 16 weeks
M. tuberculosis	Rat	Antagonistic	Ratcliffe (1954) (abstract)	Only after 150 days did tubercles progress more rapidly with 25% than with 5% protein diet; 17% gave intermediate effect
M. tuberculosis	Rat	No effect	Lange & Simmonds (1923)	High resistance on both high- and low-protein diet
M. tuberculosis	Rat	No effect	Sriramachari & Gopalan (1958)	2%, 5%, and 18% protein diet groups all relatively resistant
M. tuberculosis	Hamster	Antagonistic	Ratcliffe (1951) (abstract)	40% casein resulted in larger tubercles and more secondary spread after 16 weeks than did 6-8% casein
M. tuberculosis	Hamster	Synergistic	Ratcliffe & Merrick (1957)	Progress of disease beyond 12 weeks more rapid with 6% than 30% protein diet; 17% intermediate; eventual arrest or regression of infection with 30% protein
M. tuberculosis	Guinea-pig	Synergistic	Narasinga Rao & Gopalan (1958)	More rapid return to positive nitrogen balance with 18% than with 2% or 5% protein

TABLE 2 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
M. tuberculosis	Guinea-pig	No effect	Srirmachari & Gopalan (1958)	Level of protein did not significantly influence fatality rate and survival time of infected animals
M. tuberculosis	Guinea-pig	Synergistic	Ratcliffe (1954) (abstract)	Less rapid progression with 17% and 25% than with 5% protein diet; after 154 days more healing with 25% than with 5%; 17% protein diet intermediate
M. tuberculosis	Man (adult)	Synergistic	Co Tui et al. (1954)	High-protein diet beneficial to 12 patients with advanced disease and poor prognosis
Corynebacterium murium	Rat	No effect	Miles (1951a)	With 4%, 6%, 8%, 12%, and 16% casein diet, no effect of protein levels on survival
Pasteurella pestis	Rat	Antagonistic	Korobkov et al. (1963)	First study: Low protein intake did not adversely affect resistance and might even have increased it. Second study: Low protein clearly increased resistance
Klebsiella pneumoniae	Mouse	Synergistic	Schaedler & Dubos (1956, 1959) Dubos & Schaedler (1959)	Fatality higher with 5-8% than with 15-20% casein. Higher fatality rate, more tissue invasion with 7.5% gelatin diet than with gelatin plus 18% casein
Diplococcus pneumoniae	Rat	No statistically significant effect	Chen & Li (1930)	76% fatality with 30-50% whole wheat, 30% millet, 15% soya diet; and 59% fatality with 66% wheat, 33% milk (43 animals in each group)
D. pneumoniae	Rat	Synergistic	Wissler (1947b)	Fatality increased and agglutinin and leukocyte response decreased with approximately 2% compared with 18-22% casein protein
D. pneumoniae	Rabbit	Synergistic	Wissler (1947a)	Increased fatality, decreased agglutinin response and phagocytic activity with severe protein depletion; approximately 2% casein protein and 125-150 calories compared with 15% protein and 340 calories per day
D. pneumoniae	Chicken	Synergistic	Steffee (1950)	Death in half of controls and in 28 of 29 protein-deficient birds
D. pneumoniae, type 2	Mouse	Synergistic	Sako (1942)	Survival time doubled by increasing protein from 4,5% to 55% or 60%; 20% protein intermediate (100 animals in group)
Streptococcus pyogenes	Rabbit	Synergistic	Mannino & Schiraldi (1956)	More severe infection with 12.75% than with 34.25% protein
Rheumatic fever	Man (children)	Synergistic	Warner & Winterton (1935)	Institutional incidence reduced to a third following change from deficient to abundant protein diet

TABLE 2 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Staphylococcus aureus	Mouse	Synergistic	Schaedler & Dubos (1959)	Fatality higher with 5-8% than with 15-20% casein
Staph. aureus	Mouse	Synergistic	Dubos & Schaedler (1958)	Highest fatality with 8% casein; less with 8% casein +12% amino acids or with 20% casein
Staph. aureus	Mouse	Synergistic	Schaedler & Dubos (1956)	Higher fatality, more tissue invasion with 7.5% gelatin diet than with 7.5% gelatin plus 18% casein diet
Staph. albus	Mouse	Synergistic	Dubos & Schaedler (1959)	Higher fatality rate and earlier death with 5% casein or corn diet than with 20% casein
B-hemolytic streptococcus	Rabbit	Synergistic	Mannino & Schiraldi (1956)	More severe disease and higher fatality rate among animals fed 12% vegetable protein diet than with 34% protein, mainly casein
Pseudomonas aeruginosa	Man (children)	Synergistic	Kefalides et al. (1962)	Reduction or delay of deaths among severely burned children achieved with plasma and albumin therapy with or without gamma globulin
Natural infection	Rat	Synergistic	Taylor (1963)	Frequent infection of sterile subcutaneous pouches in different animals; controls resistant
Salmonella gallinarum	Chicken	Antagonistic	Smith & Chubb (1957)	Supplementing a wheat basal diet with 2% to 10% fish meal, 40% peanut meal, or 10% each of fish meal and dried milk lowered fatality rate, 40% fish meal gave increased fatality
S. gallinarum	Chicken	Antagonistic	Boyd & Edwards (1963)	With 15% soya-corn protein, higher fatality than with 30%
S. gallinarum	Chicken	Antagonistic	Hill & Garren (1961)	Increasing protein level from 10% to 20% or 30% with soybean or casein increased fatality rate following inoculation
Escherichia coli	Chicken	Synergistic	Boyd & Edwards (1963)	With 15% soya-corn protein, lower fatality rate than with 30%
Salmonella typhimurium	Mouse	Varied with "Salmonella Resistance Factor" (SRF)	Hill et al. (1962)	5%, 15%, and 30% protein Low protein synergistic with SRF; antagonistic without SRF at minimal vitamin levels. No protein effect with tenfold vitamin levels
S. typhimurium	Mouse	Synergistic	Pritchett (1927); Webster & Pritchett (1924)	Fatality lower with 15-17% milk protein (vitamin A content also increased by milk addition)
S. typhimurium	Mouse	Synergistic	Watson (1937) Watson et al. (1938)	Good controls; effect obtained with added milk (skim)

TABLE 2 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
S. typhimurium	Rat	Synergistic	Chen & Li (1930)	84% fatality with 30-50% wheat-millet diet and 42% with 66% wheat, 33% millet (30 animals per group)
S. typhimurium	Rat	Synergistic	Miles (1951a)	Far lower fatality with 16% than 4% protein diet, no correlation with agglutinin response
S. typhimurium	Rat	No Effect	Metcoff et al. (1948); Metcoff (1949)	Comparison of 18% with 2% casein diet
S. enteritidis	Rat	Synergistic	Robertson & Doyle (1936)	Comparison of soya with casein diet, each approximately 14% protein
S. enteritidis	Rat	Synergistic	Moroz (1959)	Fatality rate increased by decreasing level of dietary protein from 20% to 3.5%
Borrelia persica	Rat	Synergistic	Guggenheim et al. (1951)	Longer duration, higher fatality on protein-free diet compared with 18% casein diet
Rickettsia				
Rickettsia prowazekii	Rat	Synergistic	Fitzpatrick (1948)	Well controlled; comparison of 5% with 18% casein, but paired-feeding showed most of effect to be due to inanition, and this, in turn, appeared to be due to thiamine deficiency
Virus				
Infectious hepatitis (virus from human cases)	Mouse	Synergistic	Ruebner & Bramhall (1960); Ruebner & Miyai (1961)	Fatality rate doubled by low protein compared with either casein or 12 amino-acid supplement
Infectious hepatitis (virus from human cases)	Rat	Synergistic	MacCallum & Miles (1946)	Transmissible only to protein-deficient animals
Louping ill virus	Mouse, rat	Synergistic	Miles (1951b)	Virus disappeared more rapidly and antibody titers higher with 16% than with 4% casein
Mouse encephalomyelitis (Theiler)	Mouse	No effect	Kearney et al. (1948a)	Comparison of 36% with 9% casein (more paralysis when specific tryptophan deficient)
Swine influenza virus	Mouse	Synergistic	Sprunt (1948)	Fatality rate increased by chronic deficiency in older animals
Swine influenza virus	Mouse	Antagonistic	Sprunt (1949)	Fatality rate decreased by acute or chronic deficiency in immature animals
Measles	Man (children)	Synergistic	Raŭlt & Raba (personal communication, 1963)	Reduction in fatality of measles in Upper Volta after mass distribution of skim milk
Newcastle disease	Chicks	Synergistic	Squibb (1964b)	On 8% soya protein in diet, increased severity of disease indicated clinically and by specifically developed tests of RNA/DNA ratio in liver cells. No change in controls receiving 25% protein, but 41%

TABLE 2 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Newcastle disease (*continued*)				to give surfeit of protein, also produced synergism
Ectromelia virus	Mouse	Synergistic	Sugawara (1957)	With large infecting dose, longer survival time and lower fatality rate with high-protein diet
Ectromelia virus	Mouse	Antagonistic	Sugawara (1957)	With small infecting dose, longer survival time and lower fatality rate with low-protein diets
Protozoa				
Entamoeba histolytica	Guinea-pig	No effect	Carrera et al. (1952)	No difference in severity of infection between protein-deficient and control animals following intracecal introduction of trophozoites
Giardia muris *Hexamitus muris* *Trichomonas muris*	12 carnivorous wild animals of 10 species	Synergistic	Hegner (1924)	Animals receiving carnivorous diets difficult to infect with protozoa
Giardia lamblia	Cat	No effect	Hegner (1924)	Cats difficult to infect with *G. lamblia* because of carnivorous diets
Trichomonas hominis	Rat	Synergistic	Hegner & Eskridge (1935)	Rats fed "high-protein" diet eliminated organisms to greater degree than those fed a standard diet
Eimeria tenella	Chicken	Antagonistic	Britton et al. (1963, 1964)	Four-week-old chicks fed 0.5%, 10%, 15%, 20%, and 30% protein for 2 weeks before inoculation; fatality increased progressively up to 15% protein; excystation of sporozoites requires trypsin, which is reduced by protein deficiency
Plasmodium berghei	Rat	Antagonistic	Platt et al. (1960)	Malaria more severe in rats receiving high protein than when protein was low
P. lophurae	Chicken	Synergistic	Seeler & Ott (1945b)	With 1%, 4%, and 7% protein, more severe and prolonged parasitemia than with 32% protein diet
Leishmania donovani	Hamster	Synergistic	Ritterson & Stauber (1949)	Earlier emaciation and death with 10-20% than with 40% protein
L. donovani	Mouse	Synergistic	Actor (1960)	Greater parasite counts with diets of 9-30% casein than with stock diet; reversible with protein supplement
Trypanosoma gambiense	Rat	Antagonistic	Yaeger (1960)	With 19.6% protein diets survival times were 6.5 days with casein, 9.9 days with gluten, and 7.6 days with gluten plus lysine
T. cruzi	Rat	Synergistic	Yaeger & Miller (1963a)	Judged by parasitemia, tissue damage, and fatality on isonitrogenous diets fed *ad libitum*, susceptibility increased when protein quality

TABLE 2 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
T. cruzi (continued)				decreased by replacement of casein by zein; intermediate when zein supplemented with lysine and tryptophan
Helminths				
Ascaridia galli	Chicken	Synergistic	Riedel & Ackert (1950, 1951)	At 18.75% protein, fewer and smaller worms following inoculation with animal protein supplement to soybean ration
Toxocara canis	Dog	Synergistic	Platt & Heard (1965)	Animals on diet of higher protein value had egg-counts about one-fifth of those on diet of lower protein value
Ascaridia lineata	Chicken	Synergistic	Ackert & Beach (1933)	With corn-wheat-oat-peanut diet more and larger worms than with 11.2% meat meal, with or without liquid skim milk *ad libitum*
Nippostrongylus muris	Rat	Synergistic	Donaldson & Otto (1946)	More worms with 9% vegetable protein as compared with 11% vegetable plus 11% animal protein
N. muris	Rat	Synergistic	Platt & Heard (1965)	Number of eggs per unit weight of feces highest in animals on diet containing least protein
Haemonchus contortus	Sheep	Synergistic	Ross & Gordon (1933)	Heavier infestation with 5.3% than with 3.1% protein instead of pasture
Cooperia punctata *Cooperia oncophora* *Ostertagia ostertagi* *Haemonchus contortus* *Trichostrongylus axei*	Cattle	Synergistic	Goldberg (1959)	Animals on higher protein feed (legume-grass) had fewer worms of each species than those on lower protein hay forage
Oesophagostomum radiatum	Cattle	No effect	Goldberg (1959)	In above study no difference in intestinal lesions caused by this parasite
Ancylostoma	Man (adult)	Synergistic	Demarchi (1958)	Recovery from anemia more frequent in 27 patients receiving 102 g of protein than in 27 patients receiving 65 g
Mixed intestinal nematodes	Man (adult)	Synergistic	Orr et al. (1931); Orr & Gilkes (1931)	Comparison of predominantly cereal-eating with meat-eating African tribes
Ancylostoma caninum *Toxocara canis*	Dog	Synergistic	Girón (1963)	Four to ten times as many eggs excreted with 6g/kg/day of protein from tortillas than with 4, 8, or 10 g/kg/day from casein
Trichinella spiralis	Mouse	Synergistic	Taliaferro et al (1949)	Increased numbers of worms after infection of protein-deficient animals
Trichinella spiralis	Mouse	Synergistic	Kwan et al. (1965)	Infested animals fed 24% casein protein had fewer adult worms in intestine at 72 hours and encysted larvae in the muscles after 4 weeks than those fed 8% protein. Group fed 48% protein showed intermediate resistance

TABLE 2 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Schistosoma mansoni	Mouse	Mixed	De Meillon & Paterson (1958)	Earlier death and higher fatality rate with 12.8% than with 20.2% protein diet, but fewer eggs and smaller male worms, with abnormal reproductive organs
Hymenolepis diminuta	Rat	Synergistic	Mettrick & Munro (1965)	Replacement of all dietary protein by carbohydrate increased growth and development of worms
H. diminuta	Rat	Synergistic	Mettrick & Munro (1965)	After ingesting 1500-2000 eggs, only 18% of rats receiving a high-protein diet were infected compared with 46% on a stock control diet
H. nana	Mouse	Synergistic	Larsh (1950) (abstract)	Nearly twice as many cysticercoids in 9% protein group as in controls on 22% protein
H. diminuta	Rat	No effect	Chandler (1943)	More worms in animals on protein-free than on stock diet with 18% casein

Bacterial infections

A greatly increased susceptibility to infection was also recognized in children with *Mehlnährschaden*, a type of kwashiorkor once prevalent in Europe. Czerny & Keller (1906), Weigert (1907), Feer (1922), and Pfaundler & Schlossmann (1923) described a high susceptibility to pyuria, bronchitis, and bronchopneumonia. The most frequent cause of death in both kwashiorkor and marasmus is bronchopneumonia (De Silva, 1964; Pretorius & Novis, 1965). Tejada and co-workers (1956) found bronchopneumonia at necropsy in two-thirds of children dying of kwashiorkor in Guatemala.

Most authors recommend antibiotics in routine initial treatment of kwashiorkor because infection is so commonly associated with it (Béhar et al., 1958b).

There is also evidence that even less severe forms of protein malnutrition aggravate bronchopneumonia, and *vice versa*. For example, most of the 82 Bantu children with bronchopneumonia studied by Prinsloo & Pretorius (1966) had weights below the third percentile for well-nourished children, and their serum albumin concentrations averaged 3.1 g per milliliter.

A major consequence of severe burns is a rapid decrease in serum albumin due to exaggerated loss of nitrogen in the urine and in exudates from the burn itself. On the basis of a study of 321 seriously burned Peruvian children treated in rotation with saline solution and plasma; with saline, plasma, and gamma globulin; and with saline, albumin, and gamma globulin, Kefalides and associates (1962) concluded that plasma protein aided in avoiding secondary infection with *Pseudomonas aeruginosa* and other infectious agents. Gamma globulin did not appear helpful.

TABLE 3. EFFECTS OF SPECIFIC AMINO ACID DEFICIENCIES ON INFECTIOUS DISEASE

Infectious agent or disease	Host	Response	Reference	Remarks
		LYSINE		
Bacteria				
Bacillus anthracis	Rat	Synergistic	Gray (1963)	Resistance decreased in weanling rats rendered deficient, with death occurring within 2 days of subcutaneous challenge
Virus				
Newcastle virus	Chicken	Antagonistic	Squibb (1961)	Infection caused less growth depression with deficient than with control diets
Poliovirus, type 2	Mouse	No effect	Jones et al. (1947)	Deficiency did not affect course of infection in inoculated animal
Helminths				
Nippostrongylus muris	Rat	Synergistic	Theses of Barakat (1948) and Seddik (1950), cited by Platt (1957)	Deficient rats showed high fatality rate; well-fed or partially deficient animals survived
		LYSINE AND TRYPTOPHAN		
Protozoa				
Trypanosoma cruzi	Rat	Synergistic	Yaeger & Miller (1963a,b)	Parasitemia and fatality high in animals fed 20% wheat gluten (1963a) or zein (1963b) compared with those receiving 20% casein. Addition of lysine to gluten or lysine and tryptophan to zein diet restored resistance
		TRYPTOPHAN		
Virus				
Mouse encephalomyelitis (Theiler)	Mouse	Antagonistic	Kearney et al. (1948a)	Almost no paralysis in deficient animals, but nearly 100% in controls.
Poliovirus, type 2	Rat	Antagonistic	Gershoff et al. (1952)	Direct tryptophan deficiencies and amino-acid imbalance induced by excess methionine leading to relative deficiency
		LEUCINE		
Helminths				
Ascaridia galli	Chicken	No effect	Riedel (1955)	High and low leucine diets began 2 weeks before inoculation; no effect on number or length of worms
		METHIONINE		
Protozoa				
Plasmodium berghei	Mouse	Antagonistic	Taylor (1956)	Induction of methionine deficiency with ethionine reduced mean peak parasitemia
P. knowlesi	Monkey	Antagonistic	Geiman & McKee (1948)	Adding methionine to a starvation diet increased parasitemia

TABLE 4. EFFECTS ON INFECTIOUS DISEASE OF INADEQUATE DIETS PREDOMINANTLY DEFICIENT IN PROTEIN

Infectious agent or disease	Host	Response	Reference	Remarks
Bacteria				
Mycobacterium tuberculosis	Man (adults)	Synergistic	Leyton (1946)	Prisoners of war receiving supplementary food with 30 g protein had higher plasma protein and tuberculosis rate of only 1.2% compared with 15-19% in other prisoners
" Diarrhea "	Man (adults)	Synergistic	McKenzie (1940)	Analysis of 320 cases with 142 deaths suggests deficiency increases susceptibility to variety of intestinal pathogens. Severe diarrhea frequent in malnourished laborers, although pathogen not usually found. Diarrhea incidence decreased when diet generally improved
" Diarrhea "	Man (children)	Synergistic	García-Erazo (1960)	Diarrhea more severe in malnourished children
Virus				
Infectious hepatitis	Man (adults)	Synergistic	Findlay (1948)	Much more severe disease and higher fatality in malnourished persons; protein therapy beneficial
Herpes simplex	Man (children)	Synergistic	Hansen (1961)	Severe disease and deaths limited mainly to children with protein malnutrition
Herpes simplex	Man (children)	Synergistic	Becker et al. (1963)	" Striking association with severe grades of malnutrition "; 10 of 16 patients studied had kwashiorkor
Protozoa				
Entamoeba histolytica	Man (adults)	Synergistic	Westphal (1937)	Increased incidence in First World War prison camps with greater malnutrition; decreased when more food became available
Entamoeba histolytica	Man (adults)	Synergistic	Elsdon-Dew (1949)	In Durban, South Africa, acute fulminating disease common in maize-eating Bantus; rare in Indians with nutritionally better curry and rice diet, and in Europeans receiving a relatively balanced diet
Helminths				
Nematodes				
Haemonchus contortus	Sheep (lambs)	Synergistic	Fraser & Robertson (1933)	When grazed on infested pasture, fewer worms and eggs in animals previously fed milk than in those fed only grass and oats.
Ostertagia Haemonchus contortus Trichostrongylus axei	Sheep (lambs)	Synergistic	Fraser et al. (1939)	Artificially infected; more worms retained by poorly fed than well-fed group

TABLE 4. (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Haemonchus contortus *Trichostrongylus* *Ostertagia circumcincta* *Cooperia curticei*	Sheep	Synergistic	Taylor (1934)	Heavier mixed natural infection with straw or hay only than with hay plus concentrates
Haemonchus contortus *Oesophagostomum colubianum*	Sheep	Synergistic	Laurence et al. (1951)	Artificial infection; increasing maize content of hay diet improved growth, decreased mortality and ultimately reduced worm load
Bunostomum trigonecephalum	Sheep	Synergistic	Lucker & Neumayer (1947)	More worms and greater egg output with hay diet than hay plus oats and corn; diet also poor in calcium, iron, and some vitamins
Mixed nematodes	Sheep	Synergistic	White & Cushnie (1952)	Supplementation of pasture diet of ewes with oats and linseed cake resulted in lower intensity of mixed nematode infection of their lambs (but no effect on ewes)
Ancylostoma caninum (dog strain)	Dog	Synergistic	Foster & Cort (1931)	Age-acquired resistance lost on poor diet; worm and egg load decreased when good diet used
Ancylostoma caninum (cat strain)	Dog	Synergistic	Foster & Cort (1932b)	Effect only on young animals when diet containing 35 % of ground peas as only protein source compared with good diet of "hog lungs, bread and powdered milk"; older animals not infected despite poor diet
Ancylostoma caninum	Dog	Synergistic	Foster & Cort (1935)	Same diets as preceding study. Older animals also infected when poor diets prolonged. Both younger and older animals were heavily infected
Ancylostoma caninum (dog strain)	Cat	Synergistic	Foster & Cort (1932b)	Same diets as preceding two studies. Malnourished cats showed significantly heavier infection
Strongyloidiasis	Man (adults)	Synergistic	Adamson & Path (1960)	Claim that the disease is frequently associated with malnutrition in Durban, but no data presented
Nippostrongylus muris	Rat	Antagonistic	Chandler (1932)	Fewer worms and fewer eggs per worm on protein-free diet
Cestodes				
Hymenolepis nana	Mouse	Synergistic	Larsh (1950)	Many more cysticercoids with diets containing 9 % of vegetable than with those containing 11 % of vegetable plus 11 % of animal protein

The marked effect of protein deficiency on infectious disease of laboratory and domestic animals, plus strong indirect evidence of its action on the resistance of man, make surprising the scarcity of direct observations in human populations. Tuberculosis has had most attention. The concern usually has been with a general improvement of diet. In most instances, protein deficiency cannot be designated as the principal factor, although it probably was.

Co Tui and associates (1954) selected 12 patients with advanced tuberculosis who were subsisting on poor diets and raised their intake of dietary protein from 0.5 to 1.0 g per kilogram of body-weight to approximately 2 g per kilogram. The results were described as beneficial, compared with previous experience with similar patients, but the experimental design lacked true controls. Moreover, dietary factors other than protein were altered.

An observation by Leyton (1946) involved Russian and British prisoners of war held in German camps. Both received the same prison diet, but the British had additional food amounting to 30 g of protein and 1000 calories per day. The incidence of tuberculosis, as determined by roentgenographic survey, was much greater among prisoners wholly on prison fare, although there were obvious differences between the two groups other than those in diet.

The sixteen studies listed in Table 2, on experimental tuberculosis in protein-deficient mice, rats, hamsters, and guinea-pigs, show synergism as the usual result. Although some studies (e.g., those of Dubos & Pierce, 1948; Dubos et al., 1955, and Dubos & Schaedler, 1958) are not well adapted to statistical analysis because conditions were changed from one experiment to another, an overwhelming consistency in results is evident. Furthermore, most of the experimental studies cited in the table had adequate controls. However, exact protein values and the extent of associated deficiencies of vitamins and minerals were uncertain in some of the earlier investigations.

Variation in response is not always due to inadequate experimental design or poor laboratory technique. Synergism has been shown to occur regularly with tuberculosis in mice, guinea-pigs, and hamsters. The high natural resistance of rats to both bovine and human strains of *Mycobacterium tuberculosis* probably explains the repeated failure to find experimental differences between well-nourished and malnourished animals (Lange & Simmonds, 1923; Metcoff et al., 1948; Metcoff, 1949; Sriramachari & Gopalan, 1958).

Such natural host variations often make interpretation of reported results difficult. For example, in 16 weeks Ratcliffe (1951) found no difference in resistance to tuberculosis in rats and hamsters fed 6% to 8% casein diets, compared with those consuming 40% casein; but thereafter tubercles were larger in animals receiving the higher amounts of protein. A subsequent report by Ratcliffe & Merrick (1957) concluded, however,

that progress of tuberculosis in hamsters was more rapid on 6% protein diets than on diets containing 30%, and was intermediate with a 19% ration. Evidently, the 40% level in Ratcliffe's earlier study was too high for optimum response. Nevertheless, rats infected with tuberculosis showed smaller, more localized lesions, and better survival rates when fed diets containing 40% protein than did those with 15% or 25% dietary protein (Koerner et al., 1949). These results, although well controlled, are difficult to interpret since 25% and even 15% protein should be adequate for the rat. (Other effects of nutrient excesses are discussed later in this chapter.)

Ratcliffe (1954) fed diets containing 25%, 17% and 5% of protein to rats and guinea-pigs infected with *Mycobacterium tuberculosis*. The results, again, differed with host species. The previous finding of more rapid tubercle development in rats on a high-protein diet for 150 days was confirmed. However, in guinea-pigs given diets with 25% of protein, infection was arrested or regressed, showing less activity than in animals receiving 17% of protein; these, in turn, resisted infection more adequately than those receiving only 5% of protein.

It is noteworthy that guinea-pigs fed a high-protein diet recovered more rapidly from the negative nitrogen balance phase of experimentally induced tuberculosis than animals receiving less protein (Narasinga Rao & Gopalan, 1958).

In salmonellosis, also, host characteristics appear to determine the type of interaction. In rats or mice, protein-deficient diets were usually synergistic for *S. typhimurium* (Webster & Pritchett, 1924; Chen & Li, 1930; Watson, 1937; Watson et al., 1938) and *S. enteritidis* (Robertson & Doyle, 1936; Moroz, 1959). Protein deficiency proved antagonistic, however, for *S. gallinarum* in chickens (Smith & Chubb, 1957; Hill & Garren, 1961; Boyd & Edwards, 1963). Chicks given 15% of protein for two weeks had a higher fatality following infection with *Escherichia coli* than those receiving a 30% protein diet. Fatality from *S. gallinarum* infection, however, was lower in birds receiving less protein (Boyd & Edwards, 1963).

In general, mild degrees of protein malnutrition have no detectable effect on the incidence or severity of infection. The exact amount or duration of protein deficiency necessary to cause observable effects varies with both host and agent. Thus Miles (1951a) found that a 4% protein diet resulted in a much increased fatality rate in rats with *S. typhimurium* infection. In equally well designed experiments, Metcoff and associates (1948) and Metcoff alone (1949) saw no effect with diets containing either 2% or 8% of protein. Since diets and duration of deficiency were similar, it is reasonable to attribute the variation in results to strain differences within the host or the infectious agent, or both.

The extensive experiments of Schneider and his colleagues, to be discussed in Chapter 4, show clearly the importance of genetic factors. The widely varied responses in selected populations of laboratory animals with different

strains of a single *Salmonella* were explained as due primarily to the presence in the diet of a "salmonella resistance factor" (Schneider & Colburn, 1960).

Many other bacterial diseases of rodents, rabbits, and chickens are synergistic with protein deficiencies. They include infections by staphylococcus (Schaedler & Dubos, 1956, 1959), streptococcus (Mannino & Schiraldi, 1956), pneumococcus (Sako, 1942; Wissler, 1947a,b; Steffee, 1950), *Klebsiella pneumoniae* (Dubos & Schaedler, 1959), and *Borrelia persica* (Guggenheim et al., 1951). Low protein intake appeared to increase resistance of rats to *Pasteurella pestis* in one study by Korobkov and co-workers (1963). However, their second study disclosed no significant effect.

Taylor & Tejada have conducted extensive and well-controlled studies, and some of the results have been published (1966). The granuloma pouch technique of Selye (1953) was used to compare well-fed and protein-deficient mice. Injection of 1% croton oil into a sac produced by subcutaneous injection of air was normally followed by accumulation of exudate in the pouch and progressive thickening of the pouch wall. This process was impaired in protein-deficient animals, and they usually developed secondary infection, often dying of septicemia, whereas controls showed the normal response. Similar findings followed induced infection of the pouch.

Lysine deficiency has been shown to have a specific effect on resistance to anthrax (Gray, 1963). Diets containing gluten rather than casein markedly lowered resistance to subcutaneous inoculation of anthrax spores, as indicated by decreased ability of the reticulo-endothelial system to clear the infectious agent. Adding lysine to the diet partially restored resistance.

Rickettsial infections

The historical association between typhus fever and famine makes particularly noteworthy a number of laboratory studies designed to determine whether the relationship is causal or casual. Fitzpatrick (1948) found a 5% casein diet markedly synergistic in rats with typhus fever as compared with a control diet containing 18% of casein; animals given the lower-casein diet had a higher fatality rate and death occurred more rapidly. Moreover, paired-feeding studies indicated anorexia and lowered food intake to be the probable explanation. The inanition was then shown to be due largely to thiamine deficiency. It is suggested that this sequence has parallels in human populations.

Viral infections

Viral infections and protein deficiencies commonly act synergistically, whereas specific amino acid deficiencies tend to result in antagonism. Severe protein lack predisposed to greater severity and fatality of infectious hepatitis among Africans (Findlay, 1948), in children with *Herpes simplex*

(Hansen, 1961; Becker et al., 1963), and in children with measles (Raûlt & Raba, personal communication, 1963).

Strains of a virus isolated from patients with infectious hepatitis (MacCallum & Miles, 1946; Ruebner & Bramhall, 1960; Ruebner & Miyai, 1961) produced a higher fatality in swine and rats with general protein deficiency than in those on an adequate diet. Similarly, in mice and rats the virus of louping ill disappeared more promptly from animals fed 16% casein diets compared with 4% casein, and residual antibody titers were higher (Miles, 1951b).

In studying swine influenza virus in mice, Sprunt (1948, 1949) found acute or chronic protein deficiency in immature animals to be antagonistic to infection. Only a prolonged deficiency state made older animals susceptible. The explanation he gave was that young animals had no appreciable reserves, with the result that even a slight deficiency sufficed for an observable response. Older animals fed low-protein diets were not affected until their reserves were depleted.

A 30% casein diet increased both survival time and survival rate in mice infected with large doses of ectromelia virus. With a small inoculating dose the dietary effect was reversed; animals receiving a 5% casein diet survived longer and had a lower fatality rate than those fed the 30% casein diet (Sugawara, 1957).

Little information is available on the effect of individual amino acids on the course of infection. Squibb (1961b) found that the growth-depressing effect of Newcastle disease was least in chicks fed rations deficient in lysine. The data indicated that the greatest severity occurred when cellular metabolism was normal, as in well-fed, growing birds.

Problems arising from imbalance of specific nutrients, such as those of amino acids, are currently receiving more attention because of evidence that excess consumption of certain nutrients markedly alters requirements for others (Harper, 1957-58; Hsu, 1963). Excess methionine, in particular, increases the requirements for tryptophan to a point where a relative deficiency is created. Gershoff and associates (1952) explained the antagonism of excess methionine to type 2 poliovirus in mice on this basis. The methionine-induced tryptophan deficiency was further accentuated by giving the analogue 6-methyltryptophan, a result to be expected from the previous demonstration of antagonism between the virus and tryptophan deficiency.

In replicated experiments, Squibb (1964b) conditioned chicks with a balanced diet containing 25% of protein and with an imbalanced 8% protein diet. Others consumed a diet containing an excessive 41% of protein. After infection with the virus of Newcastle disease, both the group on a protein-deficient diet and the group receiving a surfeit of protein showed higher fatality rates than the control group. Lower antibody titers correlated significantly with the extreme deficiency. Two possible mechanisms were

suggested: first, that an amino acid imbalance resulted from metabolic use of protein to meet increased energy needs; or second, that increased virus multiplication occurred as a result of the extra protein. The author favored the first explanation.

Tissue-culture techniques are being used increasingly as a means to acquire more specific information on the nutritional requirements of viruses. With both adenoviruses and coxsackieviruses, active cell metabolism usually creates cellular resistance sufficient to induce viral latency. The opposite situation was reported by Johnson & Morgan (1956); the infectious agent of psittacosis became latent in poorly metabolizing cells. Bader & Morgan (1958) then showed that this agent, a bedsonia, when grown in chick embryo cells or mouse fibroblast L cells, has highly specific amino acid requirements. Nine amino acids were listed as essential and ten as not essential. Of interest, glutamine, histidine, lysine, and arginine were essential for growth of the cells but not for replication of the infectious agent. By contrast, in HeLa cell cultures of poliovirus, glutamine was the only essential amino acid; other amino acids, vitamins, NH_4^+, purines, and pyrimidines were not required (Eagle & Habel, 1956). Brown & Ackermann (1951) showed a specific metabolic need of poliovirus for methionine. Using the analogue (±)-ethionine, they were able to block viral multiplication in cell cultures.

Protozoal infections

Protozoal infections are often synergistic with protein deficiencies, although secondary infection and various environmental factors make studies in humans difficult to evaluate. This applies particularly to studies of amebiasis in prisoner-of-war camps (Westphal, 1937) and in differing social groups (Elsdon-Dew, 1949; Rajasuriya & Nagaratnam, 1962).

Carrera and co-workers (1952) were unable, however, to demonstrate a difference in resistance to intracecal *E. histolytica* trophozoites among guinea-pigs fed a diet adequate in protein, one inadequate in quantity of protein, or still another of poor protein quality.

As early as 1924, Hegner found that all of ten species of carnivores he studied were difficult to infect with *Giardia*, *Hexamitus*, and *Trichomonas* until the animals were placed on vegetable-based protein-deficient diets. This observation was confirmed experimentally when rats on high-protein diets spontaneously eliminated *Trichomonas* to a greater extent than animals on a standard diet (Hegner & Eskridge, 1935).

An antagonism between protein deficiency and coccidiosis of chickens was demonstrated by Britton and associates (1963, 1964). *Eimeria* oocysts require trypsin to achieve excystation and a release of infective sporozoites. As little as 48 hours of protein deprivation or starvation reduced available trypsin to inconsequential levels and thus protected the birds. When

trypsin was added to the oocyst inoculum, antagonism was inhibited and the original observation confirmed.

The combination of protein malnutrition and malaria results sometimes in antagonism, sometimes in synergism. In day-old chicks on 1% to 7% protein diets, *Plasmodium lophurae* produced more severe and prolonged parasitemia than in chicks fed 32% protein (Seeler & Ott, 1945b). By contrast, *Plasmodium berghei* produced more severe infections in rats when their diet was high in protein (Platt et al., 1960).

A specific dependence on methionine has been shown for several malarial parasites. The parasitemia of monkeys infected with *Plasmodium knowlesi* is markedly lessened by starving the animals, and further reduced by administration of the analogue ethionine (Geiman & McKee, 1948). Ethionine produced a similar antagonism with *Plasmodium berghei* infections in mice (Taylor, 1956). A dependence on specific amino acids and synthesis of others by malaria parasites has been reviewed by McKee (1951).

Species differences in trypanosomes determine whether there will be synergism or antagonism with protein deficiency in infected rats. *Trypanosoma cruzi* produced a more severe infection, as judged by parasite densities, tissue damage, and fatality rate, when the protein quality of the host diet was poor (Yaeger & Miller, 1963a). Supplementation of a deficient wheat gluten diet with the most limiting amino acid, lysine, substantially improved the resistance of rats to Chagas disease (Yaeger & Miller, 1963b). Similar results were obtained by supplementing a zein diet with lysine and tryptophan (Yaeger & Miller, 1963a) and a threonine-deficient diet with this amino acid (Yaeger & Miller, 1965). *Trypanosoma gambiense* infection, however, became more severe, as judged by survival time, when casein rather than wheat gluten was the source of nitrogen, although wheat gluten is a much poorer quality protein (Yaeger, 1960). *Leishmania donovani* infection of hamsters and mice showed synergism with low-protein diets, as determined by fatality rates and parasitemia (Ritterson & Stauber, 1949; Actor, 1960).

Helminthic infections

Although protein deficiency is generally synergistic with both natural and experimental helminthic infections, exceptions occur. In sheep—animals that are naturally infected with a wide range of helminths—increased protein in the diet has usually resulted in fewer worms and reduced egg production (Fraser & Robertson, 1933; Ross & Gordon, 1933; Taylor, 1934; Fraser et al., 1939; Lucker & Neumayer, 1947; Laurence et al., 1951; White & Cushnie, 1952). When infections were sufficiently severe to cause death, fatality rates in animals on deficient diets could be reduced by increasing the dietary protein. Similar results have been obtained with cattle (Goldberg 1959) and chickens (Ackert & Beach, 1933; Riedel & Ackert, 1950, 1951).

Conflicting findings in protein-deficient rats are unexplained. Chandler (1932) reported that *Nippostrongylus muris* infection resulted in fewer worms and eggs in animals on protein-free diets, whereas Donaldson & Otto (1946) observed fewer worms when an 11% protein supplement was added to a vegetable diet.

Young animals may show effects of protein restriction at levels that do not normally affect resistance of older animals. For example, Foster & Cort (1931, 1932a,b, 1935) successfully infected young dogs on protein-deficients diets with the cat strain of *Ancylostoma caninum*, but were unable to infect older animals by the same procedure. Older dogs contracted the disease only when deficiency was considerably prolonged. Dogs fed diets providing 6 g of predominantly corn protein per kilogram of body-weight per day and experimentally infected with *Ancylostoma caninum* and *Toxocara canis* contained far more worms and excreted four to eight times more eggs than did animals fed diets in which casein furnished 4, 6, or 8 g protein per kilogram of body-weight per day (Girón, 1963).

Claims are frequently made that adequate protein nutrition also protects human populations against hookworm disease and strongyloidosis (Adamson & Path, 1960). Reserve must be expressed regarding the claim made by Orr & Gilkes (1931) that more illnesses occurred among cereal-eating than meat-eating African tribes. Nutritional factors other than protein, as well as factors unrelated to diet, may well have influenced their results.

In hookworm disease of man, Demarchi (1958) compared the effects of diets supplying 65 g of protein per day with those supplying 102 g. Increased protein resulted in recovery from clinical anemia without diminishing the worm burden.

Protein deficiency had an adverse effect on mouse resistance to *Trichinella spiralis* (Taliaferro et al., 1949). Kwan and associates (1965) demonstrated that mice were more resistant to *Trichinella* when the diet contained 24% of protein from casein than when it contained 8% or 48%. Mice on the 8% protein diet had the greatest number of larvae regardless of whether infected animals were placed immediately on the test diets or fed test diets for three weeks or for six weeks prior to administration of larvae.

Schistosoma mansoni has been carefully studied in protein-deficient mice (De Meillon & Paterson, 1958), but the findings were equivocal. The combination did lead to a certain degree of synergism in that mice died earlier. On the other hand, the deficiency had a deleterious effect on the worms. Females produced fewer eggs, and more of them were abnormal. Males were smaller and developed abnormal reproductive organs. The pathologic effect was attributed to increased frequency and efficiency of egg absorption by the protein-deficient mouse; the abnormal eggs apparently more readily stimulated a granulomatous reaction. The combination of a low-protein diet and infection with *S. mansoni* caused rats to develop signs of cirrhosis of the liver as early as 12 weeks, and a nodular cirrhotic liver in 28 to 32

weeks, a result not produced by either diet alone or the infection alone (Bhattacharyya, 1965). Cirrhosis could not be induced in mice by the same regimen.

A series of observations by DeWitt (1957a,b) are discussed later under the heading of selenium (originally Factor 3), along with other effects of mineral deficiency. However, his statement that a deficiency of cystine and vitamin E produced the same mixed reactions of synergism and antagonism is noteworthy here. More worms appeared, but they did not mature sexually; therefore, the eggs responsible for the pathologic changes were not produced by the affected parasites.

Mice fed Purina chow and infected with *S. mansoni* responded poorly to treatment. Only 12.5% of *S. mansoni* worms were killed. In mice on a diet containing 31% of casein, 17% of fat, and 48% of sucrose, 95% of worms were killed by the same course of treatment (Luttermoser & DeWitt, 1961).

The findings were further tested by observations in man. Patients suffering from chronic *S. mansoni* infections and moderate nutritional deficiency derived no direct therapeutic benefit from nine months on a high-protein diet. Egg counts remained the same as in controls, despite some symptomatic improvement. When treatment with stibophen was eventually started, however, the response was more rapid than that usually observed (DeWitt et al., 1964). The observation of Bell (1964) that treatment of *S. mansoni* infection is less effective in vegetarians than in non-vegetarians may be relevant.

Synergism with protein deficiency occurs with cestode infections. *Hymenolepis nana* in mice on low-protein diets produced nearly double the number of cysticercoids demonstrable in animals having adequate protein. Administration of 1500 to 2000 *Hymenolepis diminuta* eggs resulted in worms in only 18% of rats on a high-protein diet; 46% of animals on stock diets became infested with worms (Vavilova, 1946). After feeding rats a protein-free diet or one containing 18% of casein, Chandler (1943) concluded that *Hymenolepis diminuta* obtained its protein directly from the host and was not dependent on dietary protein. More recently, Mettrick & Munro (1965) have confirmed this by feeding different selected diets for seven days and measuring the resulting dry weight and nitrogen content of the worm. Neither the quantity nor the quality of dietary protein influenced the growth of this tapeworm. Carbohydrate added to the diet or substituted for all of the protein resulted in increased growth, whereas replacement of protein by fat had no effect.

Fungal infections

Vanbreuseghem (1957) examined 15 000 children and adults in the Democratic Republic of the Congo, Rwanda, and Burundi and was impressed with the high prevalence of *Tinea capitis* in regions with especially short

supplies of animal protein. He found the disease in 45 of 49 children with kwashiorkor. Data for other groups were not presented.

Schofield and associates (1963) described *Tinea imbricata* as particularly common in New Guinea infants who failed to gain weight, a circumstance highly suggestive of protein deficiency. The disease was also stated to have disappeared in half of the infected adults when they received official government rations. It was not clear whether this was due to treatment or to improved diet, but clinical symptoms of teniasis recurred when these adults returned to village rations.

Generalizations

Unfortunately, protein malnutrition severe enough to cause synergism with infection is still widespread in populations of developing areas, especially among pre-school children. Occasionally, and irregularly, it also appears in advanced countries, particularly among the elderly.

The effect of individual amino acids on the course of infection is little known. Squibb (1961b) found the growth-depressing effect of Newcastle disease least in chicks on rations deficient in lysine. The greatest effect was with normal cellular metabolism, as in well-fed growing birds. Riedel (1955) found that dietary leucine deficiency had no effect on *Ascaridia galli* in chicks. Newcastle disease in immature White Leghorn cockerels resulted in greater growth depression when complete rations were supplied. On the other hand, only lysine-deficient rats died of *Nippostrongylus muris* infection (Barakat, 1948, and Seddik, 1950, as cited by Platt, 1957). Rats that were normally fed or only partially deficient in lysine survived.

Specific Vitamin Deficiencies

Vitamin A

Vitamin A deficiency shows synergism with almost every known infectious disease. Table 5 lists nearly fifty investigations on diseases of bacterial, viral, or protozoal origin in which this deficiency resulted in greater frequency, severity, or fatality. Antagonism was observed only in chickens infected with *Plasmodium lophurae* (Roos et al., 1946). This effect was not obtained when the same authors studied ducks.

When fatality, tissue change, or prevalence has been used as a criterion, tuberculosis has been found to be a more severe disease in a variety of hosts in the presence of a vitamin A deficiency. Species tested include: chick (Solotorovsky et al., 1961), mouse (Finkelstein, 1931-32), rat (Sriramachari & Gopalan, 1958), and man (Getz et al., 1951). Since the experimental studies included no pair-fed controls, a reduced food intake was a possible factor.

TABLE 5. EFFECTS OF VITAMIN A DEFICIENCY ON INFECTIOUS DISEASE

Infectious agent or disease	Host	Response	Reference	Remarks
Bacteria				
Mycobacterium tuberculosis	Chicken	Synergistic	Solotorovsky et al. (1961)	At intakes that allowed occasional survival of deficient animals, added dietary vitamin A increased survival by 26%
M. tuberculosis	Mouse	Synergistic	Finkelstein (1931-32)	Following intraperitoneal inoculation, higher fatality rate and earlier death in deficient animals
M. tuberculosis	Rat	Synergistic	Sriramacharia & Gopalan (1958)	Following intraperitoneal inoculation, more extensive lesions in deficient animals
M. tuberculosis	Man	Synergistic	Getz et al. (1951)	Statistically significant correlation with low vitamin A diets in 28 of 1100 men developing tuberculosis
Salmonella typhimurium	Mouse	Synergistic	Ørskov & Moltke (1928)	Intravenously introduced bacteria not destroyed in liver, spleen, and lymph nodes of deficient animals so that second, often fatal, wave of infection followed
S. typhimurium *S. typhimurium*	Mouse Rat	Synergistic (due to lowered food intake)	Kligler et al. (1945)	Inanition and much higher fatality rate in both vitamin-A-deficient and pair-fed controls than with *ad libitum* intake of complete diet
S. enteritidis	Rat	Synergistic	McClung & Winters (1932a)	Following intraperitoneal inoculation, 9% survival of animals on vitamin-A-deficient diet compared with 95% for same diet plus vitamin A
S. typhimurium	Rat	Synergistic	Robertson & Tisdall (1939)	Increased fatality in deficient animals reduced by supplementation with carotene
Pseudomonas aeruginosa	Rabbit	Synergistic	Greene (1933)	Spontaneous infections in vitamin-A-deficient animals but not in controls or D-deficient animals
Bacterium leptisepticum	Rabbit	Synergistic	Greene (1933)	Deficient animals more susceptible to intranasal infection (animals also vitamin D deficient)
Diplococcus pneumoniae	Rabbit	Synergistic	Greene (1933)	Deficient animals more susceptible to intranasal infection
S. typhimurium	Rat	Synergistic	Lassen (1930, 1931)	Liver and spleen of young rats on vitamin-A-free diet unable to destroy orally or subcutaneously introduced infectious agent; therefore, severe and often fatal secondary bacteremia
S. typhimurium	Mouse	Synergistic	Webster & Pritchett (1924)	Fatality rate of 70-80% in orally infected mice fed diet lacking adequate vitamins; 10% fatality rate when 5% butter fat added
Bacillus anthracis	Rat, Rabbit	Synergistic	Werkman (1923b)	Well-nourished animals normally immune; deficient animals susceptible

TABLE 5 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Klebsiella pneumoniae	Rat	Synergistic	Boynton & Bradford (1931)	Intraperitoneal inoculation after 4-10 weeks on vitamin-A-free diet; more deaths than in controls
Respiratory infections	Rat	Synergistic	Daniels et al. (1923)	Naturally acquired purulent fatal respiratory infections frequent in rats with xerophthalmia; reversed by vitamin A administration
Natural infections	Rat	Synergistic	Sherman & Burtis (1927-28)	Rats fed vitamin-A-deficient diet for 1 month at 4 weeks of age still showed increased susceptibility at 3 months of age despite good diet
Natural infections	Rat	Synergistic	McCollum (1917)	Naturally acquired infections developed much more frequently in vitamin-A-deficient animals
Natural infections	Rat	Synergistic	Drummond (1919)	Naturally acquired infections developed much more frequently in vitamin-A-deficient animals
Natural infections	Rat	Synergistic	McCarrison (1931)	Naturally acquired infections not a problem in 2000 well-fed rats over 2-year period, but frequent in deficient animals. Deficiency of other nutrients may also have been involved to lesser extent
Natural infections	Rat	Synergistic	Green & Mellanby (1928, 1930)	Severe naturally acquired infections of various organs and tissues in A-deficient animals reversed by vitamin A administration. No infections in controls
Natural infections	Man	Synergistic	Bloch (1927-28)	Of 109 patients with xerophthalmia, 68 had severe infections, 23 had diarrhea, and 1 had conjunctivitis
Natural infections	Man	Synergistic	Blegvad (1923), cited by Lassen (1931), Blegvad (1924)	In 165 persons with xerophthalmia, bronchopneumonia, bronchitis, pyuria, otitis media, and coryza were frequent; mortality from bronchopneumonia was 21.5% in 434 persons with xerosis
Natural infections	Man (infants)	Synergistic	Bloch (1924, 1927-28)	Respiratory, genito-urinary, and other infections frequent, persistent, and often fatal in vitamin-A-deficient children with xerophthalmia
Natural infections	Man (infants)	Synergistic	Blackfan & Wolbach (1933)	Eight of 13 children with xerophthalmia or keratomalacia died of secondary infection
Natural infections	Man (infants)	Synergistic	Clausen (1935)	Frequency of otitis media, pneumonia, and other severe infections doubled in children with A-deficient diets (317 in study)
Natural infections	Man (children)	Synergistic	Oomen (1958)	Almost no xerophthalmia cases free of respiratory infections

TABLE 5 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Saprophytic bacteria	Rat	Synergistic	Bradford (1928)	Normally saprophytic bacteria increased in number and became pathogenic in animals fed vitamin-A-deficient diet
Saprophytic Gram-negative bacteria	Rat	Synergistic	Turner et al. (1930)	Normally saprophytic bacteria increased in number and became pathogenic in animals fed vitamin-A-deficient diet
Rickettsia				
Rickettsia prowazekii	Rat	Synergistic	Zinsser et al. (1931)	Well-nourished animals difficult to infect intraperitoneally, but readily so with xerophthalmia induced by deficient diet
Virus				
Poliomyelitis	Rat	Synergistic	Weaver (1946)	Shorter incubation time after oral infection of A-deficient animals; no difference with other inoculation sites
Newcastle disease	Chicken	Synergistic	Squibb (1961)	Increased fatality in chicks with borderline vitamin A deficiency
Infectious bronchitis	Chicken	Synergistic	Panda et al. (1962b)	Birds fed 3600 and 10 000 IU of vitamin A per lb of diet, experienced greater weight gains during pre-infective and active disease than those fed 1200 IU
Infectious bronchitis	Chicken	No effect	Gratzl et al. (1963)	Chicks fed 5 times the physiologic need (40 000 IU per kg body-weight) from 2 days and infected intranasally at 6 weeks showed no difference from controls in course of infection
Protozoa				
Eimeria tenellum and *acervulina*	Chicken	Synergistic	Erasmus et al. (1960)	Faster recovery with higher levels of vitamin A in diet
Eimeria acervulina and *necatrix*	Chicken	Synergistic	Panda et al. (1962a,b)	Birds fed 3600 and 10 800 IU of vitamin A per lb of diet experienced greater weight gains during pre-infective and active disease than those fed 1200 IU
E. tenellum	Chicken	No effect	Waldroup et al. (1963)	Level of vitamin A given before or after infection did not affect fatality from cecal coccidiosis or weight gain 3-6 days later
Trypanosoma equiperdum	Rat	Synergistic	Reiner & Paten (1932-33)	Vitamin-A-deficient rats died sooner than those fed complete diets
Plasmodium lophurae	Duck	No effect	Roos et al. (1946)	No effect of deficiency on severity of parasitemia
P. lophurae	Chicken	Antagonistic	Roos et al. (1946)	Parasitemia less severe when deficient

TABLE 5 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
T. cruzi	Rat	Antagonistic	Yaeger & Miller (1963)	After 13 weeks on vitamin-A-deficient diet, rats significantly more susceptible to *T. cruzi* than pair-fed controls
Helminths				
Ascaridia lineata	Chicken	Synergistic	Ackert et al. (1927, 1931)	Worms longer and more numerous in vitamin-A-deficient animals
Ascaris lumbricoides	Pig	No effect	Clapham (1934b)	Deficient animals had initially heavier infections, but all animals eventually had marked infections
Parascaris equorum	Rat	Synergistic	Clapham (1933)	Susceptible to infection when mildly deficient, but not when well nourished
Heterakis gallinae	Chicken	No effect	Clapham (1934a)	Mild deficiency did not affect size or number of worms
Toxocara canis *Toxascaris leonina*	Dog	Synergistic	Wright (1935)	Deficient animals exposed to superinfections; five times more worms at autopsy than controls
Ascaris lumbricoides	Pig	Synergistic	Hiraishi (1927)	Human strain infected only 1 of 30 well-nourished and 29 of 30 deficient animals
Trichinella	Rat	Synergistic	McCoy (1934)	Two and a half times more larvae in muscles of experimentally infected deficient rats and no development of immunity; younger rats most affected
Diphylidium caninum	Kitten	Synergistic	MacKay (1921)	Three kittens fed deficient diet developed heavier unintentional infections than 2 controls
Strongyloides ratti	Rat	Synergistic	Lawler (1941)	A-deficient animals had (*a*) larger worm counts 10 days after consuming 2000 larvae, (*b*) possibly slower elimination of worms, and (*c*) poorer development of immunity
Nippostrongylus muris	Rat	Synergistic	Spindler (1933-34)	Lack of vitamin A in the diet greatly lowered resistance to superinfections, as judged by egg production and larvae recovered at autopsy
Schistosoma mansoni	Rat	Synergistic	Krakower et al. (1940)	Artificially infected well-fed animals destroy worms in lungs and liver in 5 to 7 weeks; destruction minimal or absent in deficient animals
Onchocerca volvulus	Man	Synergistic	Rodger (1957, 1960)	Eye lesions in patients with this infection more common in individuals or populations with low vitamin A intake

Ørskov & Moltke (1928) and Lassen (1930, 1931) found an increased susceptibility to salmonellosis in mice and rats on diets deficient in vitamin A. This they attributed to failure of the animals to destroy bacteria in the liver, spleen, or lymph nodes. McClung & Winters (1932a) reported a 9% survival of rats with salmonellosis on vitamin-A-deficient diets, compared with 95% for animals receiving added vitamin A. Differences of similar magnitude were reported for mice infected with *S. typhimurium* (Webster & Pritchett, 1924; Pritchett, 1927) and rats with *Klebsiella pneumoniae* (Boynton & Bradford, 1931). In experimental salmonellosis, Kligler and co-workers (1945) observed an increased fatality rate in mice and rats rendered vitamin-A-deficient compared with those receiving complete diets *ad libitum*. They concluded that food restriction and possible protein deficiency caused this effect, since pair-fed controls succumbed with equal frequency.

Greene (1933) conducted extensive studies on rabbits deficient in vitamin A or vitamins A and D. Those with insufficient vitamin A were less resistant to natural infection with *Pseudomonas aeruginosa*, whereas vitamin-D-deficient and well-fed animals resisted this organism. Chickens fed a vitamin-A-deficient diet were more susceptible to *P. aeruginosa*, although other differences in diet may have been involved (Niilo & Bezeau, 1961). Animals with a deficiency of both vitamins A and D succumbed to intranasal infection with *Bacterium lepisepticum* more readily than did controls; vitamin-A-deficient animals succumbed more readily to *Diplococcus pneumoniae*. Well-nourished rats are resistant to anthrax. Werkman (1923b) and Werkman and associates (1924b), however, found that animals with insufficient vitamin A were susceptible to this disease and to diphtheria. The views of these two groups of workers on antibody and leukocyte response are discussed in Chapter 4.

Many worthwhile observations are from reports of natural epidemics among rats maintained on vitamin-A-deficient diets for other purposes (McCollum, 1917; Drummond, 1919; Daniels et al., 1923; Green & Mellanby, 1928, 1930; McCarrison, 1931). In the experience of Sherman & Burtis (1927-28), rats fed vitamin-A-deficient diets for one month, commencing at four weeks of age, showed an increased susceptibility to infection, which still persisted at three months of age, although the animals were then receiving good diets. In vitamin-A-deficient rats, not only did susceptibility to known pathogens increase, but normally saprophytic bacteria increased in numbers and became pathogenic (Bradford, 1928; Turner et al., 1930).

Similarly, children and adults with xerophthalmia are highly susceptible to natural infections, particularly respiratory and genito-urinary disease (Blegvad, 1924; Bloch, 1919, 1924, 1927-28; Blackfan & Wolbach, 1933; Clausen, 1935; Oomen, 1958).

Squibb (1961a) found that vitamin-A-deficient birds inoculated with the virus of Newcastle disease had a greater fatality than chicks with adequate

vitamin reserves; they were exposed to the double risk of death from avitaminosis and death from infection.

Weaver (1946) showed a shortened incubation period for poliomyelitis after oral administration of the virus to vitamin-A-deficient rats. Since a similar effect did not occur after intracerebral, intraperitoneal, subcutaneous, or intracardiac inoculation, experiments were instituted to test the possible effect of vitamin A deficiency on permeability of various parts of the alimentary tract. When the virus was administered orally or acquired through contact with diseased animals, both the vitamin-deficient and the control animals developed paralysis; when the virus was placed in the upper larynx, stomach, or colon, more controls escaped paralysis of two or more extremities than did deficient animals. In six-week-old chickens whose rations were already adequate, a fivefold increase in vitamin A had no effect on the course of infectious bronchitis induced by administration of the appropriate virus (Gratzl et al., 1963).

Coccidiosis, a protozoal disease, was more severe and protracted in chickens with vitamin A deficiency (Erasmus et al., 1960; Panda et al., 1962a, b). *Trypanosoma equiperdum* infection brought about a higher fatality in deficient rats than in those on complete diets (Reiner & Paton, 1932-33). Although vitamin A deficiency had no apparent effect on the course of malaria in the duck, it was associated with lower levels of parasitemia in chicks (Roos et al., 1946).

After four weeks on a vitamin-A-deficient diet, rats showed a slight increase in susceptibility to *T. cruzi* infection; however, pair-fed controls were equally susceptible, suggesting that inanition was responsible for the lowered resistance (Yaeger & Miller, 1963c). After 13 weeks on the vitamin-A-deficient diet, rats were significantly more susceptible to *T. cruzi* infection, and to spontaneous bacterial infections than were pair-fed control rats.

In the only available study of a rickettsial infection in vitamin-A-deficient animals, Zinsser and co-workers (1931) had difficulty in infecting rats intraperitoneally with *Rickettsia prowazeki* unless this deficiency was first induced.

Synergism of vitamin A deficiency with a wide variety of helminthic diseases is general. Larger and more numerous ascarids in chickens have been reported (Ackert et al., 1927, 1931), although a different species of helminths, *Heterakis gallinae*, and milder degrees of deficiency produced no detectable interaction (Clapham, 1934a). Vitamin-A-deficient dogs exposed to superinfection with *Toxocara canis* and *Toxascaris leonina* had five times as many worms at autopsy as dogs on an adequate diet and exposed at the same time (Wright, 1935).

Ascaris lumbricoides in pigs gave an initially heavier infection in the presence of a deficiency, but eventually all animals acquired heavy worm burdens (Clapham, 1934b). For most young pigs, infection with the human strain was possible only when vitamin A deficiency was present (Hiraishi,

1927). In the presence of a mild vitamin A deficiency, rats, which are usually resistant to infection with *Parascaris equorum*, acquired significant worm burdens after ingesting infective eggs (Clapham, 1933).

McCoy (1934) made a comprehensive study of the effect of vitamin A deficiency on resistance of rats to trichinosis. Not only did adult worms persist longer in the intestinal tract, but, two weeks after infection, more than two and a half times as many larvae were recovered from muscles of the vitamin-deficient animals as from well-fed controls. Furthermore, controls had complete immunity to a second infection 30 days after the first whereas deficient rats acquired no such protection.

Observations of three deficient and two well-fed kittens showed that naturally acquired *Diplidium caninum* infection was more intense in animals fed a vitamin-A-deficient diet (MacKay, 1921). Rats infected with *Strongyloides ratti* (Lawler, 1941) or *Nippostrongylus muris* (Spindler, 1933-34) had higher worm burdens and greater egg production when vitamin A deficiency had been induced; in the first instance at least, immunity developed more slowly. Finally, in well-nourished rats fed *Schistosoma mansoni*, worms reaching liver and lungs were destroyed within five to seven weeks; worm destruction in animals on vitamin-A-deficient diets was inhibited, or ceased completely (Krakower et al., 1940).

To summarize, no nutritional deficiency is more consistently synergistic with infectious disease than that of vitamin A. One of the first recognized features of avitaminosis A, increased susceptibility to infection, has had strong confirmation.

Other fat-soluble vitamins

Table 6 lists studies on deficiencies of vitamin D and vitamin E. Even with a deficiency of vitamin D sufficient to produce rickets, resistance to specific infections was not affected in at least half the investigations cited. Synergism was observed in two studies of experimental salmonellosis in rachitic rats. In both instances, the reduced resistance of vitamin-deficient animals was restored by adding vitamin D to the diet (Ross & Robertson, 1930-31; Robertson & Ross, 1932; McClung & Winters, 1932b). One report of young pigs on barley diets states that animals with rickets readily acquired a fatal salmonellosis (Manninger, 1928a,b). The same micro-organism produced no symtoms in well-nourished animals of the same age. (This study is not listed in Table 6 since the nature of the deficiency is uncertain.)

More frequent and more severe whooping cough in rachitic children is another example of synergism (Clausen, 1935).

Studies of tuberculosis in mice (Finkelstein, 1931-32), salmonellosis (Lassen, 1931) and *Klebsiella pneumoniae* infection in rats (Boynton & Bradford, 1931), *Pseudomonas aeruginosa* in rabbits and poliomyelitis type 2

in mice (Foster et al., 1949) did not show any increased severity in rachitic as compared with control animals. However, vitamin-D-deficient dogs were

TABLE 6. EFFECTS OF VITAMIN D AND VITAMIN E DEFICIENCIES ON INFECTIOUS DISEASE

Infectious agent or disease	Host	Response	Reference	Remarks
		Vitamin D		
Bacteria				
Bordetella pertussis	Man (infants)	Synergistic	Clausen (1935)	Pertussis severe in 8 of 10 children with rickets and 17 of 38 without
Mycobacterium tuberculosis	Mouse	No effect	Finkelstein (1931-32)	Survival of intraperitoneally inoculated animals fed diet deficient in fat-soluble vitamins unaffected by irradiated ergosterol
Salmonella typhimurium	Rat	Synergistic	Ross & Robertson (1930-31), Robertson & Ross (1932)	Rachitic rats much more susceptible than controls; resistance restored by irradiation of diet or exposure of animals to sunlight
S. enteritidis	Rat	Synergistic	McClung & Winters (1932b)	Fatality from intraperitoneal inoculation after 7 weeks on D-deficient diet was 63% as compared with 27% when cod liver oil was added to the diet
S. typhimurium	Rat	No effect	Lassen (1931)	No increased fatality in infected animals with severe rickets
Bronchopneumonia	Dog	Synergistic	Mellanby (1919)	Rachitic animals more susceptible
Klebsiella pneumoniae	Rat	No effect	Boynton & Bradford (1931)	No difference in susceptibility to intraperitoneal inoculation between rachitic and control animals
Pseudomonas aeruginosa	Rabbit	No effect	Greene (1933)	Unlike vitamin-A-deficient animals, those with vitamin D deficiency no more susceptible than controls
Virus				
Distemper	Dog	Synergistic	Mellanby (1919)	Rachitic animals more susceptible to naturally acquired infection
"Coryza"	Rat	Synergistic	György et al. (1926)	When spontaneous epidemic broke out, fatality rate was 39% in animals on a rachitic diet and zero in controls
Poliovirus, type 2	Mouse	No effect	Foster et al. (1949)	Virus injected into animals fed 5 combinations of phosphorus and calcium levels; no difference in number of deaths with or without vitamin D
Helminths				
Ascaridia lineata	Chicken	No effect	Ackert & Spindler (1929)	Variable and inconclusive results

TABLE 6 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
		Vitamin E		
Bacteria				
Acid-fast organisms from leprosy patients	Hamster Rat	Synergistic	Mason & Bergel (1955)	Well-nourished animals could not be infected; inoculation of vitamin-E-deficient animals intraperitoneally or subcutaneously resulted in lesions containing acid-fast bacilli
Virus				
Vesicular stomatitis	Mouse	Synergistic	Sabin & Duffy (1940)	Animals fed deficient diets after weaning were somewhat retarded in developing resistance to intranasal inoculation
Helminths				
Trichinella spiralis	Rat	Antagonistic	Zaiman (1940)	After large doses of larvae, terminal muscle counts were lower in animals fed vitamin-E-free diet
Schistosoma mansoni	Mouse	Mixed	DeWitt (1957)	In animals fed diets deficient in vitamin E and cystine, fewer pathologic changes because, although worms were more numerous, they were smaller, did not mature sexually, and did not produce the large numbers of eggs primarily responsible for tissue damage

more susceptible to spontaneous bronchopneumonia and distemper (Mellanby, 1919). György and associates (1926) also reported a higher fatality rate among rachitic laboratory rats during an epidemic of "coryza".

Studies by Ackert & Spindler (1929) on *Ascaridia lineata* in chickens on a vitamin-D-deficient diet gave variable and inconclusive results. In some trials, worms were smaller in size, but more numerous; in others, the effect was not clear-cut.

Hamsters and rats are not susceptible to human leprosy. According to a report unconfirmed by other workers, administration of diets deficient in vitamin E resulted in growth of acid-fast bacilli after intraperitoneal or subcutaneous inoculation of material from patients with lepromatous leprosy (Mason & Bergel, 1955).

Deficiency of vitamin E retarded normal "maturation resistance" to intranasal inoculation of the virus of vesicular stomatitis in weanling mice; susceptibility to intracerebral inoculation continued in both deficient and control animals (Sabin & Duffy, 1940).

In rats fed 2500 *Trichinella spiralis* larvae, fewer worms were recovered from muscles of animals deficient in vitamin E than from controls during periods ranging from six days to four months (Zaiman, 1940).

Schistosoma mansoni were smaller and did not develop sexually in mice with diets low in vitamin E (DeWitt, 1957a,b). Although the number of worms was greater in the vitamin-deficient animals, the net pathologic effect was considered less because the production of large numbers of eggs normally responsible for tissue damage did not occur.

To summarize, deficiencies of vitamins D and E are synergistic with infectious disease under some conditions, although often no effect is observed, especially with vitamin D deficiency.

Ascorbic acid

Deficiency of ascorbic acid is synergistic with all infectious diseases studied, with the exception of a single report of antagonism in monkeys with malaria. A noteworthy feature of Table 7 is the absence of any investigation of a viral disease in scorbutic animals. Since guinea-pigs and primates are the only laboratory animals with a dietary requirement for vitamin C, experiments are limited to these species.

Compared with controls, scorbutic guinea-pigs had a greater susceptibility to tuberculosis (Leichtentritt, 1924; Mouriquand et al., 1924; McConkey & Smith, 1933; Birkhaug, 1938, 1939; Gangadharam & Sirsi, 1953; Boyden & Andersen, 1955) and diphtheria (Berzin, 1955). In addition, skin reactions to both diphtheria toxin (Bieling, 1925, 1927) and tuberculin (Birkhaug, 1939a, b) were less marked in scorbutic animals.

Scorbutic guinea-pigs were more susceptible than normal animals to pneumococci (Findlay, 1923a,b; Werkman, 1923b; Schmidt-Weyland & Költzsch, 1927-28; Wámoscher, 1927), to anthrax bacilli (Werkman, 1923a), streptococci (Jackson & Moody, 1916; Findlay, 1923a; Rinehart et al., 1934), to colon bacilli (Findlay, 1923b), to salmonella (Grant, 1926), to the rickettsia of typhus fever (Zinsser et al., 1931; Pinkerton, 1949), and to *E. histolytica* (Sadun et al., 1951). On the other hand, vitamin C deficiency had no clear-cut effect in guinea-pigs infected with *Schistosoma mansoni* (Krakower et al., 1944). Worm burden was unaffected, and the occurrence of normally fertilized ova with defective shells seemed to have little significance. However, a secondary bacteremia of the host sometimes led to bacterial infection of the worms.

In one of two studies on ascorbic acid deficiency and infections of monkeys, oral infection with *Spirillum sputigenum* was more severe in the deficient animals (Kelly, 1944). The second study, the only one of an intracellular protozoan, is also the only reported instance of antagonism. Experimental parasitemia with *P. knowlesi* was less marked in deficient animals; it increased rapidly after addition of ascorbic acid to the diet (McKee & Geiman, 1946).

The findings in laboratory animals suggest that persons with scurvy would be expected to have more than the usual number of attacks of

TABLE 7. EFFECTS OF VITAMIN C DEFICIENCY ON INFECTIOUS DISEASE

Infectious agent or disease	Host	Response	Reference	Remarks
Bacteria				
Mycobacterium tuberculosis	Guinea-pig	Synergistic	Leichtentritt (1924)	More weight loss and pathologic changes and shorter survival times in scorbutic than in vitamin-supplemented animals after inoculation
M. tuberculosis	Guinea-pig	Synergistic	McConkey & Smith (1933)	When animals were fed infected sputum daily, tuberculous intestinal ulcers developed in 26 of 37 scorbutic and 2 of 35 non-scorbutic animals
M. tuberculosis	Guinea-pig	Synergistic	Bieling (1925)	Smaller tuberculin reaction in scorbutic guinea-pigs
M. tuberculosis	Guinea-pig	Synergistic	Gangadharam & Sirsi (1953)	Greater pathologic change, less growth, shorter survival time in scorbutic than in vitamin-supplemented infected animals
M. tuberculosis	Guinea-pig	Synergistic	Boyden & Andersen (1955)	Longer survival time, lower fatality rate in animals receiving diets supplemented with alfalfa and grass than in those on diets supplemented with sugar beet; difference attributed to lower vitamin C intake with latter
M. tuberculosis	Guinea-pig	Synergistic	Birkhaug (1939, 1938)	Daily oral vitamin C intake correlated with urinary excretion and adrenal concentration of the vitamin and with increased body-weight and reduced extent of the lesion
M. tuberculosis	Man	Synergistic	Getz et al. (1951)	Of 1100 men followed for 1 month to 5 years significantly more tuberculosis developed in those with habitually low ascorbic acid intake
M. tuberculosis	Guinea-pig	Synergistic	Mouriquand et al. (1924)	Mortality after tuberculous infection greater in animals with acute scurvy than in controls
Spirillum sputigenum	Monkey	Synergistic	Kelly (1944)	Oral infection more severe in scorbutic animals
Corynebacterium diphtheriae	Guinea-pig	Synergistic	Bieling (1927)	Reduced skin reactions and resistance to large doses of toxin in scorbutic animals
C. diphtheriae	Guinea-pig	Synergistic	Berzin (1955)	Deficient animals more susceptible
C. diphtheriae	Man (infants)	Synergistic	Hess (1917, 1920)	In infants with scurvy, nasal diphtheria more common, often became infected despite negative Schick test
Pseudomonas aeruginosa	Chicken	Synergistic	Niilo & Bezeau (1961)	Major outbreaks more common in deficient flocks
Staphylococcus aureus	Man (children)	Synergistic	Hess (1917, 1920)	Furunculosis more common in hospitalized children with scurvy

TABLE 7 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Diplococcus pneumoniae	Guinea-pig	Synergistic	Schmidt-Weyland & Kóltzsch (1927-28)	Droplet inhalation produced much more infection in scorbutic animals
D. pneumoniae	Guinea-pig	Synergistic	Wámoscher (1927)	Increased fatality rate from spontaneous pneumonia in scorbutic animals
D. pneumoniae Bacillus anthracis	Guinea-pig	Synergistic	Werkman et al. (1924a)	Higher fatality rate in scorbutic animals
D. pneumoniae Streptococci and colon bacilli	Guinea-pig	Synergistic	Findlay (1923a)	Decreased resistance to intraperitoneal infection in scorbutic animals
Streptococci	Guinea-pig	Synergistic	Jackson & Moody (1916)	Gram-positive cocci more common in scorbutic animals; intracardiac or intravenously injected streptococci produced multiple lesions in scorbutic but not in well-fed animals
Natural bacterial infections	Man (children)	Synergistic	Bloch (1927-28)	More frequent and more severe in children with scurvy
Salmonella typhimurium	Guinea-pig	Synergistic	Grant (1926)	Orally induced infection much more severe in scorbutic animals
Rickettsia				
Rickettsia prowazekii	Guinea-pig	Synergistic	Zinsser et al. (1931)	Rickettsia more numerous and widely distributed in scorbutic animals
R. prowazekii	Guinea-pig	Synergistic	Pinkerton (1949)	Claim that infection more severe in deficient animals based on own unpublished data
Virus				
"Grippe"	Man (children)	Synergistic	Hess (1917, 1920)	Infections more common in hospitalized children with scurvy
Protozoa				
Plasmodium knowlesi	Monkey	Antagonistic	McKee & Geiman (1946)	Less parasitemia in deficient animals; overwhelming increase following ascorbic acid administration
Entamoeba histolytica	Guinea-pig	Synergistic	Sadun et al. (1951)	Greater infectivity when inoculated in scorbutic than in well-fed animals; disease more severe and fatality rate higher
Helminths				
Schistosoma mansoni	Guinea-pig	Mixed	Krakower et al. (1944)	After exposure to concentrated cercaria, normal development of parasites and fertilization of ova except that schistosome egg shells were defective in deficient animals

"grippe", furunculosis, and other ordinary infections. Hess (1917, 1920) found this to be true. Bloch (1927-28) also recognized a more frequent occurrence of spontaneous infections in children with scurvy. More recently, Getz and co-workers (1951), in Philadelphia, Pa., USA, attempted to relate occurrence of tuberculosis to diet among 1100 young adults, mainly Negroes. Observations ranged from a month to five years. Significantly more tuberculosis developed in persons with low dietary intakes of ascorbic acid, but other likely factors were not satisfactorily explored.

Severe ascorbic acid deficiency tends regularly to lower the resistance of man to most infectious diseases. Sound clinical studies are few; the existing evidence is mainly from epidemiologic observations rather than experimental trial. The flood of clinical reports of a supposed benefit deriving from an increase in levels of ascorbic acid in persons already well nourished had their origin in the intemperate enthusiasm that followed initial recognition of the importance of vitamins in human nutrition. None of these studies is included here, because they lack adequate controls or are otherwise unreliable.

To summarize, ascorbic acid deficiency is regularly synergistic in its interaction with specific infections.

Vitamin B-complex

Table 8 summarizes studies of diets deficient in several or all of the B-complex vitamins. In general, they antedate most experimental investigations on the effects of individual B-vitamin deficiencies. Better-controlled experiments using specific deficiencies have largely superseded these studies, but they still illustrate the high fatality among animals that have both a B-complex deficiency and an infection.

Salmonella in the rat (Ross & Robertson, 1930-31; Lassen, 1931), *Clostridium perfringens* and *Staphylococcus aureus* in the dog (Rose, 1927-28; Rose & Rose, 1936), *Mycobacterium lepraemurium* in the rat (Lamb, 1935), and *Streptococcus pyogenes* in the monkey (Saslaw et al., 1942) were all more than usually lethal when the animals were maintained on a B-complex-deficient diet.

More worms than usual were found in B-complex-deficient chickens fed ascarid ova (Zimmerman et al., 1926; Ackert & Nolf, 1931); the worms of *Ascaridia lineata* were smaller, whereas those of *Ascaridia perspicillum* were larger.

The egg production of *Hymenolepis diminuta* was apparently markedly inhibited in rats fed diets lacking B-complex vitamins, although thiamine deficiency alone did not affect fecal egg counts (Hager, 1941). The average number of worms per animal was also decreased by diets deficient in B-complex vitamins (Addis & Chandler, 1944).

Results suggesting antagonism have been obtained in four other studies.

TABLE 8. EFFECTS OF DEFICIENCY OF B-COMPLEX VITAMINS ON INFECTIOUS DISEASE

Infectious agent or disease	Host	Response	Reference	Remarks
Bacteria				
Salmonella typhimurium	Rat	Synergistic	Ross & Robertson (1930-31)	Earlier death in orally infected rats deprived of B-complex
S. typhimurium	Rat	Synergistic	Lassen (1931)	Severe degrees of B-complex deficiency gave moderately but significantly increased fatality
Ulcerative cecitis	Rat	Antagonistic	Bloomfield & Lew (1943)	Spontaneous infection in 3 of 16 B-complex-deficient animals and 7 of 14 controls
Clostridium perfringens	Dog	Synergistic	Rose (1927-28)	High fatality rate in animals receiving no B-complex vitamins in diet
Staphylococcus aureus	Dog	Synergistic	Rose & Rose (1936)	At 13-33% of normal B-complex requirements, *S. aureus* infection more severe as judged by duration of positive blood cultures and fatality rate compared with pair-fed controls
Streptococcus pyogenes	Monkey	Synergistic	Saslaw et al. (1943)	Much higher fatality rate in B-complex-deficient animals
Mycobacterium lepraemurium	Rat	Synergistic	Lamb (1935)	Intracardiac inoculation; higher fatality rate in B-complex-deficient animals compared with *ad libitum*-fed controls (diets also low in protein)
Rickettsia				
Rickettsia prowazekii	Rat	Synergistic	Fitzpatrick (1948)	When one-tenth adequate B group in diet, 9 of 11 rats died following infection; when double adequate B group in diet, only 3 of 11 rats died
Virus				
Influenza, type A	Monkey	Synergistic	Saslaw et al. (1943)	Much higher fatality rate in B-complex-deficient animals
Poliovirus, type 2	Rat	No effect	Weaver (1945)	Variety of inoculation sites used; no change in severity of reaction in B-complex-deficient rats as compared with controls
Encephalomyelitis	Mice	Antagonistic	Schneider et al. (1957)	Animals 100% susceptible on a stock diet but only 15% on synthetic diet with 18% casein and minimal vitamins for growth and maintenance; effect largely reversed by addition of biotin, folic acid, and vitamin B_{12} in combination or singly
Protozoa				
Trypanosoma equiperdum	Rat	Antagonistic	Reiner & Paton (1932-33)	Infected B-complex-deficient animals survived slightly but significantly longer than controls

TABLE 8 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Helminths				
Ascaridia lineata	Chicken	Mixed	Ackert & Nolf (1931)	Worms small, but more numerous, in B-complex-deficient group compared with controls
Ascaridia perspicillum	Chicken	Synergistic	Zimmerman et al. (1926)	Worms in B-complex-deficient animals more numerous and larger 3 weeks after feeding ova
Hymenolepis diminuta	Rat	Antagonistic	Hager (1941)	Elimination of B-complex vitamins markedly inhibited egg production (thiamine deficiency alone had no effect)

Genetically homozygous mice were 100% susceptible to acute disseminated encephalomyelitis when fed a stock laboratory regimen. On a synthetic diet containing a minimal list of vitamins adequate for growth and maintenance, only 15% of the animals were susceptible (Schneider et al., 1957). Supplementation of the synthetic diet with biotin, folic acid, and vitamin B_{12} restored susceptibility to 70%.

Deaths from *Trypanosoma equiperdum* infection in B-complex-deficient rats seemed slightly delayed (Reiner & Paton, 1932-33). Ulcerative cecitis affected fewer B-complex-deficient rats than litter-mate controls when a natural infection appeared spontaneously in a rat colony (Bloomfield & Lew, 1943). An infectious agent was never identified; it was postulated that a viral invader was superimposed on a prevailing endemic salmonellosis.

To summarize, except for the above examples and an additional instance in which response to poliomyelitis was the same in deficient and control animals (Weaver, 1945), a deficiency of B-complex vitamins has proved regularly synergistic with infectious disease. As synthetic vitamins became available and purified diets were developed, research inevitably turned to the study of deficiencies of individual B-vitamins.

Thiamine

The investigations listed in Table 9 demonstrate that thiamine deficiency is regularly synergistic with infectious disease of bacterial origin, and often antagonistic with that of viral etiology. The deficiency appears to inhibit the course of systemic protozoal infections, whereas intestinal protozoal disease is worsened.

In India in 1918-19, McCarrison observed that pigeons fed polished rice rapidly developed more severe symptoms with *Salmonella choleraesuis* infection than did controls. He considered his most important finding the

TABLE 9. EFFECTS OF THIAMINE DEFICIENCY ON INFECTIOUS DISEASE

Infectious agent or disease	Host	Response	Reference	Remarks
Bacteria				
Salmonella typhimurium	Pigeon	Synergistic	McCarrison (1918-19)	Following spontaneous infection, symptoms developed more rapidly and were more severe and fatality rate was higher in thiamine-deficient birds. "Liberal diet of mixed grains" conferred "comparative immunity"
S. typhimurium	Pigeon	Synergistic	Barlow (1930)	More severe bacteremia and anemia in animals fed polished rice diet
S. typhimurium	Mouse, rat	Synergistic	Guggenheim & Buechler (1946a)	Increased fatality rate after oral infection in (*a*) deficient mice compared with pair-fed and *ad libitum* controls; (*b*) both thiamine-deficient and pair-fed compared with *ad libitum* controls
Escherichia coli *S. enteritidis*	Pigeon	Synergistic	Findlay (1923b)	On diets producing signs of beriberi, deaths occurred 9-36 hours after intraperitoneal inoculation; *ad libitum* controls survived
Small cocci and coliform bacilli	Macacus monkey	Synergistic	McCarrison (1919-20b)	Nine of 11 animals fed autoclaved rice diets developed dysentery and diarrhea; 8 controls did not
Anthrax	Pigeon	Synergistic	Corda (1923)	Birds on polished rice susceptible to beriberi and anthrax; protected by added vitamin B_1 (in asparagus)
Shigella	Man	Synergistic	Burgess (personal communication, 1962)	Dysentery frequently lethal to thiamine-depleted persons in a Japanese prisoner-of-war camp; when more thiamine became available, deaths decreased
Bacillus anthracis	Pigeon	Synergistic	Werkman (1923a)	Well-nourished birds normally immune; deficient birds susceptible
Diplococcus pneumoniae	Mouse	Synergistic	Wooley & Sebrell (1942)	Fatality rate following intranasal infection greater in deficient animals than in *ad libitum* or pair-fed controls
D. pneumoniae	Rat	Synergistic	Robinson & Siegel (1944)	Increased fatality rate in deficient animals following intratracheal inoculation compared with controls fed *ad libitum*. Inanition had no effect
D. pneumoniae	Rat	Synergistic	Wertman & Groh (1959)	Compared with *ad libitum* or pair-fed controls, higher fatality after infection in deficient animals
Mycobacterium lepraemurium	Rat	Synergistic	Badger et al. (1940)	Incubation shorter, fatality higher, on deficient diet
Borrelia persica	Rat	Synergistic	Guggenheim & Halevy (1952)	With severe deficiency and 50 % reduction in food intake, increased fatality; with mild deficiency and 30 % reduction, fatality not affected

TABLE 9 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Rickettsia				
Rickettsia prowazekii	Rat	Synergistic	Fitzpatrick (1948)	Diet complete except for reduced thiamine, 88% fatality rate
Bedsonia				
Psittacosis	Pigeon	Synergistic	Pinkerton & Swank (1940)	5% of 400 previously healthy pigeons developed severe infection spontaneously after 6-12 days deficiency
Virus				
Poliovirus, type 2	Mouse	Antagonistic	Toomey et al. (1943-44)	Following infection both B_1-deficient and B_1-overfed animals had less paralysis and longer life-span than normally fed animals
Mouse encephalomyelitis (Theiler) Poliovirus, type 2	Mouse	Antagonistic	Rasmussen et al (1944a)	Lower incidence of infection in deficient animals; some deficient survivors given B_1 became paralysed. Similar effect, but much less marked, in pair-fed controls
Poliovirus, type 2	Mouse	Antagonistic	Foster et al. (1942)	Deficiency prolongs incubation, reduces incidence of paralysis and lowers fatality
Poliovirus, type 2	Mouse	Antagonistic	Foster et al. (1944a,b)	(*a*) Deficient diet for 35 days prior to inoculation: 21% of deficient animals and 58% of controls died, but 14% of deficient animals injected with normal brain tissue also died (*b*) Antagonism more marked with B_1-deficient than with pair-fed animals, despite some increased resistance of latter
Poliomyelitis	Monkey (*Macacca mulatta*)	No effect	Clark et al. (1945)	No change in resistance of deficient animals to poliovirus
Poliomyelitis	Cotton rat	No effect	Weaver (1945)	No change in resistance of deficient animals to poliovirus
Vesicular stomatitis Eastern, Western, and St Louis encephalomyelitis	Mouse	Synergistic	Sabin (1941)	Resistance to intranasal inoculation developed more slowly in young animals fed polished rice diets with fresh, rather than autoclaved, yeast
Vesicular stomatitis	Mouse	Synergistic	Sabin & Duffy (1940)	Development of resistance to intranasal inoculation greatly retarded in young animals nursed by B_1-deficient mothers or weaned at 14 days and placed on deficient diet
Western equine encephalomyelitis	Mouse	Antagonistic	Kearney et al. (1948b)	With severe thiamine deficiency, death of inoculated animals appeared due to the vitamin deficiency rather than to encephalomyelitis
Avian encephalomyelitis	Chicken (1-day-old)	Synergistic	Cooperman et al. (1946)	One-day-old chicks show greatest resistance to infection on diet with optimum thiamine (50% paralysed);

TABLE 9 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Avian encephalomyelitis (*continued*)	(2-week-old)	Antagonistic		progressively less resistance as B_1 deficiency increased (97% paralysed) Reversal of above effect with 63% of 2-week-old chicks paralysed as compared with 90% of those on optimum intake
Protozoa				
Plasmodium gallinaceum	Chicken	Mixed	Rama Rao & Sirsi (1956)	Moderate thiamine deficiency retarded parasite multiplication. Complete depletion had same effect, but host was adversely affected and died earlier. High doses of B_1 accelerated parasitemia and hastened death
P. lophurae	Duck Chicken	No effect	Roos et al. (1946)	Deficiency had no effect on degree of parasitemia or duration of infection
P. lophurae	Chicken	Synergistic	Seeler & Ott (1946)	Parasite counts highest in severely deficient chicks and lowest at levels approaching minimum requirements; when fed excess B_1, parasite counts fell between those of minimally and severely deficient groups
Leishmania donovani	Mouse	No effect	Actor (1960)	Deficiency had no effect on number of leishmania, compared with either *ad libitum* or pair-fed controls
Trypanosoma equiperdum	Rat	Antagonistic	Reiner & Paton (1932-33)	Infected deficient animals live longer than controls on normal diet
T. brucei	Pigeon	Synergistic	Sollazzo (1929)	Animals not normally susceptible become so when fed polished rice diet
T. cruzi	Rat	Synergistic	Yaeger & Miller (1960a)	Parasitemia greater, lesions more extensive, and parasites more common in heart tissue of B_1-deficient animals than in *ad libitum*, pair-fed, or extra thiamine-fed controls
Entamoeba histolytica	Monkey	Synergistic	McCarrison (1919-20a)	Chronic amebiasis became clinically severe in 5 of 11 animals fed poorly consumed polished rice diets; no disease in 12 controls, although 3 proved to be carriers
Giardia muris	Mouse	Antagonistic	Scholtens (1962)	Fewer trophozoites in deficient animals compared with controls given same diet plus B_1 or laboratory chow
Eimeria nieschulz	Rat	Synergistic	Becker & Dilworth (1941)	Significant decrease in oocyst yield in deficient animals
Helminths				
Nippostrongylus muris	Rat	Synergistic	Watt (1944)	Slightly more larvae recovered following primary infection of moderately thiamine-deficient

TABLE 9 (*continued*)

Infectious agent or disease	Host	Response	Reference	Remarks
Nippostrongylus muris (continued)				animals; with superinfection, 25% of larvae recovered from deficient animals compared with 10% from controls
Ascaris lumbricoides	Mouse	Synergistic	Dolin et al. (1958, 1961)	All deficient animals died from doses of eggs not lethal to controls
Ascaris lumbricoides	Guinea-pig	Synergistic	Dolin et al. (1958, 1961)	1500 eggs killed all animals on stock diet and only 38% of those on thiamine supplement

"comparative immunity" conferred on control birds fed diets of mixed grains. In subsequent papers he reported a more severe amebiasis in monkeys receiving autoclaved polished rice diets (1919-20a). Tuberculosis of lungs and viscera, and septicemia from various infectious agents, also proved more common in monkeys made deficient by this regimen (1919-20b). Many small cocci and coliform bacilli were found in blood cultures of thiamine-deficient monkeys, and animals with chronic amebiasis developed acute clinical disease with induction of the deficiency.

Other studies on pigeons soon followed. Birds on thiamine-deficient diets proved more susceptible to *S. typhimurium*, *S. enteritidis*, *Esch. coli* (Findlay, 1923b; Barlow, 1930), and *B. anthracis* (Werkman, 1923a). Whether death was from infection or from acute beriberi is not made clear. In other early work (Corda, 1923), pigeons fed polished rice diets proved highly susceptible to anthrax; death was avoided by the addition of asparagus to the diet as a source of thiamine.

Rats fed low-thiamine diets *ad libitum* showed similar results. Infection with *Diplococcus pneumoniae* (Robinson & Siegel, 1944), *Mycobacterium lepraemurium* (Badger et al., 1940), and *Borrelia persica* (Guggenheim & Halevy, 1952) were all synergistic with severe deficiency. Mild deficiency did not affect the fatality from *Borrelia persica* infection. In contrast to pantothenate deficiency, severe thiamine deficiency did not render rats susceptible to *Corynebacterium kutscheri* (Seronde et al., 1956).

Wooley & Sebrell (1942) were among the first to use both *ad libitum* and pair-fed controls. Control mice infected intranasally with *Diplococcus pneumoniae* had a lower fatality ratio than thiamine-deficient animals similarly infected in experiments using both types of control. The results clearly indicate that the synergistic interaction of thiamine deficiency and bacterial infection is more than something attributable to diminished food intake. This was subsequently confirmed in rats infected with *Diplococcus pneumoniae* (Robinson & Siegel, 1944; Wertman & Groh, 1959).

Physicians who survived Japanese prisoner-of-war camps in the Second World War vividly describe the many deaths among thiamine-depleted

persons who contracted clinical bacillary dysentery (Burgess, personal communication, 1962). Systematic observations were never published. Whether these deaths were caused by acute beriberi precipitated by dysentery, or by lowered resistance to bacterial infection remains uncertain. A reasonable assumption is that the two acted synergistically.

In viral diseases, thiamine deficiency is more often antagonistic than synergistic. The effect of thiamine deficiency in inducing greater resistance to poliomyelitis in mice is especially well established. Foster and co-workers (1942) showed that thiamine-deficient mice infected with type 2 poliovirus had a prolonged incubation period, less frequent paralysis, and a lower fatality than mice on unrestricted diets. Further studies (Foster et al., 1944a,b) gave quantitative proof of this protective action, which was maximal in mice on diets 40% deficient in vitamin B_1 for 35 days. At 12 days after virus inoculation, deaths were one-third those in controls. In mice kept on normal diets, paralysis was present in 64% of survivors within 21 days and in 83% after 46 days, whereas 8% of thiamine-deficient mice showed paralysis at 21 days and 17% at 46 days. Results were similar when animals were restricted to 40% of their usual dietary consumption in order to simulate the reduced food intake of thiamine-deficient animals. Limited food intake was thus seen to have a definite protective effect which, however, was considerably less than the effect directly attributable to vitamin B_1 deficiency.

These findings were confirmed by Rasmussen and associates (1944a) and Toomey and co-workers (1943-44), who further demonstrated that protection increased as the proportion of dietary thiamine was further decreased. Questionable protection was also noted with excess thiamine. No decreased resistance to poliovirus could be demonstrated in thiamine-deficient monkeys (Clark et al., 1945) or cotton rats (Weaver, 1945).

With a number of other viruses, variations in this clear-cut expression of antagonism were encountered. Thiamine-deficient mice infected with the virus of Western equine encephalomyelitis did not die with clinical symptoms of encephalomyelitis, but with atonia and tremors typical of thiamine deficiency. The interval between infection and death was similar to that of mice on optimum diets and dying from typical Western equine encephalomyelitis; it was distinctly shorter than for uninoculated thiamine-deficient mice. Examination of brains revealed multiplication of virus along with marked histologic evidence of encephalomyelitis. Clinically, therefore, the deficiency of thiamine was protective against symptoms of encephalomyelitis, but pathologically encephalomyelitis progressed uninterruptedly. Apparently, the encephalomyelitis also aggravated the thiamine deficiency, with resultant earlier death (Kearny et al., 1948b).

An age-dependent reversal effect was noted in two instances. When one-day-old chicks were placed on thiamine-deficient diets and infected with avian encephalomyelitis, paralysis developed in from 50% to 97% of animals,

the frequency depending on the severity of the deficiency. However, when two-week-old chicks previously maintained on optimum diets were infected and placed on a thiamine-deficient diet, the interaction was in the opposite direction, namely, antagonistic. Paralysis developed in 63% of deficient chicks, but in 90% of those on an optimum diet (Cooperman et al., 1946).

Sabin and co-workers showed a reversal of what they call "maturation resistance" in mice two to four weeks old and exposed to vesicular stomatitis virus. Intracerebral inoculation produced infection in both old and young mice; only young mice were susceptible to intranasal inoculation (Sabin & Olitsky, 1938). Mice on diets deficient in vitamin B-complex, thiamine, vitamin E, or total calories had a delayed development of this normal resistance to peripheral infection, with differences most clear-cut at four weeks of age. The delay occurred both when suckling mothers were placed on deficient diets and when the young mice were weaned on these diets (Sabin & Duffy, 1940). Further experiments extended the observations to a similar delay in development of maturation resistance to peripheral inoculation with the viruses of Eastern equine encephalomyelitis, Western equine encephalomyelitis, and St Louis encephalitis (Sabin, 1941).

In addition to rickettsiae generally, and viruses occasionally, the filterable agent of psittacosis also shows synergism with thiamine deficiency. Once classed as a virus, although always recognized as possessing irregular features, this infectious agent is now characterized as a member of the newly designated bedsoniae. The whole range of water-soluble vitamins was tested in extensive studies using mouse fibroblast cultures infected with psittacosis (Bader & Morgan, 1961). Pigeons that were previously asymptomatic carriers of the infectious agent succumbed to acute illness 6 to 12 days after being placed on thiamine-deficient diets (Pinkerton & Swank, 1940). Tissue cultures showed the psittacosis agent to be highly dependent on thiamine for growth. For maximal growth, pantothenate, niacin, pyridoxine, and choline were also needed, but not biotin, inositol, folic acid, or riboflavin.

Thiamine and pyridoxine deficiency of tissue cultures have proved antagonistic to the viruses of mumps and influenza (Cushing & Morgan, 1952). In chick-embryo tissue cultures, the thiamine analogue oxythiamine completely inhibited growth of mumps virus and partially inhibited growth of influenza virus. The vitamin analogue was added 24 hours after introduction of the viruses; hemagglutination titers were the means of evaluation.

In addition to its effect on clinical amebiasis in monkeys, referred to above, thiamine deficiency has also been observed to increase susceptibility to a number of other protozoa: *Trypanosoma cruzi* in rats (Yaeger & Miller, 1960a), *Trypanosoma brucei* in pigeons (Sollazzo, 1929), and *Plasmodium lophurae* in chickens (Seeler & Ott, 1946). In the last two studies, excess thiamine also increased parasitemia, although the effect was much less with an excess of the vitamin than with a deficiency.

Both moderate thiamine deficiency and complete depletion retarded multiplication of *Plasmodium gallinaceum* in the chicken (Rama Rao & Sirsi, 1956); depletion, however, so seriously affected the host as well that earlier death resulted. Doses of thiamine too small to aid parasite multiplication were sufficient to improve host resistance and postpone death; large doses accelerated parasitemia and hastened death.

Fewer trophozoites of *Giardia muris* were present in mice on thiamine-deficient diets than in controls on the same basic diet but supplemented with thiamine or fed laboratory chow (Scholtens, 1962). Similarly, oocyst yield decreased in thiamine-deficient rats infected with the sporozoa *Eimeria nieschulzi* (Becker & Dilworth, 1941). Combined thiamine and pyridoxine deficiency reduced oocyst counts even below those usual in thiamine-deficient animals, whereas pyridoxine deficiency alone increased the counts. After recovery from infection, rats had an immunity to it although they remained thiamine deficient.

Thiamine-deficient mice were killed by doses of *Ascaris lumbricoides* eggs not lethal in control animals (Dolin et al., 1958; Dolin, 1961). Findings with guinea-pigs were similar, in that all thiamine-deficient animals succumbed to doses of 1500 eggs, compared with a fatality ratio of 38% for animals on the same diet with the addition of a supplement of thiamine.

From the observations cited, thiamine deficiency is seen to worsen most infectious diseases. Antagonism is the commonest interaction, however, in systemic viral and protozoal infections of laboratory animals suffering from severe thiamine deficiency.

Riboflavin

Riboflavin deficiency is synergistic with most infectious diseases, as shown by the studies summarized in Table 10. Kligler and co-workers (1944) demonstrated that riboflavin-deficient mice were more susceptible to spontaneous salmonellosis in an animal colony than were mice having adequate supplies of this nutrient. Decreased food intake was probably a factor, since susceptibility in pair-fed controls was intermediate between that of *ad libitum* controls and that of deficient animals. Wooley & Sebrell (1942), investigating effects of riboflavin deficiency on *Diplococcus pneumoniae* infections in mice, and Wertman and co-workers (1957), studying the same infection in rats, found deficient animals more susceptible than either *ad libitum* or pair-fed controls, both of which had an equal susceptibility.

The single known study on riboflavin deficiency in man concerns pellagrous patients who suffered from frequent stomatitis due to *Streptococcus pyogenes*. However, other B-complex vitamins may also have been involved (Riddle et al., 1940). The justification for considering the circumoral lesions to be associated specifically with riboflavin deficiency was their disappearance following administration of this vitamin alone.

TABLE 10. EFFECTS OF RIBOFLAVIN DEFICIENCY ON INFECTIOUS DISEASE

Infectious agent or disease	Host	Response	Reference	Remarks
Bacteria				
Salmonella Sp.	Mouse	Synergistic	Kligler et al. (1944)	Deficient animals more susceptible to spontaneous infections than *ad libitum*-fed controls; pair-fed controls had intermediate susceptibility
Salmonella dysenteriae	Rat	No effect	Sporn et al. (1950)	No increase in deaths of deficient animals following oral inoculation
Streptococcus pyogenes *Micrococcus pyogenes* var. *aureus*	Man	Synergistic	Riddle et al. (1940)	Infected lesions around mouth of "pellagrous" patients cleared by treatment with riboflavin
Diplococcus pneumoniae	Mouse	Synergistic	Wooley & Sebrell (1942)	Deaths following intranasal infection greater in deficient animals than in *ad libitum* or pair-fed controls
D. pneumoniae	Rat	No effect	Robinson & Siegel (1944)	No effect on resistance to intratracheal inoculation
D. pneumoniae	Rat	Synergistic	Wertman et al. (1957)	Animals receiving deficient diet highly susceptible compared with *ad libitum* or pair-fed controls
Rickettsia				
Rickettsia prowazekii	Rat	Synergistic	Fitzpatrick (1948)	63% fatality with diet very low in riboflavin; no fatality in controls
Murine typhus fever	Rat	Synergistic	Pinkerton & Bessey (1939, 1942)	Heavier infection of Kupffer cells of liver and rapid death in deficient rats; no deaths among controls
Virus				
Mouse encephalomyelitis (Theiler)	Mouse	No effect	Rasmussen et al. (1944b)	No change in resistance of deficient animals to intraperitoneal or intracerebral inoculation
Poliovirus, type 2	Mouse	Antagonistic	Rasmussen et al. (1944b)	Slight but consistent increase in resistance of deficient animals to intracerebral inoculation
Protozoa				
Trypanosoma cruzi	Rat	Synergistic	Yaeger & Miller (1960b)	Peak of parasitemia developed earlier in deficient animals
Helminths				
Nippostrongylus muris	Rat	Synergistic	Watt (1944)	Higher fatality rate in deficient animals after primary infection with 2500 larvae; after secondary infection, 60% of larvae recovered from deficient animals compared with less than 6% from controls