

## DETERMINANTS OF THE EFFECTS OF NUTRITION ON INFECTION

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### Introduction

Nutritional factors can modify the characteristics of both host and infectious agent. An organism's response to nutritional influences is conditioned by such innate host features as genetic make-up, age and physiologic state, and by the presence of a complicating illness.

The direct effect of malnutrition on host resistance is well documented. Nutritionally induced changes in antibody formation, phagocytic activity, tissue integrity, inflammatory response, intestinal flora, endocrine metabolism, and non-specific protective mechanisms have been studied intensively by investigators in a variety of fields. Their findings are reviewed below.

To understand better the antagonism sometimes existing between a specific nutrient deficiency and an infection, attention is also given to the metabolic requirements of infectious agents.

### Innate Characteristics of Host and Agent

Host characteristics, including resistance to infection, are basically the result of genetic variation and environmental influence. The important consideration is that resistance is dependent on an interaction of the two, an interaction that may be recent or remote. In interpreting the nutritional influences on innate resistance, it is thus necessary to take into account the age, sex, and physiologic and pathologic states of a person.

The more that is learned of environmental effects, and especially diet, the more significant these influences appear in accounting for differences among people that were once considered wholly inborn. Resistance may be expected to be highest when a favorable inheritance is combined with good nutrition; and greatest susceptibility is anticipated in persons with poor heredity and severe malnutrition. Between these two extremes, dietary

factors often become highly important. Physiologic variables can influence nutritional status and hence resistance to infection. For example, owing to the increase in nutritional requirements during pregnancy and lactation, a diet that was previously satisfactory often becomes barely adequate, and hence susceptibility to infection is increased.

To tabulate the many pathologic states capable of diminishing resistance of the host would not serve any useful purpose. The subject has been extensively reviewed in *Natural Resistance and Clinical Medicine*, by Perla & Marmorston (1941). Typical examples are the debilitating effect of cancer (so extensive that the eventual cause of death is commonly a secondary infection), the infections directly associated with accidental or surgical trauma, and the respiratory infections so common in persons with pneumoconiosis.

In summary, the innate resistance of animal hosts to infection is determined by many factors. The interaction of the genotype with a constantly changing environment leads to the result that the organism is progressively renewing itself yet constantly aging. As a consequence, nutritional influences on innate resistance must of necessity be interpreted in the light of a changing individual.

The microbic agents of naturally occurring infections are also affected by genetic variations, a basic biologic fact sometimes ignored in experimental studies. Laboratory animals, themselves often highly inbred, are frequently infected with a relatively homogeneous strain of micro-organism. Under such circumstances, the infectious agent may be either so virulent or so attenuated that the outcome cannot be influenced by dietary manipulation. Similarly, the host may be genetically so susceptible or so resistant that neither his nutritional state nor variation in virulence of the agent will affect the outcome. Conclusions derived from experiments conducted under these conditions, therefore, may have little or no direct application to what happens in heterogeneous general populations of animal or man.

That both innate resistance of the host and virulence of the infectious agent are governing factors in determining the effects of dietary deficiency on resistance is well illustrated by the classical experiment of Schneider (1950) (Fig. 3). He chose three host genotypes corresponding to selected resistant, selected susceptible, and unselected strains of mice, and exposed each of the three to three different cultures of salmonella—one composed entirely of a virulent genotype, another that was uniformly avirulent, and the third a mixed culture containing both virulent and avirulent genotypes. Taking survival or death as the criterion, the nine genetic combinations of host and agent, each tested with an adequate and an inadequate diet, gave rise to one of three results:

1. When natural resistance was high in relation to the virulence of the agent, disease did not result no matter how the diet was varied.

FIG. 3. THE EFFECT OF A NATURAL (N) AND A SYNTHETIC (S) DIET ON SURVIVAL FOLLOWING INFECTION IN NINE DIFFERENT GENETIC CIRCUMSTANCES

		Host Genotype		
		Inbred, selected, resistant	Random-bred (outbred) non-selected	Inbred, selected, susceptible
Pathogen-Genotype	Uniformly virulent	N-Died S-Died	N-Died S-Died	N-Died S-Died
	Mixed virulent and avirulent	N-Survived S-Survived	N-Survived Dietary effect S-Died	N-Died S-Died
	Uniformly avirulent	N-Survived S-Survived	N-Survived S-Survived	N-Survived S-Survived

From H.A. Schneider (1950) *Strategic concepts in epidemiology*. In: *Biological Foundations of Health Education, Proceedings of the Eastern States Health Education Conference, April 1-2, 1948*, New York, Columbia University Press, p. 145.

2. When natural or constitutional resistance of the host was low in relation to the virulence of the agent, severe infectious disease ensued regardless of nutritional factors.

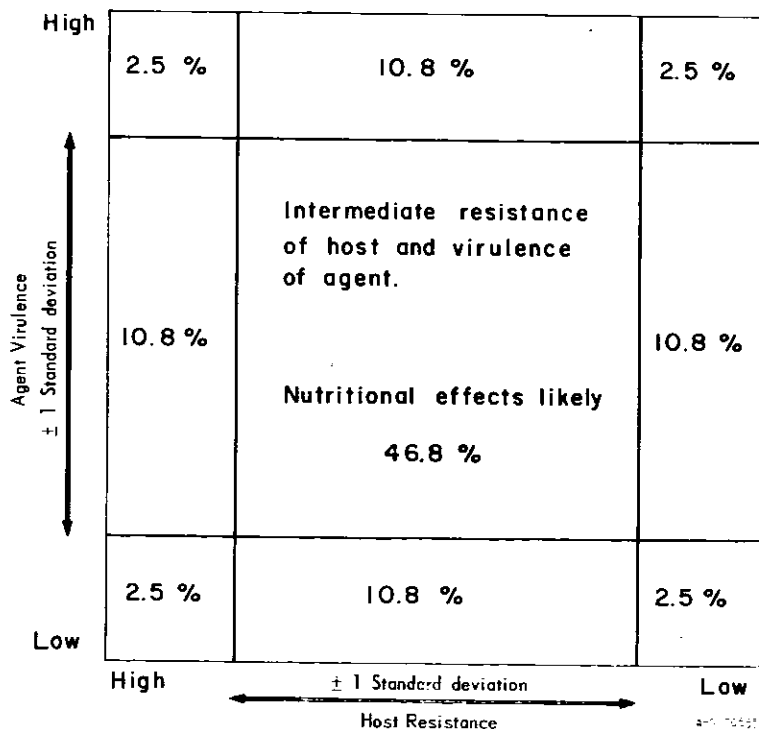
3. When there was an equilibrium between natural or constitutional resistance and the virulence of the agent, nutritional factors did affect the outcome of the infection.

The fact that only one of the nine test groups presented in Fig. 3 showed this effect does not mean that this proportion reflects the usual situation in nature. From a public health point of view, the contrary is true. The results were correctly interpreted by the author, but they have been misused to derogate the importance of nutrition in resistance to infection. In only one of the test groups did the situation correspond to that characteristic of a general population; this was also the only one showing an effect of diet.

With a normal distribution of agent virulence and innate host resistance in a population, as usually results when there is an ecologic balance, the most frequent situation will be an intermediate agent virulence combined with an intermediate host resistance. If normal distributions are assumed both for virulence of the infectious agent and for host resistance, and if the

extremes of virulence, avirulence, resistance and susceptibility are arbitrarily defined as lying outside the range  $\pm 1$  standard deviation, it is possible to construct the distribution diagram shown in Fig. 4. From this it can be seen that diet should then influence the severity of infectious disease in nearly 50% of cases, whereas the four extreme combinations in Fig. 3 together account for only 10% of cases.

FIG. 4. SCHEMATIC REPRESENTATION OF PROBABLE DISTRIBUTION, IN NATURE, OF AGENT VIRULENCE AND HOST SUSCEPTIBILITY, AS SHOWN IN FIG. 3, INDICATING THEORETICAL PERCENTAGES OF POPULATION SHOWING DIET EFFECT



A shift in the distribution of either agent virulence or host resistance would result in greater or smaller numbers of cases in which no nutritional effect would be expected. If the range virulence—avirulence is arbitrarily taken as one and a half or even two standard deviations on either side of the mean, a more likely circumstance in nature, the proportion of a population affected by diet would be even greater than indicated in Fig. 4.

On theoretical grounds, the deduction clearly follows that the nutritional state of an organism is often the deciding factor in a particular infection. Although there will undoubtedly be some cases in nature in which nutri-

tional status will have no effect on the outcome of infection, the tendency in laboratory experiments is for such situations to occur with exaggerated frequency. Unfortunately, no other investigator of the effect of malnutrition on resistance has explored the reasons for observed results as thoroughly as Schneider (1950, 1956).

As already pointed out, the failure in many instances to demonstrate an effect of nutrition on infection was due to the fact that the virulence of the infectious agent or the innate susceptibility of the host was too extreme to permit any other result. This observation has important practical implications. Experiments on the interaction of nutrition and infection that give a negative result are usually much less relevant to practical problems in public health than are those that show a significant relationship. On the other hand, the data presented in the preceding chapter suggest that, even when conditions are sufficiently favorable to end in demonstration of an effect of diet on resistance, some nutrient deficiencies are more likely than others to exert that influence. Furthermore, a deficiency must always be relatively pronounced in order to produce a significant result.

At the beginning of this discussion, the statement was made that genetic variations in virulence of infectious agents are closely related to variations in their metabolic requirements. The particular characteristics of the agent often determine that a given deficiency has a greater effect on agent metabolism than on mechanisms of host resistance. Furthermore, the degree of antagonism between an infectious agent and a nutrient deficiency varies greatly among different genetic strains of a micro-organism, as suggested by the tables in Chapter 3.

Some infectious agents are today mainly restricted to the tropics and subtropics, the same regions in which malnutrition is commonly prevalent. This enhances the opportunity for interaction. It also makes difficult the separation of the synergistic effects of infection and malnutrition from influences such as coexisting ignorance, poverty, and poor environmental sanitation. For example, *Entamoeba histolytica* is widely distributed in man, but causes serious disease mainly in tropical areas.

In summary, the outcome of a potential interaction between malnutrition and infection depends in part on innate host factors, such as age and genotype, and in part on abnormal physiologic states, metabolic disorders, and acquired immunity. Interaction also depends on the genetic constitution of the infectious agent. If the agent is uniformly highly virulent or uniformly avirulent, the effect of diet may be minimal. In the same way, the innate resistance of the host may be so slight or so great that diet is of secondary importance. In the ecologically balanced populations characteristically present in nature, the situation is an intermediate level of both virulence of agent and resistance of host, with the result that diet often determines the outcome. Dietary factors thus have greater significance in nature and in public health practice than some laboratory experiments suggest.

### Synergistic Action of Nutritional Deficiencies

#### Antibody formation

The antigen-antibody relationship and its associated specific immunity is the best known and most extensively studied mechanism of resistance to infection. Most infectious agents either contain or discharge one or more protein molecules capable of stimulating production of specific antibodies by a host. Rarely, the antigenic substance is a carbohydrate. Antibodies have the capacity to bind or neutralize antigen; the infectious agent is thereby rendered more susceptible to phagocytosis or other resistance mechanisms, or is directly damaged so that its ability to harm the host is reduced or neutralized. Since, historically, recognition of the importance of immunity, of essential nutrients, and of nutritional deficiency disease came at much the same time, the influence of nutritional deficiencies on antibody production attracted early attention.

#### *Vitamin deficiencies*

One of the first attempts to demonstrate an effect of B-complex deficiency on antibody production was through injection of killed typhoid bacilli into rats; it was unsuccessful for reasons still not clear (Zilva, 1919). In other early experiments, Werkman (1923a,b) and Werkman and associates (1924a,b) induced deficiencies of vitamin A, ascorbic acid, or B-complex in rats, rabbits, and pigeons and failed to show that the vitamin deficiency had any effect on subsequent antibody response. Erythrocytes, killed typhoid bacilli, anthrax bacilli, and pneumococci were among the antigens used. Agglutinins, precipitins, hemolysins, and bacteriolysins were measured. In view of subsequent investigations, the failure to observe a nutritional effect on antibody production is difficult to understand; some of the deficiencies were sufficiently severe to have affected resistance. Vitamin C would not, of course, have been expected to have an effect, since none of the animals used has an obligatory requirement for ascorbic acid.

Scorbutic guinea-pigs were found to have markedly reduced skin reactions to *Corynebacterium diphtheriae* toxin introduced either intracutaneously or subcutaneously (Arkwright & Zilva, 1924). This was confirmed by Bieling (1925), who viewed it as a partial explanation of the previously observed greater susceptibility of scorbutic guinea-pigs to large doses of diphtheria toxin. Even in guinea-pigs with a minimal deficiency, hemorrhage and necrosis were more marked at the site of injection; and survival time was reduced by half (King & Menten, 1935).

When killed typhoid bacilli were injected into rats fed a diet deficient in vitamins A and D, both agglutinin and bacteriolysin response were less than in controls (Blackberg, 1927-28). With living organisms, the difference

between deficiency and control groups was somewhat less marked. In 1933, Greene reported that vitamin-A-deficient rabbits responded with lower average antibody titers to injection with sheep or ox erythrocytes, but with no loss of agglutinin response to *Salmonella typhi*.

More than a decade passed before attention focused again on this relationship. Stoerk & Eisen (1946) and Stoerk and co-workers (1947) showed that pyridoxine deficiency in rats brought about a striking reduction in growth, and also in antibody response to sheep erythrocytes. Protein, thiamine, riboflavin, or pantothenic acid deficiency, which reduced growth, did not influence antibody production. Agnew & Cook (1949) conducted experiments with pyridoxine-deficient rats inoculated with either sheep erythrocytes or killed cultures of typhoid bacilli containing the "H" antigen. They found a significantly lower antibody response than in either *ad libitum* or pair-fed controls.

In comprehensive investigations of vitamin deficiency and antibody formation, Axelrod and co-workers found a strong reduction in hemagglutinin response to human erythrocytes in rats deficient in pyridoxine and pantothenic acid. Riboflavin-deficient animals exhibited titers that were variable but lower than usual (Axelrod et al., 1947). Pair-fed controls indicated an effect directly attributable to the specific deficiency and not to inanition from reduced consumption of food (Ludovici et al., 1949). The eventual conclusion was that deficiencies of pantothenic acid, pyridoxine, or pteroylglutamic acid severely reduced antibody production in rats, that riboflavin, thiamine, biotin, vitamin A, or niacin-tryptophan deficiencies had a moderate effect, and that deficiencies of vitamins D and B<sub>12</sub> had no demonstrable influence (Ludovici & Axelrod, 1951a,b; Axelrod, 1952, 1953).

The known biochemical role of several of these nutrients accounts for the decrease in protein synthesis and lowered antibody response. Doubt was raised as to whether the antibody effect would hold when the antigen was a virus. Studies with influenza virus in rats confirmed the previous general result. Both pantothenic acid and pyridoxine deficiencies caused strong and specific diminution of antibody production, but thiamine deficiency had no effect (Axelrod & Hopper, 1960). Diphtheria toxoid produced similar results when injected intraperitoneally (Axelrod & Pruzansky, 1955; Pruzansky & Axelrod, 1955). Impairment was also observed with biotin or vitamin D deficiency.

In an excellent summary of these studies, Axelrod (1958) emphasized that simple inanition failed to modify antibody production and that vitamin deficiencies interfered equally with primary and secondary responses. Subsequently, both young and mature guinea-pigs were rendered pyridoxine-deficient, either by administering the antagonist deoxypyridoxine or by feeding a highly purified pyridoxine-free diet. The result with diphtheria toxoid was decreased formation of circulating antibody and a less marked early Arthus-type skin hypersensitivity (Axelrod et al., 1961).

Several other laboratories studied these problems at about the same time, with various results. Rats fed one-tenth of presumed optimal amounts of thiamine, pantothenic acid, pyridoxine, niacin, and riboflavin exhibited no effect on the production of complement-fixing antibodies following large doses of the rickettsiae of murine typhus fever. However, small amounts of antigen resulted in a depressed antibody response in rats deficient in pantothenic acid and thiamine (Wertman & Sarandria, 1951a,b). Subsequent experiments showed impairment in rats deficient in riboflavin, as well as in vitamin B<sub>12</sub> and folic acid, when compared with either pair-fed or *ad libitum* controls (Wertman & Sarandria, 1952; Wertman, Crisley & Sarandria, 1952) and also in niacin-tryptophan-deficient animals (Wertman, Smith & O'Leary, 1954). Despite inconclusive data, claims have been made that administration of pantothenic acid improved antibody response when typhoid-paratyphoid vaccine was administered to rabbits (Meyer et al., 1955b, 1956) and also in patients with natural paratyphoid infection (Meyer et al., 1955a).

Zucker & Zucker (1954) observed that as many as half of the rats consuming a diet deficient in pantothenic acid died spontaneously from *Corynebacterium pneumoniae*, an organism not pathogenic to well-nourished rats. Inanition as a contributing factor was ruled out by pair-feeding. The break in resistance occurred even before physical signs of deficiency were apparent (Seronde, 1954). Further study showed that young rats deprived of pantothenate gradually lost their species resistance to a strain of *Corynebacterium kutscheri* originally isolated from a spontaneous pseudotuberculous lesion in a deficient animal (Zucker et al., 1955; Seronde et al., 1955). Neither low calories nor low thiamine intakes altered the ability of young rats to form agglutinins following administration of a vaccine made from a killed culture of the organism (Zucker et al., 1956; Seronde et al., 1956). No detectable agglutinins, however, were formed in pyridoxine-deficient animals.

In pantothenic acid deficiency, the capacity to form agglutinating antibodies was lost by some deficient animals and impaired in others, with the net effect an intermediate result about the same as that observed with deficiencies of thiamine and pyridoxine. It was concluded, however, that the ability to produce agglutinins does not influence the degree of resistance of rats to the live organism (Zucker et al., 1956).

Chicks fed diets partially deficient in either vitamin A, pantothenic acid, or riboflavin had a significantly lower agglutinin response to *Salmonella pullorum* than did controls (Panda & Combs, 1963). The deficiencies had no effect on thymus, spleen, and adrenal weights, although the weight of the bursa of Fabricius averaged less than in controls. Average eight-week gains in weight were approximately 1500 g for controls and for vitamin-A-deficient birds, and 1377 g and 1115 g, respectively, for those fed diets deficient in pantothenic acid or riboflavin. The results suggest that the need

for these nutrients was greater for optimum antibody production than for good growth.

Research workers in the USSR have had similar results. In guinea-pigs given thiamine, precipitin antibody response to *Ascaris* larvae was greater than in animals on a stock diet (Dolin et al., 1958; Dolin, 1961). Leutskaja (1964a) immunized chickens with an antigen from *Ascaridia galli* and obtained antibody levels 25% to 50% lower in vitamin-A-deficient animals than in well-fed controls.

Feller and associates (1942) kept five adult male patients on diets deficient in vitamin A or C and measured neutralizing antibodies and complement-fixation titers *in vitro* against influenza virus from infected mouse lung. In these studies the vitamin deficiencies had no observed effect.

Severe ascorbic acid depletion in guinea-pigs (Klimentova & Frjazinova, 1965) failed to influence the production of complement-fixing antibodies against *Rickettsia prowazeki* var. *typhi*, or the production of diphtheria antitoxin as determined in regional lymph nodes or blood serum. However, the administration to rabbits of 10  $\mu$ g of vitamin B<sub>12</sub> per kilogram of body-weight every other day or 25  $\mu$ g per kilogram every five days significantly increased the average tetanus antitoxin titers of animals immunized with toxoid, compared with the response in controls (Tashmukhamedov, 1965).

Pigs on a vitamin-A-deficient diet after early weaning had a reduced antibody response to *Salmonella pullorum* until the deficiency was corrected (Harmon et al., 1963a). Similar results were produced by diets deficient in pantothenic acid, pyridoxine, and riboflavin (Harmon et al., 1963b). A vitamin-A-deficient diet did not, however, influence antibody formation against swine influenza virus in mice, although supplementation of the deficient diet with vitamin A increased resistance to the disease (Underdahl & Young, 1956).

Hodges and co-workers have demonstrated conclusively that vitamin deficiency in man can interfere with antibody response. Adult young men consuming a diet deficient only in pantothenic acid exhibited impaired antibody production in response to tetanus antigen but not to typhoid antigen. The addition of the antagonist omega-methylpantothenic acid abolished rather than augmented the effect. This led to speculation that the antagonist may have acted as an active vitamin for this particular function (Hodges et al., 1962c).

Pyridoxine deficiency, however, with or without administration of the antagonist, slightly impaired antibody response to both tetanus and typhoid antigens (Hodges et al., 1962d). Five young men were given a diet deficient in both pantothenic acid and pyridoxine, plus the antagonists, until clinical signs of deficiency appeared. At this stage, blood serum did not agglutinate either tetanus or typhoid "O" antigen, and only an insignificant reaction occurred with typhoid "H" antigen. The antibody rise was normal after the deficient vitamins were included in the diet. By contrast, all five men,

when still deficient, responded strongly to immunization with poliomyelitis antigens (Hodges et al., 1962e). There was no impairment of antibody production in subjects in whom only minor combined pyridoxine and pantothenic acid deficiency was allowed to develop.

Few studies have measured antibody response in man under conditions of natural vitamin deficiencies. Morey & Spies (1942) compared agglutinating titers following administration of a suspension of several strains of *Pasteurella tularensis* in 17 patients with mild deficiency disease with those in 31 patients showing signs of severe pellagra or moderately severe multiple vitamin-B-complex deficiency. The response was slower and maximum titers were much lower in the markedly deficient patients. Jayalakshmi & Gopalan (1958) described several previously reported false-negative tuberculin reactions in malnourished persons and added 20 cases of their own.

The possibility of diminished response to protective immunization among poorly nourished people must be taken into account by public health authorities, but is not of itself sufficient reason to postpone needed immunization.

#### *Protein and amino acid deficiency*

The interference of protein and amino acid deficiencies with antibody production is well documented. An early observation of differences in disease incidence between well-nourished and poorly nourished Africans led Orr and co-workers (1931) to conduct a comprehensive study of agglutinin reactions in malnourished sheep. They tested 80 castrated animals, divided into 16 groups, for the hemolyzing power of blood serum on rabbit red blood cells, its bacteriolytic effect on *Escherichia coli* and *Salmonella choleraesuis*, and its agglutinating properties with *Brucella abortus* and *S. schottmuelleri*. Responses were inversely proportional to the physical and nutritional status of animals maintained on protein-deficient diets. An improvement was observed with protein supplementation or when the animals were shifted to green pasture.

Madden & Whipple (1940) systematically gathered experimental evidence on the consequences of lowering plasma proteins. Dogs depleted by plasmapheresis exhibited a reduced ability to form specific antibodies and were more susceptible to infection (Miller et al., 1940). This could be reversed by feeding more protein and discontinuing the plasmapheresis. A dynamic equilibrium was shown to exist between circulating plasma proteins, including gamma globulins, and the proteins of the tissue cells (Whipple & Madden, 1944; Yuile et al., 1951; Bent et al., 1952).

Cannon (1942, 1945a) was among the first to emphasize the need for dietary protein in the synthesis of the antibody-containing gamma globulin fraction of blood plasma. Both young and adult rabbits rendered hypoproteinemic (Cannon et al., 1943) had a decreased capacity to produce

agglutinins to *Salmonella typhi* and *S. paratyphi*. Attention was paid to the speed of secondary immunologic response as being a more significant index of resistance than the mere presence of circulating antibodies (Cannon, 1945b).

Wissler and associates (1946b) found protein-depleted rats less capable of rapid antibody formation. Restoration of dietary protein increased antibody formation. These observations were extended by Wissler (1947b) to include intradermal injection of pneumococcus type 1 into protein-depleted rabbits. Not only was agglutinin response poorer in the deficient animals, but local lesions spread more rapidly and caused death. Similar results were obtained with rats, although the differences were not as marked (Wissler, 1947a). Lowered hemolysin titers to sheep cells in protein-deficient rats could be restored by isocaloric substitution of carbohydrate by food protein, protein hydrolysates, or synthetic amino acid mixtures (Wissler et al., 1946a,b).

Using 100 weanling rats fed various diets and exposed to *Pasteurella tularensis*, Berry and co-workers (1945) concluded that the average agglutinin titer was reduced by 50% in females and 75% in males maintained on basal diets not supplemented with casein. The ability of rats to form antibodies against sheep red blood cells or Friedländer's bacillus decreased progressively with length of time on a low-protein diet (Benditt et al., 1949). Gemeroy & Koffler (1949) also reported diminished precipitin response to beef serum in protein-depleted rabbits. In summarizing these findings, Cannon (1949, 1950) emphasized the potential clinical and public health importance of reduced antibody response due to moderate to severe protein deficiency.

The only known study in which the antigen was other than bacterial is that of Klimentova & Frjaznova (1963), who measured complement-fixing antibodies for *Rickettsia prowazeki* var. *typhi* in protein-deficient rats. Antibody reduction was three-fold in the blood and four- to six-fold in the regional lymph nodes of protein-deficient animals.

Little information exists about the effect of inanition on antibody formation because it is rarely possible to separate inanition from deficiency of protein or other nutrients. Ruckman (1946) has reported the development of neutralizing antibodies against the virus of Western equine encephalomyelitis to be significantly, although not markedly, impaired either in starved or protein-deficient mice.

Antibodies were once assumed to originate from preformed serum protein components specifically modified by contact with antigens. Studies of the direct synthesis of antibodies from isotopically labeled amino acids (Green & Anker, 1954; Gros et al., 1952; Taliaferro & Talmage, 1955; Yuile et al., 1951) indicate that a delay in production may be due to the need for antibody-synthesizing enzymes rather than to the formation of precursors. In either case, specific amino-acid or over-all protein deficiency would be the factor interfering with antibody production.

Cannon (1942, 1945a, b, 1949) and Cannon and co-workers (1944) presented evidence that synthesis of antibodies occurred in phagocytes with the following five essential amino acids particularly involved: lysine, methionine, tryptophan, threonine, and leucine. He and his associates proposed that a template protein was synthesized and retained in particular macrophages, the result being a rapid response to a reinforcing dose of antigen.

In *Nippostrongylus muris* infection of rats, lysine deficiency restricted an increase in the gamma globulin of blood serum. When compared, lysine but not methionine deficiency was found to produce not only an altered gamma globulin synthesis, but also loss of resistance, as shown by egg counts, hemoglobin levels, and other evidence of infection (Barakat, 1950). An extension of the theory of antibody formation (Schweet & Owen, 1957) led to the concept that antigens, in penetrating the nuclei of certain phagocytes, produce a primarily genetic change by altering the deoxyribonucleic acid molecule. As a consequence, new ribonucleic acid templates are formed and carried by further multiplication into daughter cells. This is an elaboration of the template concept of Cannon (1942).

The sequence of cell types involved in the process was studied in rats (Cannon et al., 1944; Wissler et al., 1957), typhoid bacilli, sheep erythrocytes, and *Klebsiella pneumoniae* being used as antigens. The development in two to four days of specifically sensitized antibody-forming cells from macrophages could be blocked by radiation, by protein depletion, and by specific amino acid analogues. Many other workers have demonstrated synthesis of antibodies within the reticuloendothelial system (Livieratos et al., 1954; Stavitsky, 1954) and shown that it could be blocked by amino-acid deficiencies (Cannon, 1945a,b; Stavitsky, 1957). The latter investigators used deficiencies produced by the amino acid analogues parafluorophenylalanine and gamma-ethylamidoglutamic acid.

Almost no work has been done on the extent to which human populations actually suffer through failure of antibody formation attributable to protein deficiency. The earliest clinical reference we have found was that of Krebs (1946), who described a single malnourished person with serum proteins of 3.1 g/100 ml and a serum albumin level of 2.0 g/100 ml. This person showed no antibody response to commercial typhoid vaccine. With a high protein diet, however, gamma globulin levels rose from 0.15 to 0.68 g/100 ml.

Gell (1948) had an opportunity to test the antibody response of 57 severely malnourished survivors of a German concentration camp at the close of the Second World War. Although he chose such unlikely antigens as tobacco mosaic virus and avian red cells, responses were much less among the malnourished than among 16 well-nourished British soldiers.

Wohl and associates (1949) followed the antibody response to typhoid immunization of 102 patients in a Philadelphia hospital; 88 had original serum albumin levels below 4.0 g/100 ml. The markedly slower response in patients with low albumin was improved when they were given a protein

supplement. Despite the many patients studied and the apparently clear-cut improvement in antibody production with protein supplementation, this report has largely gone unnoticed.

Balch (1950) reported no relation between amounts of serum protein, antitoxin response to diphtheria toxoid, and development of infection in 25 "grossly depleted" patients with terminal illnesses. This study has uncertain value because only 10 of the 25 subjects had total serum proteins below 6.0 g/100 ml and only seven had serum albumin values below 2.7 g/100 ml. Furthermore, his discussion leans heavily on undocumented statements that infection was not especially prevalent among malnourished civilian or prisoner-of-war populations in the Second World War.

Other seemingly negative studies should be mentioned. In infants on *ad libitum* diets, Dancis and co-workers (1953) found no difference in antibody response to a single injection of diphtheria toxoid whether the protein content of the diet was 10% or 20% of the caloric intake. Obviously, neither group of infants was protein deficient, since they consumed 3.0 g and 5.5 g, respectively, of protein per kilogram of body-weight per day.

A similar criticism applies to the finding that the ability to produce antitoxin after diphtheria toxoid was the same in 15 seriously wounded patients as in 11 healthy controls (Havens et al., 1954). Serum total protein levels were at the relatively high average of 6.8 g/100 ml in the experimental group and 7.4 g/100 ml in the controls. Only one patient had serum protein below 6.4 g/100 ml. Albumin levels averaged 3.3 g/100 ml in the wounded patients compared with 4.3 g/100 ml in controls.

Recent papers entitled "Antibody response in children with protein malnutrition", by Pretorius & De Villiers (1962), and "Serum antibody response of malnourished children as compared with well-nourished children", by N.A. Fernández (1960), report no differences in antibody response. The studies were carefully conducted to compare the agglutinin response to "H" and to "O" antigens of *Salmonella typhi* among children treated for kwashiorkor. They are particularly misleading, however, since they deal with patients receiving vigorous dietary therapy and hence no longer deficient in amino acids available for protein synthesis. Unpublished INCAP studies of antibody production in children under treatment for kwashiorkor gave similar results.

The ability of children with untreated kwashiorkor to form antibodies is clearly impaired or even completely inhibited. When Budiansky & Da Silva (1957) maintained children with severe protein malnutrition on a poor diet throughout an experimental period, antibody response to typhoid vaccine was inhibited. In another experiment, Olarte and associates (1956) obtained the same results when diphtheria toxoid was the antigen. Similarly, Reddy & Srikantia (1964) showed that four of seven children with kwashiorkor given 30 g of protein per day and one of three receiving 50 g failed to develop antibodies to typhoid-paratyphoid vaccine. Of those showing an

antibody response, titers were higher with 50 g than with 30 g of dietary protein.

Brown & Katz (1965) compared the antigenic response of children with kwashiorkor admitted to a pediatric ward with that of children convalescing from tuberculosis and well nourished at the time of study. They found no impairment of antibody response to attenuated type 1 oral poliovirus vaccine or of clinical response to smallpox vaccination (Brown & Katz, 1966b). Details of diet are not given. They did note a complete absence of seroconversion at periods ranging from 5 to 14 days after administration of these vaccines in eight children with kwashiorkor compared with a positive response in four of six controls. In subsequent work, children whose only sign of malnutrition was retarded growth showed impaired conversion of Mantoux tests after being given BCG (Harland & Brown, 1965; Harland, 1965). Immunologic response to live measles vaccine administered with gamma globulin appeared normal (Brown, personal communication, 1965). Brown and Katz (1966a) have since described the results of administering the 17-D strain of yellow fever vaccine to eight children with kwashiorkor. None of the eight developed antibodies, while six well-nourished control children had a pronounced antibody response following the same procedure.

When visiting pediatricians in a number of developing areas, we have been told that children with kwashiorkor do not ordinarily react to tuberculin skin tests until after dietary treatment and partial recovery, even when tuberculous joints or pulmonary tuberculosis are found to be present. Until such impressions are confirmed by additional systematic and controlled studies, we suggest only that clinicians be aware of this possibility. The scientific relevance of the clinical impression arises from the importance attached to the skin test as an indicator of cellular immunity as distinguished from the more commonly measured antibody response.

The well-planned studies of Hodges and co-workers should be given far more weight than the negative results that have been reported. Three pairs of healthy men were fed diets containing approximately 0.1, 1.0, and 2.0 g of protein per kilogram of body-weight. One man from each pair received egg yolk, or egg yolk and skim milk solids, as sources of protein, the other, only skim milk solids. The men receiving the lowest protein intakes were in negative nitrogen balance. Antibody response to tetanus and typhoid "O" and "H" antigens was poor in the man receiving the low-protein diet of skim milk solids, but not in his colleague who ate yolk protein. However, as the quantity of egg yolk protein was further increased, the magnitude of antibody response declined (Hodges et al., 1962a). Large doses of gamma globulin administered intravenously to three other subjects approximately doubled the gamma globulin concentration in the serum, but resulted in a poorer antibody response to typhoid fever "H" and "O" antigens and to Asian influenza vaccine than in control subjects, although the response to tetanus toxoid was unaffected (Hodges et al., 1962b).

Golebiowska and associates (1965) reported that gamma<sub>2</sub> globulins had a tendency to decrease in undernourished Polish children, although this was not the case for gamma<sub>1</sub> globulin or for the production of iso-agglutinins.

In summary, protein deficiency, if sufficiently severe, inhibits normal antibody response. A widespread, but mistaken, impression among clinicians is that protein deficiency sufficient to cause this effect is not seen in human populations. On the contrary, not only is antibody production impaired in the large numbers of children who develop kwashiorkor in the developing countries, but there is also strong evidence that impaired antibody formation is frequent among persons with debilitating illnesses in all countries.

### Phagocytic activity

Phagocytosis is generally viewed as the second major defense against infectious disease. Cells capable of phagocytic activity have a similar site of origin in the reticulo-endothelial system to those responsible for antibody production. Phagocytic cells are primarily the fixed macrophages located in the liver, spleen, and other reticulo-endothelial tissues, and, secondly, the wandering microphages, among which the polymorphonuclear leukocytes are most prominent. Despite a more or less common origin, phagocytic activity and antibody formation are affected differently by nutritional state.

Under some circumstances, although production of antibodies is reduced resistance to infection is maintained (Zucker et al., 1956). In others, poor resistance is evident even when antibody formation seems unimpaired (*Miles 1951a; Kahn et al., 1957*). *Severe undernutrition and concurrent* depletion of protein reserves will eventually lead to a marked atrophy of the liver, spleen, bone marrow, and lymphoid tissues, from which phagocytes originate (Cannon et al., 1944; Livieratos et al., 1954). In the severe protein malnutrition of kwashiorkor, concurrent infection sometimes results in little, or complete lack, of the anticipated leukocytosis (Trowell et al., 1954; Béhar et al., 1956).

Well-controlled animal experiments support the findings described in man. Asirvatham (1948) found that protein-deficient rats lost their ability to respond by leukocytosis to turpentine abscesses. Bone marrow smears showed atrophy of tissue with depletion of myeloid and lymphoid elements. After 18 days of re-feeding, leukocyte response was restored.

Prolonged protein deficiency in rabbits sufficient to cause hypoproteinemia resulted in poor antibody response to *Diplococcus pneumoniae* (Wissler, 1947a) and, also, in an inability of polymorphonuclear leukocytes to phagocytize the coccus. Similar, but less pronounced, effects were noted in protein-deficient rats (Wissler, 1947b).

When Steffee (1950) infected roosters with *Diplococcus pneumoniae* in numbers sufficient to cause death in half the control animals, 28 of the

29 protein-depleted ones died. Total food intake was equal in the two groups. The capacity of the depleted animals to clear the circulating blood of *Diplococcus pneumoniae* was greatly reduced, a result interpreted as due in part to ineffectiveness of the Kupffer cell macrophages of the liver.

Guggenheim & Buechler (1946b, 1948, 1949) stated that protein-deficient diets "invariably" impaired phagocytic regeneration in rats and that the effect was readily reversed by restoring dietary protein. Rats were fed different amounts or kinds of protein and inoculated intraperitoneally with 0.5 ml of a 24-hour broth culture of *Salmonella typhimurium*. Leukocyte counts and the phagocytic index were lower and bacterial counts higher in animals receiving a 3% casein diet than those fed 6%, 9% or 18% of casein. Maize protein at a level of 9% had about the same effect as the 3% casein diet; 9% of protein from egg or meat produced a result more nearly equivalent in bactericidal and phagocytic activity to that observed with 18% casein.

At lower levels of protein intake the effect of protein quality on phagocytic activity is readily measurable in laboratory animals. This demonstration lends urgency to clinical studies of the effect of protein malnutrition in young children in less developed areas. The need is for knowledge of the mechanisms actually operating in these human populations and responsible for the observed greater susceptibility to infection.

The situation in marasmus and inanition is not wholly comparable with that of specific protein malnutrition. In patients in the last stages of prolonged wasting diseases, no decrease was seen in numbers of circulating neutrophils nor in the phagocytic power of the cells against staphylococci (Balch & Spencer, 1954). In marasmus, amino acids mobilized from muscle cells are known to become available to the liver and presumably to the reticulo-endothelial system as well.

Decreased macrophage activity has been reported in deficiencies of vitamin A and ascorbic acid. This is significant in view of the frequent synergism of these deficiencies with a specific infectious disease. Ørskov & Moltke (1928) found that vitamin-A-deficient mice had a reduced ability to remove and destroy *Salmonella* injected intraperitoneally. As a result, the infectious agent accumulated in liver, spleen, and peripheral lymph nodes and frequently gave rise to severe and often fatal septicemia. In his long series of experiments with *Salmonella* in vitamin-A-deficient animals, Lassen (1930, 1931) repeatedly observed the same behavior. He also demonstrated decreased macrophage activity, as measured intraperitoneally and *in vitro*. A combined deficiency of vitamins A and D in rats, sufficient to produce an effect on phagocytosis, also prevented growth of the host (Cottingham & Mills, 1943).

Previously, Findlay & Maclean (1925) observed that in rats fed a diet deficient in vitamins A and D to the point where the animals developed keratomalacia, the blood lost its capacity to kill staphylococci *in vitro*. The

blood leukocyte count was not significantly reduced and the blood serum was apparently not bactericidal for staphylococci; the results were attributed to decreased capacity of leukocytes to achieve phagocytosis.

Turner & Lowe (1930-31) reported no effect of vitamin A deficiency in rats on numbers or proportions of white blood cells in circulating blood, but this means little because they measured neither the response of leukocytes to infection nor phagocytic activity. Among 25 prisoners in Uganda jails receiving only the prison diet and an equal number who received 30 ml of cod liver oil daily for 14 days, Hennessey (1932-33) failed to demonstrate a significant difference in leukocyte counts after subcutaneous injection of *Escherichia coli*. Since both groups were "outwardly healthy" and dietary intake was not recorded, this can hardly be considered a study of the effect of vitamin A deficiency.

Indian workers (Hassan et al., 1947) have reported an inverse relationship between plasma vitamin A levels and mean neutrophilic leukocyte counts in 60 male medical students. They postulated a protective physiologic leukocytosis in vitamin A deficiency, but their data do not justify this conclusion. The average leukocyte counts in five groups, in order of decreasing plasma levels of vitamin A, were 7350, 7130, 7150, 7430, and 8633 per cubic millimeter. The marginally higher average values in students with the lowest plasma levels of vitamin A would seem more likely to have been due to greater frequency of infection in this group.

Scurvy in guinea-pigs has been associated with decreased numbers of granulocytes and a lessened phagocytic power. Irritating substances such as titanium dioxide failed to produce the inflammatory exudate, clouded by pus cells, so characteristic of normal animals (Lawryncowicz, 1931; Nungester, 1951). Leukocytes also showed a marked tendency to rupture and fragment (Nungester & Ames, 1948). The injection of supplemental ascorbic acid into normal mice enhanced phagocytic activity of leukocytes in peritoneal exudates, as indicated by tests with staphylococci (Marcus et al., 1953).

Vitamin B-complex deficiencies also reduce white blood cell activity. Monkeys on diets deficient in B vitamins developed a striking granulocytopenic leukopenia and a concomitant markedly lowered resistance to natural infections (Saslaw et al., 1943). Phagocytosis of *Micrococcus candidus* was reduced by 35% to 40% in rats fed a basal diet deficient in protein, minerals, and B-complex vitamins (Berry et al., 1945). Activity was not restored by adding casein or casein plus minerals to the basal diet, but only by addition of B-complex vitamins.

Wertman and co-workers (1953) described a leukopenia, due mainly to fewer lymphocytes, in thiamine-deficient, B-complex-deficient, and pair-fed rats suffering from inanition. The bone marrow showed a marked relative lymphocytopenia with a proportionate increase in granulocytes. Subsequently, Wertman & Groh (1959) reported no obvious effect on the cap-

acity of leukocytes from thiamine-deficient or inanition control rats to phagocytize *Diplococcus pneumoniae*, despite a greater susceptibility of the deficient animals to this micro-organism.

Riboflavin deficiency also resulted in leukopenia in rats (Shukers & Day, 1943; Wertman et al., 1952, 1957; Wertman & Sypherd, 1960); but, again, the same result occurred in controls suffering from inanition. Comprehensive studies of blood cell response in rats with these deficiencies were initiated in an effort to explain the greater susceptibility to *Diplococcus pneumoniae* (Wertman & Groh, 1959; Wertman & Sypherd, 1960). Two important changes were believed to offer a sufficient explanation: first, a sharply reduced complement activity and, second, a reduced ability of phagocytes to migrate to the site of infection. Although no decrease in numbers of circulating leukocytes was observed, leukocytes were reduced in peritoneal exudates and in exudates from other inflamed areas.

Pyridoxine deficiency, which has such a markedly deleterious effect on antibody production, also reduced numbers of effective phagocytic cells. Mushett and associates (1947) fed chicks, puppies, and monkeys either a pyridoxine-deficient diet or the metabolic analogues deoxypyridoxine and methoxypyridoxine. Chicks developed hypoplasia of the lymphoid elements of the spleen, and dogs and monkeys, leukopenia and microcytic anemia. In accord with other authors, these investigators attributed the leukopenia to atrophy of the reticulo-endothelial system, especially lymph nodes and thymus. Autopsies gave supportive evidence, except that in dogs the ratio of spleen weight to body-weight was increased. Their experiments with pair-fed controls indicated that the leukopenia in rats fed a pyridoxine-deficient diet was due to the vitamin deficiency, not to the associated inanition. Thus, lack of pyridoxine appears more specific in its effect on leukocytes than lack of thiamine or riboflavin.

From experimental studies on monkeys and from clinical observations, Doan (1946) came to the conclusion that folic acid deficiency produces cellular inadequacy in mammalian bone marrow of sufficient extent to interfere with the production of leukocytes and largely to nullify the protective action of antibodies. Wertman and co-workers (1956) found that rats deficient in either folic acid or vitamin B<sub>12</sub> had leukopenia and that the migrating power of their leukocytes in response to an irritant was diminished. Other investigators have had difficulty (Lichstein et al., 1946) in maintaining folic acid deficiency in monkeys (*Macaca mulatta*) because of frequent leukopenia, severe dysentery, and high mortality. On deficient diets, the animals developed a striking granulocytopenia and had a high mortality from spontaneous infections and from experimental infection with group C hemolytic streptococci or influenza virus (Saslaw et al., 1943).

In these studies, reduced phagocytic activity was a more constant and important consequence of malnutrition than leukopenia. A clue to the mechanism is provided by the numerous intracellular enzymes identified in

phagocytes (Bazin, 1956; Cohn & Hirsch, 1960a,b; Hirsch & Cohn, 1964; Braunsteiner et al., 1964; Cohn & Wiener, 1963a,b; Saito & Suter, 1965). DeDuve (1959, 1964) has suggested that in many cell types lysosomes may play a defensive role. Histochemical observations have shown a transfer of basic protein from lysosomes to engulfed micro-organisms within the phagocytic vacuoles (Cohn & Hirsch, 1960a,b). The process by which acid phosphatase and other enzymes concentrate in lysosomes is still a problem. Tejada and associates (1964) found decreased alkaline phosphatase activity of leukocytes in kwashiorkor. A marked loss of acid phosphatase has also been described in the leukocytes of guinea-pigs treated with excess vitamin A (Janoff & McCluskey, 1962).

In summary, existing evidence indicates that a number of nutritional deficiencies, particularly of protein, vitamin A, and ascorbic acid, when sufficiently severe, can interfere with phagocytosis by leukocytes. This action would be expected because of the associated interference with the intracellular enzymes that digest micro-organisms and with the production of antibodies, which are an essential feature of the opsonocytophagic process. These deficiencies, and those of pyridoxine, folic acid, and vitamin B<sub>12</sub>, as well as primary inanition and inanition secondary to thiamine, riboflavin, or other nutrient deficiencies, may also interfere with the ability of the liver, spleen, and bone marrow to produce macrophages and microphages. Aschkenasy (1957), in experiments on rats, found that protein deficiency induced anemia and leukopenia because it deprived the hematopoietic organs of amino acids for new cell formation. The extent to which nutrient deficiencies, through their action on numbers and activity of phagocytes, have clinical and public health significance remains undetermined.

#### **Non-specific protective substances**

Blood serum and body fluids of normal animals have a capacity to kill or inhibit growth of many infectious agents independently of antibodies or phagocytosis. Several non-specific protective substances have been identified.

#### *Properdin*

Properdin is a euglobulin found in the blood serum of all normal animals thus far tested (Pillemer et al., 1954, 1955, 1956). It appears to be associated in some way with natural resistance to many diseases of bacterial, viral, and even protozoal origin (Hunter & Hill, 1958; Finkelstein et al., 1959; Hinz, 1956; Schubart et al., 1964; Blum, 1964). The presence of magnesium is essential to its action. Properdin seemingly functions by combining selectively with polysaccharides of high molecular weight (Wardlaw et al., 1955).

Following administration of bacterial lipopolysaccharides (Landy & Pillemer, 1956), properdin titers were lower in germ-free rats (Gustafsson & Laurell, 1960) and higher in germ-free mice. Removal of properdin eliminated the bactericidal activity of rat blood serum as tested against a variety of bacterial agents (Wardlaw & Pillemer, 1956). Properdin is not wholly independent of the antigen-antibody system since complement is necessary for its activity.

The properdin system is vulnerable to nutritional deficiency at several points — in the formation of the compound itself, in its need for appropriate complement, and in its demand for magnesium. To date, the only published confirmation of nutritional effect is the report of a marked reduction in properdin in rat serum in the presence of a deficiency of pantothenic acid sufficient to interfere with growth and with enzyme activity of the liver (Wiss et al., 1957). Thiamine deficiency sufficient to interfere with growth had no such effect, which would indicate that the results with pantothenic acid were not due to an accompanying inanition. The action of other nutritional deficiencies on the properdin system needs investigation.

### *Interferon*

Interferon is a natural product of animal cells that protects them from attack by a number of viruses (Burke & Isaacs, 1960; Isaacs, 1961, 1963; Isaacs & Hitchcock, 1960; Friedman et al., 1962; Wagner, 1963; Neva & Weller, 1964; Finter, 1964a,b; Glasgow, 1965a,b; Merigan, 1967). It supplements other mechanisms of resistance to viral infection and presumably accounts for some of the resistance to a second viral infection when one virus is already present in cells. Its mechanism of action appears to be through uncoupling of oxidative phosphorylation. Glucose is still metabolized to lactic acid, but the process no longer produces the normal amount of adenosine triphosphate (ATP). Since viruses are unable to multiply within a cell unless plentifully supplied with ATP, the blocking mechanism theoretically interferes with viral replication without depriving the cell of sufficient ATP for its own needs.

The release of interferon appears to be a general reaction of cells to virus infection. Since it is a protein molecule, its formation may well be depressed in nutritional states in which protein synthesis is impaired, but as yet there is no information on this possibility.

Recently, interferon has been found in cells as an apparent response to *Escherichia coli* endotoxin and to infection with *Serratia marcescens*, *Salmonella typhimurium*, *Brucella abortus*, and *Rickettsia tsutsugamushi* (Youngner & Stinebring, 1964; Stinebring & Youngner, 1964). Even RNA and such products of RNA hydrolysis as adenosine monophosphate are effective in stimulating interferon production (Sigel, 1964).

### *Lysozymes*

The existence in body fluids of enzymes that destroy pathogenic microorganisms, at least *in vitro*, is recognized, although these products are usually regarded as of minor significance. It is known, for example, that the circulating levels of a lysozyme-type of enzyme are significantly higher in guinea-pigs sensitized with heat-killed tubercle bacilli than in normal animals. This could not be related to either skin reactivity or leukocytic activity in response to purified protein derivative of the tubercle bacillus (PPD) (Janicki & Patnode, 1961). Lysozyme isolated from egg white has shown *in vitro* and *in vivo* activity in mice against pathogenic staphylococci (Ermol'eva et al., 1964). The authors state that this lysozyme preparation has been used successfully in the USSR for curing antibiotic-resistant staphylococcal carriers.

In addition to destroying bacteria, lysozymes are said to act on viruses *in vivo* and *in vitro* (Ferrari et al., 1959). An inhibitor effective against the viruses of poliomyelitis, herpes simplex, and Rous sarcoma has been demonstrated in the genital tract of women, but its nature has not been determined (Pannu & Sigel, 1963).

There is little doubt that enzymatic activity of this nature can be decreased or abolished as a result of nutritional deficiency. The greatly reduced lysozyme activity in the tears of two children with xerophthalmia "increased remarkably with five to seven days of cod-liver-oil therapy" (Anderson, 1933). Such findings may be of significance in view of the frequency of secondary ophthalmia, as well as systemic infections, in cases of xerophthalmia. Decreased secretion of lysozyme into the gastrointestinal lumen has been observed in vitamin A deficiency in man (Sullivan & Manville, 1937), although the bowel wall itself contained a higher concentration of lysozyme than normally observed.

The saliva of malnourished persons, in contrast to that of persons who were well nourished, had little or no bacteriolytic activity against a variety of bacterial agents, including *Vibrio cholerae*. Saliva from cholera patients showed a similarly reduced activity (Dawson & Blagg, 1950).

Cohn & Wiener (1963a,b), analysing selected hydrolases from rabbit peritoneal macrophages, found a two- to three-fold increase in lysozyme when such cells were stimulated by injection of killed BCG. They also noted a similar increase in acid phosphatase and lipase activities under these circumstances.

Inhibition of reproduction of trypanosomes during early stages of infection by a humoral substance has been described (Braude, 1963; Raffel, 1961; Pérez-Tamayo, 1961).

A normally occurring serum factor,  $\beta$ -lysin, is bactericidal for such Gram-positive bacteria as *Bacillus anthracis* and *B. subtilis* (Donaldson et al., 1964).

Another factor that reacts with the group-specific polysaccharide C-substances of pneumococci (C-reactive protein) is found in a number of other infections (Braude, 1963).

### *Other*

Even intestinal helminths may be inhibited by non-specific protective substances secreted into the gut. Well-nourished horses were capable of elaborating a specific growth-inhibiting substance against *Nippostrongylus muris* (Schwartz et al., 1931), but production was largely suppressed by nutritional deficiencies. Similarly, an extract of mucus from adult dogs and hogs caused early death of the fowl nematode *Ascaridia lineata* when tested *in vitro*. Antigen-antibody mechanisms were ruled out by autoclaving the extracts before the test; no nutritional studies were conducted (Eisenbrandt & Ackert, 1941). The effectiveness of mucus as an inhibitor of *Ascaris galli* in chickens increased with age of the animals, but this was perhaps attributable to larger numbers of goblet cells per area of intestinal wall. The phenomenon was not studied in malnourished chickens (Frick & Ackert, 1941).

A well-worked-out, specific enzymic effect of an infectious agent comes from demonstration that protein deficiency reduces production of trypsin, which, in turn, is necessary for the excystation of oocysts of *Eimeria* in chicks (Britton et al., 1964). Severe protein deficiency or starvation markedly reduced trypsin production in 48 hours, with closely correlated amelioration of the infectious process. A direct relationship was then shown by adding trypsin to the *Eimeria* oocysts before feeding. Prompt restoration to full infectivity occurred as the sporozoites were released from the oocysts.

In summary, the significance of the various non-specific mechanisms of resistance to infection just cited is difficult to assess. The occurrence of interferon and of properdin in living cells is well established and potentially important clinically. Lysozymes, although known much longer, appear of marginal importance. Recorded investigations on these substances are few, and the subject has attracted little recent interest.

A non-specific substance inhibiting intestinal nematode infections has been described by several investigators. Other such substances may well exist.

### **Non-specific destruction of bacterial toxins**

Neutralization of bacterial toxins in the course of resistance to infectious processes is ordinarily through combination with specific antitoxin generated by the animal host. There is some evidence of other mechanisms. Werkman and co-workers (1924b) found that rats suffering from deficiencies of B-complex vitamins or vitamin A were more susceptible than controls to

diphtheria toxin, although antitoxin production was unaffected and the rate of disappearance of injected toxin was normal.

Dubos and co-workers (1955) reported that, after 48 hours, fasting mice were susceptible to *Klebsiella pneumoniae* endotoxin when given 10% of the LD<sub>50</sub>, whereas animals on an adequate ration were susceptible only to 50% of the LD<sub>50</sub>. This sensitivity was reversed by 48 hours of good diet. The authors concluded that the effect was independent of ordinary immune mechanisms, since large differences were noted a few hours after injection and long before an immune response could have occurred.

Guinea-pigs starved or treated with carbon tetrachloride were more susceptible to intravenously injected *Klebsiella* endotoxin than normal animals. Since this was a first exposure and deaths in treated animals occurred within a few hours, these findings cannot have been due to a difference in antitoxic immunity. The authors provide no explanation of their results (Formal et al., 1960).

Fatty degeneration of the myocardium in guinea-pigs inoculated with diphtheria toxin is manifested biochemically by a depressed rate of oxidation of long-chain fatty acids in heart muscle, excessive accumulation of triglycerides, and a striking decrease in concentration of myocardial carnitine (DL-gamma-trimethylamino-beta-hydroxybutyrate), a compound known to stimulate long-chain fatty acid oxidation (Wittels & Bressler, 1964). Exogenous carnitine had a restorative effect *in vitro* on the depressed rate of palmitic acid oxidation by myocardial homogenates of the toxin-treated animals, suggesting a biochemical basis for resistance to this bacterial toxin.

### Tissue integrity

Dietary inadequacies have long been assumed to diminish resistance to infection by reducing the integrity of various tissues. Nutrient deficiencies frequently result in gross epithelial lesions. Examples are the metaplastic hyperkeratosis due to vitamin A deficiency; the dermatitis, cheilosis, and angular stomatitis from riboflavinosis and pyridoxine deficiency; the characteristic dermatosis and mucosal atrophy of pellagra; the spongy gums and subcutaneous hemorrhages of scurvy; and the atrophy of skin and gastrointestinal mucosa of severe protein deficiency.

The mucosa of the gastro-intestinal tract of vitamin-A-deficient cotton-rats was more readily penetrated by poliovirus than the mucosa of control animals (Weaver, 1946). Similarly, Seidmon & Arnold (1931-32) found a significant increase in numbers of *S. typhimurium* in livers of rats deficient in vitamin A or B when cultures were made 30 to 60 minutes after inoculation. Stryker & Janota (1941), however, were unable to duplicate these results when *S. enteritidis* was administered to vitamin-A-deficient rats by stomach tube.

From work with guinea-pigs infected with *M. tuberculosis*, Grant (1926) suggested that an adequate balance of dietary calcium, vitamin C, and vitamin D was essential to normal resistance of the intestinal wall against entrance of bacteria into the blood-stream from the lumen.

Nutritional deficiency conceivably has an influence on resistance to infection through one or more of the following pathologic tissue changes (Horwitt, 1955):

- (1) alterations in intercellular substance;
- (2) reduction or absence of secretion of mucus;
- (3) increased permeability of intestinal and other mucosal surfaces;
- (4) accumulation of cellular debris and mucus, resulting in a favorable culture medium;
- (5) keratinization and metaplasia of epithelial surfaces;
- (6) loss of ciliated epithelium of the respiratory tract;
- (7) nutritional edema with increased fluid in tissues;
- (8) reduced fibroblastic response; and
- (9) interference with normal tissue replacement and repair.

The evidence now available does not permit judgment as to the relative significance of these several changes.

Lynch (1957) used a special synthetic diet containing gum arabic and various salts to produce fulminating amebiasis in guinea-pigs. The result was first attributed to alteration of the bacterial flora. Feeding massive doses of bacteria to simulate the change had no effect, however, on the severity of amebiasis in control animals. He concluded that the thinning and vacuolation of the intestinal mucosa in animals receiving the diet were a more likely explanation.

The "maturation resistance" to viruses described by Sabin (1941) was apparently due to a mechanism preventing the spread of virus from nasal mucous membrane to the brain. Four different viruses, infective by intracerebral inoculation, were also infective by intranasal inoculation at two to four weeks of age, but not thereafter. Deficiency of vitamin B-complex, thiamine, vitamin E, or total calories delayed the development of this "maturation resistance" to intranasal inoculation.

In summary, some of the tissue changes characteristic of nutritional deficiencies influence resistance to infection, but their relative importance is not well known and is sometimes over-emphasized.

### **Wound healing and collagen formation**

Wound healing, fibroblastic response to local trauma, walling-off of abscesses, and collagen formation are all reactions closely related to nutri-

tional deficiencies. Too often evaluation of synergism and antagonism between nutrition and infection is restricted to the acute manifestations of infectious disease. The impact in terms of total resulting disability is frequently dependent on the rapidity with which the infection can be localized and contained without serious clinical manifestations.

The walls of induced sterile subcutaneous abscesses in protein-deficient rats are much thinner than in well-nourished animals and, when spontaneously or experimentally infected, show much less fibroblastic response (Taylor and Tejada, 1966). As a consequence, a fatal septicemia is frequent. Protein deficiency, and particularly lack of methionine, interferes not only with the conversion of procollagens, but also with the tensile strength of collagen fibers (Dunphy et al., 1956; Dunphy, 1957; Williamson et al., 1951). Fasting for seven days also reduces the procollagen content of guinea-pig skin (Gross, 1958). Skin collagen synthesis, as measured by the uptake of radioactively labeled glycine, was considerably decreased in rats fed diets deficient in amino acids (Nimni et al., 1962).

Early experimental observations by Menkin and co-workers (1934) showed the importance of ascorbic acid in the formation of collagen. Subsequent biochemical studies identified the dominant role of ascorbic acid in the synthesis of the amino acids from which collagen is formed (Gould 1960, 1961, 1963; Dunphy et al., 1956). Two amino acids are almost unique to collagen, hydroxyproline (Gould & Woessner, 1957) and hydroxylysine (Sinex & Van Slyke, 1955). The skin of wounds of scorbutic guinea-pigs contained no hydroxyproline, which can be taken as indicating the absence of procollagen, but when ascorbic acid was restored to the diet, hydroxyproline was rapidly produced (Gould & Woessner, 1957).

Some confusion arose because, in spite of the biochemical evidence of collagen deficiency, sections of the scorbutic skin examined microscopically appeared to contain abundant collagenous material. This was due to accumulation of mucopolysaccharides, which resemble collagen histologically (Robertson & Hinds, 1956). Bavetta and associates (1961) designed experiments to measure the rate of collagen biosynthesis in rats fed different amounts of protein, with and without ascorbic acid supplementation. There was a highly significant increase in initial collagen formation in rats consuming the higher-protein diet and the added ascorbic acid. There is, as yet, little to connect nutritionally induced changes in collagen formation with resistance to infection, but the relationship should be investigated in such diseases as tuberculosis.

#### **Altered intestinal flora**

The micro-organisms ordinarily inhabiting the intestinal tract of man have in recent years been credited with increasing physiologic and pathologic significance. Since the initial observations of Metchnikoff (1908), many

studies in man and in laboratory animals eventually established that the diet of a host can have a profound effect on the intestinal flora. Evidence increases that changes in the so-called "normal" flora make the host more susceptible to a number of pathogenic agents and that others that are usually harmless become pathogenic in the presence of malnutrition.

Studies in swine have shown that the relative numbers of fecal *Escherichia coli*, of other Gram-negative bacilli, of enterococci, and of clostridia are influenced by the type of diet. Diets rich in animal protein and calcium gave increased numbers of atypical *Clostridium perfringens*, from a normal of about 200 per gram to about 500 000 per gram, as well as greater numbers of Gram-negative bacilli (Mansson & Olsson, 1961a,b,c). Enterococci and *Clostridium perfringens* were fewer after addition of citric acid to the diet (Mansson & Olsson, 1962). The investigators reviewed the work of others who also found a marked dietary influence on intestinal flora.

The *in vitro* growth of *Streptococcus faecalis* was halted by specific deficiencies of certain amino-acids (Shockman et al., 1958). Lysine was essential to culture media for growth of both cell wall and cell protein. Deficiencies of valine, histidine, and threonine, however, interfered with protoplast synthesis; but cell wall production was stimulated to the extent that the usual size of bacteria was doubled.

Lack of dietary protein is believed responsible for disturbances of the microbial pattern and overgrowth of intestinal bacteria in kwashiorkor (Smythe, 1958). The weight of stools from children with kwashiorkor may be as much as four to five times normal figures (Hansen et al., 1962). The bacteria of the lower intestinal tract also tend to migrate to higher levels in patients with sprue (Frazer, 1949), and probably in severe malnutrition associated with other conditions.

After sulfonamides and antibiotics came into common use, many studies were reported of their effect on the flora of the intestinal tract. Although no review is attempted here, it is to be noted that such therapy regularly induces changes in the relative proportions of intestinal bacteria of various types, and that total numbers of bacteria increase (Bridges et al., 1952, 1953). Spatial distribution within the intestinal tract may also be altered (Anderson et al., 1956).

For the most part, these therapeutic agents act by blocking either nutrients or metabolic reactions essential to the bacteria. The selective effect on the intestinal flora is of the same general order as that produced by primary dietary deficiencies. Whether or not these changes in intestinal micro-organisms influence susceptibility to infection is still an open question.

Some recognized pathogens probably find a more favorable environment in the intestine of the malnourished host, with the result that micro-organisms not normally pathogenic increase in numbers to such an extent that they may cause diarrhea or other symptoms. Dubos and co-workers (1963c) have recently summarized the evidence that "certain components of the indigenous

flora play a useful role in increasing resistance against virulent pathogens, and also against less virulent, but nevertheless deleterious microbial species which would otherwise become established in the intestinal tract". The microbial species responsible for this protective effect have not as yet been identified.

Dubos and co-workers (1963a,b) have pointed out that the intestinal bacteria of man range biologically from those so well adapted that they are never pathogenic to others that are always pathogenic. They consider the lactobacilli and the anaerobic bacilli included in the genus *Bacteroides* to be the truly normal flora of the intestinal tract of mice and of men, and their presence to be generally beneficial. Other common intestinal bacteria include enterococci, clostridia and Gram-negative enterobacilli (particularly *Escherichia coli*, the *Proteus* group, and *Pseudomonas*)—micro-organisms that ordinarily are not so numerous as the lactobacilli and *Bacteroides*. Dubos and co-workers (1963c) regard them as usually harmless invaders, which, under selected circumstances, may become pathogenic.

The animals of a mouse colony gained weight more rapidly and utilized protein more efficiently than did stock animals when they were kept essentially free of *Esch. coli*, the *Proteus* group of bacilli, and *Pseudomonas aeruginosa*, and when the greater proportion of the intestinal flora was restricted to organisms commonly classified as lactobacilli and *Bacteroides* (Dubos & Schaedler, 1960, 1962a,b; Dubos et al., 1963a,b; Schaedler & Dubos, 1962). When penicillin was administered, lactobacilli disappeared and enterococci and Gram-negative enterococci increased explosively. A striking observation was that *Esch. coli*, long thought to be a normal component of the microbiota, became abundant and was accorded a role in subsequently observed pathologic effects (Ashburner & Mushin, 1962). *Clostridia* (Lev & Forbes, 1959) and enterococci (Anderson et al., 1956) are among other bacteria presumably having a part in deleterious effects arising from the ordinary intestinal flora.

An inhibition of the protective action of the normal enteric flora was the explanation offered for the increased susceptibility of guinea-pigs to experimental shigellosis after either four days' starvation or antibiotic therapy with streptomycin, erythromycin, or nystatin (Formal et al., 1958). Control animals had a non-fatal infection; test animals had a prompt and high fatality. Starvation for a maximum of 36-48 hours resulted in chronic non-fatal disease.

The last chapter of this monograph emphasizes the high proportion of diarrheas among malnourished pre-school children in less developed countries where no known bacterial pathogen can be identified. It seems likely that some diarrheas are caused by infectious agents not normally pathogenic in the well-nourished child and not necessarily identified by present microbiologic methods. Gordon and associates (1957) found that feeding excessive numbers of even such normally desirable bacteria as *Lactobacillus*

*acidophilus* provoked diarrhea in children with severe protein malnutrition.

The dramatic increase in numbers of Gram-negative bacilli and *Clostridia* in the stools of babies fed a formula of cow's milk rather than breast milk may have significance even for the well-nourished child (Gyllenberg et al., 1957). It has been suggested that *Lactobacillus bifidus*, so regularly a feature of the intestinal flora of breast-fed infants, confers some degree of protection against the establishment of harmful strains of *Esch. coli* (Ross & Dawes, 1954) and other bacterial pathogens (Petuely, 1957; Gyorgy et al., 1962). Rose & Gyorgy (1955) have shown that, when children are fed breast milk, certain mutants of *L. bifidus* rapidly suppress other intestinal micro-organisms. At the University of Zagreb, in Yugoslavia, an experimental study is currently under way in which young children are fed large quantities of *L. bifidus* in order to determine whether or not the flora thus produced is beneficial in controlling enteric infections. The results are not yet available.

In the guinea-pig, six different diets each produced definite qualitative changes in the bacterial flora (Crecelius & Rettger, 1943). A number of intestinal micro-organisms benefit others by synthesizing the nutrients required, or, conversely, they may compete for the same limited supply of nutrients (Rosebury et al., 1954; Rosebury & Sonnenwirth, 1958). That some intestinal bacteria are truly symbiotic, to the benefit of the host, is suggested by the poorer growth commonly observed in germ-free animals when compared with that of normal animals on the same diet.

Newton & DeWitt (1961) found that germ-free guinea-pigs did not grow as well as animals conventionally reared, even though food consumption relative to body-weight was greater. A lack of bacterial flora was thought to hamper good nutrition. The germ-free animals, in addition to being smaller, had serum protein levels 25% less than those conventionally fed. Levenson & Tennant (1963) summarize the nutritional contribution of a normal flora as delaying the full effect of starvation, cirrhosis, and vitamin B deficiency in conventional as compared with germ-free animals. Scurvy is apparently augmented in conventionally reared guinea-pigs because their intestinal bacteria use the available vitamin C.

Experimental suppression of the enteric bacterial flora by antibiotics has resulted in decreased resistance to parenteral infection (Dineen, 1961). Observations on germ-free animals suggested that the normal flora somehow enhances the ability of the host to cope with infection. For example, germ-free guinea-pigs were susceptible to infection with *Sh. flexneri* alone; but, if first infected with *Esch. coli*, the animals survived (Formal et al., 1961).

Of possible relevance is the recent report that the normal flora has a significant impact upon ability of the host to mobilize and concentrate leukocytes in an area of injury (Abrams & Bishop, 1965). Quantitative comparison of aseptic, starch-induced peritonitis in germ-free mice and animals conventionally reared disclosed that extravascular migration of leukocytes, in response

to the sterile irritation, was significantly greater in animals harboring a living microflora.

An intestinal flora more favorable to growth of guinea-pigs resulted when part of the sucrose of the basal diet was replaced by 15% of gum arabic, 2.5% of potassium acetate, and 0.5% of magnesium oxide (Roine & Elvehjem, 1950). In general, most of the observed competitive microbic interactions seem to have occurred because of a pH unfavorable to growth of the specific host, or more commonly from action of an antibiotic substance (Florey, 1945, 1946; Rosebury, 1962). Some reports support the theory of such a competitive interaction as a possible factor in resistance, for example, to diphtheria (Mühelenbach, 1939) and to shigellosis (Friedman & Halbert, 1960).

The many different colicins are antibiotics that have attracted attention for at least forty years. They are derived from *Esch. coli* and other Gram-negative enteric bacilli (Fredericq, 1957). A pure culture of *S. enteritidis* from mouse feces prevented the growth *in vitro* of *S. typhi*, *Sh. dysenteriae*, and *Sh. flexneri* (Topley & Fielden, 1922). Later it became evident that *Esch. coli* can suppress *Vibrio cholerae in vitro* even in media favoring the vibrio. When the experiment was repeated *in vivo*, using isolated bowel loops in rabbits, no evidence of antagonism was obtained (Barua et al., 1963). Another example is the identification by Flippin & Mickelson (1960) of a non-pathogenic *Esch. coli* strain with an antagonistic action against a contaminating *Salmonella* introduced into egg-white medium. In a study in Egyptian villages, the probability of finding coliform strains capable of inhibiting *Shigella* was greatest when they were derived from the feces of patients with shigellosis. Next in order were those from family contacts, and last those from neighbors or persons having no direct contact with the disease (Robbins et al., 1958).

The use of non-pathogenic coliform bacteria as an adjunct to antibiotic therapy was tried by Stewart and co-workers (1964). They selected a strain of *Esch. coli* resistant to paromomycin or neomycin, the particular antibiotics to be used, and then introduced the organisms into the gut along with the antibiotic, treating two patients infected with *Sh. sonnei*, five with *S. typhimurium*, and one with enteropathogenic *Esch. coli*. From this experience they concluded that the method might be useful in maintaining a coliform flora during prolonged antibiotic therapy, but that it did not contribute to elimination of the pathogens. Finegold and co-workers (1965) suggest studying these phenomena by selective inhibition of the various major elements of the intestinal flora with carefully selected drugs.

The increased gastro-intestinal motility and accompanying diarrhea associated with some deficiency states is another example of an alteration in the numbers and behavior of intestinal flora and of parasites. Loughlin & Mullin (1955) thought that ascarids and other helminths were less numerous in protein and vitamin A deficiencies because of "gastro-intestinal hurry",

that is, the more rapid passage of food through the digestive tract. On the other hand, it has been suggested that in chickens with vitamin A deficiency weakening of peristalsis may result in intestinal stasis and an accumulation of ascarids (Ackert & Nolf, 1931; Ackert et al., 1931).

The diets of herbivores are stated to lead, at times, to intestinal stasis, thereby favoring toxin production by *Clostridium perfringens* (Roberts, 1938; Parry, 1948).

Larsh (1945) presented evidence that the lowered resistance resulting from alcohol feeding or increasing age in *Hymenolepis nana* infections of mice was associated with decreased intestinal emptying time. He was able to produce the same effect with morphine (Larsh, 1947a).

Amebiasis became a fulminating disease in guinea-pigs fed a special synthetic diet that altered and increased the intestinal flora (Lynch, 1957). The result could not be duplicated by feeding well-nourished animals massive doses of the bacteria making up the greater part of the altered flora, from which the conclusion followed that the shift in flora was not the primary cause of the greater susceptibility. Hegner (1924) was impressed by the rarity of intestinal protozoa in many carnivores. Experimentally, he found that rats fed diets high in animal protein were less favorable hosts for *Giardia muris*, *Trichomonas muris*, and *Hexamitus muris* than animals subsisting mainly on vegetable proteins and carbohydrates.

The foregoing discussion demonstrates clearly that several forms of malnutrition alter types, numbers, and distributions of intestinal bacteria. Less is known of the effect this may have on resistance to pathogenic agents acting primarily on the intestine. Nevertheless, a number of convincing examples confirm a decreased resistance to intestinal infections brought about by nutritionally induced alterations of the gastro-intestinal flora. The severity of protozoal and helminthic infections of the intestine is frequently determined by dietary changes. Alterations in gastro-intestinal motility secondary to malnutrition may also play a part.

### Endocrine imbalance

Endocrine activity is an integral part of the biologic mechanisms involved in resistance to infection; and malnutrition can produce endocrine abnormalities. The effect of protein deficiencies on endocrine function has been specifically reviewed by Leathem (1958). In laboratory animals, most endocrine responses are reduced once body proteins are so depleted that labile protein reserves are at a premium. In children with kwashiorkor, the urinary excretion of 17-hydroxy-steroids is lowered (Castellanos & Arroyave, 1961), and adrenal gland size is reduced (Stirling, 1959; Tejada, 1955). Kwashiorkor and less specific forms of severe protein malnutrition in adults also tend to induce panhypopituitarism and associated atrophy of other endocrine glands (Zubirán et al., 1955). Marasmus or starvation stimulates

the stress reaction, one manifestation being an increase in cortisone production.

Pyridoxine deficiency in rats reduces thyroid activity, as indicated by diminished  $^{131}\text{I}$  uptake (Hsu et al., 1959; Hsu, 1963). That iodine deficiency results in endemic goiter and possible hypothyroidism is well known.

The administration of thyroid hormone following infection of rats with pneumococci or streptococci (Sidorkina, 1950) apparently increased the number of survivors, and the time between inoculation and death was greater for the animals that died. Although a beneficial effect has been claimed for thyroid hormone in patients with scarlet fever, confirmation is lacking (Zimanyi, 1948). In mildly hyperthyroid chickens (Todd, 1949), *Ascaridia galli* attained a significantly greater length than in normal or mildly hypothyroid hosts. The opposite was true for *Heterakis gallinae*.

Several investigators have indicated that hypothyroidism has an adverse effect on the course of tuberculous infection (Joll, 1932; Fishberg, 1932; Webb, 1916; Delore, 1926; Lisser, 1934; Lurie & Ninos, 1956; Lurie et al., 1959). However, the reasons why an abnormal hormonal state alters the host's resistance to tuberculosis are not known. Lurie and co-workers (1956, 1959) have demonstrated that hypothyroid rabbits have a lowered resistance to tuberculous infection, which suggests that this hormonal disorder may alter the inflammatory response of the host. The uptake and release of  $^{131}\text{I}$  has been shown to be reduced by pneumococcal septicemia in the rat (Shambaugh & Beisel, 1966). This was accompanied by a marked fall in the protein-bound iodine and circulating free thyroxine. The failure of serum thyroid-stimulating hormone levels to change appeared to be due to decreased pituitary response. Similar effects occurred with human subjects infected with *Pasteurella tularensis* or vaccinated with a living attenuated strain of Venezuelan equine encephalitis virus (Shambaugh & Beisel, 1967). Presumably, nutritionally induced endocrine changes could enhance these effects.

Chronic adrenal insufficiency in man, or Addison's disease, and experimental adrenalectomy of animals markedly diminish resistance to infection and to stress in general (Kinsell, 1955). The mode of action is not clear, but the defect is correctable by adequate corticoid therapy (Selye 1946, 1949, 1950, 1951). Conversely, adrenocorticotrophic-hormone (ACTH) or corticoid therapy favors extension of many infections by diminishing the protective inflammatory response (Kligman et al., 1951; Selye, 1951; Spink, 1957). The breakdown of tuberculous lesions, the spread of staphylococcal disease, the greater severity of varicella, and even the development of fatal vaccinia are all recognized hazards of such therapy. Top (1964) summarizes clinical experience in the statement that corticoids produce a reduction of symptoms but with danger of causing spread of infection in viral hepatitis, histoplasmosis, epidemic keratoconjunctivitis, measles, infectious mononucleosis, mumps, pneumococcal pneumonia, trichinosis, and typhoid fever.

There is also clinical and experimental evidence that some protozoan diseases, such as trypanosomiasis (Wolf et al., 1951), malaria (Kass et al., 1951), and amebiasis (Eisert et al., 1959), may be aggravated by steroid therapy. Cruz and associates (1966) have reported five cases of fatal strongyloidiasis developing during, or shortly after, a course of corticosteroid therapy.

The increased susceptibility to Coxsackie virus of mice treated with cortisone has been demonstrated by Behbehani et al. (1962). Growth hormone had the opposite effect. Administration of cortisone has been reported to reduce the extent of acquired immunity of mice to typhoid bacilli, as evidenced by a greater concentration of the infectious agent in organs and tissues (Zhitova & Kudryashova, 1965). Where there is latent corynebacterial infection, a natural occurrence in mice, a single injection of 10 mg of cortisone may precipitate the active disease, pseudotuberculosis (Fauve et al., 1964.)

Germuth and associates (1951) reported that an inadequate amount of ACTH or cortisone inhibited formation of antibodies and development of the Arthus phenomenon in rabbits. Antibody suppression has been noted in guinea-pigs (Kepinow, 1922), rats (Wyman, 1929; Ivanov, 1963), and mice (Weiser et al., 1941) under similar treatment. Meyer and co-workers (1964) were unable to demonstrate an effect of cortisone on the production of precipitins and natural hemagglutinins in thymectomized and bursectomized chicks.

In convincing studies, Hirsch & Church (1961) found that polymorphonuclear leukocytes collected from rabbits given large doses of cortisone exhibited a normal capacity to engulf and kill certain staphylococci and enteric bacteria. The granulocytes of such animals also contained normal amounts of the antimicrobial agents, lysozyme, phagocytin, and histone. They concluded that the decreased resistance to infection occurring with high doses of glucocorticoids was not associated with significant changes in either the opsonic or the bactericidal activity of serum.

Adrenalectomy has been described as considerably decreasing properdin levels in rats (Biró et al., 1964). The effect is increased by deoxycorticosterone, decreased by prednisolone, and abolished by aldosterone or cortisone.

Numerous claims of benefit from cortisone in the treatment of a variety of acute infections have been made, but mainly in the early enthusiasm for cortisone, when it was tried for almost every pathologic condition. Such claims were usually based on poorly controlled and inconclusive studies or on misinterpretation of a relief of inflammatory symptoms as control of infection.

Nevertheless, when cortisone was tested in mice for an effect on macrophage activity, as judged by splenic uptake of colloidal thorium dioxide, and on the capacity of leukocytes of the peritoneal cavity to take up *Staphylococ-*

*cus aureus*, both actions were significantly enhanced (Marcus et al., 1953). There is some evidence, moreover, that cortisone in mice is able to reduce the effect of a number of bacterial endotoxins (Boyer & Chedid, 1953). The protective action was observed only when cortisone was administered before the toxin (Geller et al., 1954; Berry & Smythe, 1963).

This action may be related to the protective influence of cortisone on endotoxin-induced disturbances of carbohydrate metabolism or to the ability of cortisone to mitigate tissue damage (Berry & Smythe, 1959). Another possibility is that cortisone slows the spread of infection by inducing tissue edema. Rabbits became more resistant to vaccinia virus by reason of increased tissue fluids produced either by hypertonic salt solution or by administration of estradiol. Spread of both virus and India ink particles from the site of infection was slowed down by edema (Taylor & Sprunt, 1943).

A possible relationship between resistance to avian malaria and gonadal hormones is indicated by studies of ducks infected with *Plasmodium lophurae* (Trager, 1948). In egg-laying ducks with active ovaries the number of parasites introduced into the circulating blood was limited. In males, and in females with inactive ovaries, multiplication of trophozoites was observed. Sadun (1948) noted that both the gonadal hormones, testosterone and estradiol, increased the resistance of chickens to *Ascaridia galli* infection.

Addis (1946) showed that *Hymenolepis diminuta* in the male rat is ordinarily dependent on testosterone for normal growth, although progesterone can be substituted. The administration of hydrocortisone, testosterone, and, especially, progesterone to guinea-pigs inoculated with *Entamoeba histolytica* resulted in a greater frequency of hepatic abscesses than in untreated animals (Biagi et al., 1963).

Secondary infection is notably a common and severe complication of diabetes, although less now that long-acting insulin preparations have become available. High nitrogen losses were once a common result of the brief periods of ketosis so frequent when diabetics were dependent on regular insulin. Pollack (1955) suggests that maintenance of positive nitrogen balance with protamine-zinc insulin, despite some glucosuria, is the key to the greater resistance to infection characteristic of diabetics under modern management.

According to Cruickshank & Payne (1949), the bactericidal power of leukocytes is impaired in alloxan diabetic rabbits. In a later study, Cruickshank (1954) attributed the greater susceptibility to a peripheral circulatory failure, which inhibited migration of leukocytes to the infected tissues. Furthermore, the leukocytes of diabetic patients have a lower capacity to form lactic acid from glucose than do leukocytes of normal controls, a situation corrected by insulin (Martin et al., 1953). This is believed to be a significant consideration in view of the bactericidal action of lactic acid.

Kligman and co-workers (1951) mentioned the fungus *Trichophyton mentagrophytes* among cutaneous infections to which the cortisone-treated host is more susceptible. Fungal infections have not been mentioned previously in this monograph because of lack of published evidence that nutritional deficiencies affect them.

Pulmonary mucormycosis, an infection now recognized with increasing frequency, provides at least one exception. The disease is almost always associated with metabolic disorders, most frequently diabetes mellitus (Baker, 1956). Control of the diabetes appears to be the principal requirement for survival of patients with this mycosis (Harris, 1955). Rabbits with alloxan diabetes developed nasal, pulmonary, and cerebral lesions of mucormycosis after inoculation with the causative organism *Rhizopus oryzae*, even when inoculation of the fungus preceded the experimental diabetes by several days (Bauer et al., 1956). A less pronounced fungal susceptibility was also produced in rabbits by hyperglycemia induced by glucose infusion. The presence of degenerative changes in rabbit polymorphonuclear leukocytes in both types of hyperglycemia may be an important factor in pathogenesis of infection. It is noteworthy that thyroidectomy has been shown to increase the susceptibility of rats to this organism (Paplanus & Sheldon, 1965).

In summary, all mechanisms of resistance to infection are affected, in varying degrees, by the endocrine status of the host. Endocrine activity, in turn, is altered by many nutritional factors, including such common clinical deficiencies as those of protein or iodine. Starvation or marasmus, by producing a stress reaction, also affects endocrine balance. Part of the reduced resistance to infection characteristic of malnourished persons is almost certainly mediated through endocrine changes. It is probable that a better endocrine balance is sometimes partly responsible for the increase in resistance that follows an improvement in nutritional status. Moreover, specific endocrine disorders such as diabetes and Addison's disease must be controlled if the constant hazard of death from superimposed infection is to be avoided.

### Response to drug therapy

Under special conditions, dietary imbalance markedly alters the therapeutic effectiveness of a number of drugs. Stibophen therapy of *Schistosoma mansoni* infection in mice was more effective after the mice were given supplemental vitamin K (Bueding et al., 1947). The observed effect was probably not attributable to direct nutritional mechanisms, because the level of vitamin K was one thousand times greater than the ordinary requirement for mice.

A similar non-specific nutritional effect on stibophen therapy of *S. mansoni* infection of mice was reported by Luttermoser & DeWitt (1961). A

purified synthetic diet with a protein content of 8% to 30% was compared with standard animal chow. Up to 95% of parasites were killed by stibophen therapy in animals on the purified diet, compared with 12.5% in mice on the stock diet. The maximum fatality occurred when the protein content was low. The special diet had no effect on infections of untreated mice.

Subsequent studies in man showed that a high-protein diet had no direct effect on chronic *S. mansoni* infection. When stibophen treatment was started, however, the therapeutic response was more rapid among patients who had received a high-protein supplement for eight months (DeWitt et al., 1964).

*Plasmodium gallinaceum* infection of chicks responded 4 to 20 times more readily to treatment with sulfadiazine or metachloridine when the birds were on a purified casein diet rather than on a regular stock diet (Taylor & Greenberg, 1955). No effect was observed when infections were treated with quinine, chloroquine, atabrine, or other antimalarials. In subsequent studies, the accentuated therapeutic effect was eliminated by increased amounts of soy bean in the diet. An extract of tertiary amines, presumed responsible for the phenomenon, was eventually isolated (Greenberg et al., 1959). Pyridoxine supplements inhibited the therapeutic action of both quinine and atabrine on *P. lophurae* and *P. cathemerium* infections of birds (Seeler, 1945).

### Antagonistic Action of Nutritional Deficiencies

Antagonism occurs when deficiency of a nutrient has a greater effect on the infectious agent than on the host. Sometimes the particular nutrient is required only by the agent, and not by the host. Under other circumstances, metabolic disturbances are induced that affect the agent more than the host, because the more complex host organism has alternative metabolic pathways.

Antagonism *in vivo* is best understood from such tissue culture studies as those in which thiamine-deficient mouse fibroblasts proved more susceptible to the filterable agent of psittacosis than cells from well-fed animals (Bader & Morgan, 1961). Oxythiamine-induced thiamine deficiency in minced chick embryo inhibited mumps virus and partially suppressed influenza virus (Cushing & Morgan, 1952). Deoxypyridoxine-induced pyridoxine deficiency in minced chicken embryo suppressed development of both mumps and influenza viruses, and methionine deficiency produced by ethionine blocked poliovirus replication in mouse fibroblasts (Brown & Ackermann, 1951; Brown 1952). Under the same experimental conditions, Mohajer & Gabliks (1966) found that the methionine analogue 1-ethionine had no significant effect on the biosynthesis of two strains of poliovirus (Mahoney and Lansing) in HeLa cells, whereas in primary monkey kidney cells it markedly inhibited the biosynthesis of the Lansing strain of poliovirus.

Both ethionine and glycine methylester suppressed the replication of vaccinia virus in human Chang liver cells (Gabliks et al., 1967). The inhibitory effect of ethionine was partly reversed by an excess of methionine, but glycine addition did not prevent the effect of glycine methylester. Since an excess as well as a deficiency of either methionine or glycine inhibited vaccinia virus replication more than they inhibited cell growth, it appears that the balance of the amino acids in the intracellular pool can affect the magnitude of viral biosynthesis.

Many compounds currently used in chemotherapeutic and antibiotic management of infectious disease are closely related chemically to specific nutrients; they act by blocking normal participation of these nutrients in the metabolism of the agents. An example of current research of this type is the observation that a combination of 5-bromodeoxyuridine and *N*-methylisatinthiosemicarbazone is effective in eliminating vaccinia virus from cultured HeLa cells (Furusawa et al., 1965). To assure the necessary margin of safety, the metabolic requirements of the host for the blocked nutrients must be significantly lower than those of the agent.

Knowledge gained in the course of experimental studies on antagonism has been applied usefully in the search for more effective chemotherapeutic agents; for example, pyrimidines are required for the synthesis of nuclear material, and the systematic search for related compounds led to the discovery of paludrine (Curd et al., 1945).

Experimental production of antagonism in animals has not resulted in any direct contribution to control of infection in man. Deficiencies severe enough to produce antagonism almost always lower resistance to secondary infections, which then become a significant cause of death. Furthermore, antagonistic relationships usually require a highly specific and severe deficiency, obtained only under experimental conditions and rarely seen under natural conditions in either animal or human populations. Thus, the generally inadequate diets characteristic of less developed regions typically lead to synergism rather than antagonism.

From data summarized in the preceding chapter and viewed in relation to specific nutrients, certain generalizations about antagonism are possible:

1. Antagonism occurs more frequently with specific than with generalized nutritional deficiencies. The few instances of antagonism following acute fasting may have been due to a specific caloric effect, but none has been recognized as due to multiple deficiencies. This fits in with the concept that antagonism occurs because an infectious agent suffers from lack of a specific nutrient. In multiple deficiencies, any possible antagonistic effect is usually overwhelmed by synergism resulting from loss of host resistance, brought about by one of the mechanisms already discussed.

2. Among nutrients essential to man, certain ones are not essential to microbial metabolism, and their deficiency has not produced antagonism.

The lack of antagonism with vitamin A deficiency, for example, contrasts strongly with the frequent occurrence of synergism. In a comprehensive review of the literature by Guirard & Snell (1962) on nutritional requirements of micro-organisms, particularly the bacteria, vitamin A is conspicuous by its absence from the list of essential nutrients. The only report of antagonism that we have found was that *P. lophurae* parasitemia was less severe in vitamin-A-deficient chickens than in normally fed birds (Roos et al., 1946).

3. The nutrient deficiencies resulting in antagonism are those essential to microbial nutrition. In our tables, and in Guirard & Snell's (1962) review, these nutrients are: the vitamin B group, about half of which were originally discovered because they were essential for micro-organisms; a list of amino acids ranging from none to as many as 18, according to the particular organism; and a number of mineral ions, including K, PO<sub>4</sub>, SO<sub>4</sub>, Mg, Mn, Fe, Zn, Na, Ca, Mo, and Cl.

4. Micro-organisms need certain nutrients not known to be essential to the human host; as a consequence, they provide metabolic pathways susceptible to therapeutic attack. Examples are para-aminobenzoic acid, purines, peptides, pyrimidine bases, polyamines, and a long series of unidentified growth factors still under study (Guirard & Snell, 1962). Naturally, this circumstance is most frequent among infectious agents whose action is relatively independent of host metabolism. The prospect of finding nutrients specifically essential to a micro-organism diminishes with its increasing host dependence. Much of the success of antibiotic and synthetic antimicrobial agents depends on interfering with chemical processes unrelated to human metabolism (Davis & Feingold, 1962). One example of such an antagonistic effect is the dependence of malaria parasites on para-aminobenzoic acid.

### Host factors

As already made clear, antagonism usually occurs because certain infectious agents are more dependent on specific metabolites than are their hosts. Animal hosts may ordinarily achieve biochemical homeostasis through multiple metabolic pathways. The simpler systems of micro-organisms are more often dependent on single metabolic pathways. Microbial metabolism thus can be selectively blocked by inducing specific deficiencies. These can be produced most readily and effectively by use of anti-metabolites.

The tables in the preceding chapter show that antagonism is most frequent among infectious agents highly dependent on host metabolism. From a practical standpoint, the diseases they cause are the ones most resistant to modern therapeutic measures. Infectious agents relatively independent of host metabolic processes can be successfully attacked by

means that do not directly affect the host's metabolic processes. Because information about such interactions aids understanding of the more complex metabolic interactions of host-dependent micro-organisms, a summary is now presented of pertinent views in this rapidly developing field of microbial metabolism.

Although reports are few, the possibility of nutritional interference with invasiveness and spread of infectious agents should also be considered. A good example is that fasting rabbits are more difficult to infect with vaccinia virus than are normal animals, a result attributed to accumulation of interstitial fluid (Sprunt, 1942).

### Agent factors

Several reviews summarize important developments in bacterial metabolism. Davis & Feingold (1962) related metabolic research to prospects for developing new antimicrobial agents. Their main purpose was to find antimetabolites capable of blocking metabolic pathways required by the agent but not by the host. A variety of existing experimental evidence was introduced to illustrate the means by which substitute metabolites can be incorporated into complex chemicals to alter the function of infectious agents. From this came a hypothesis of "false feed-back inhibition" to explain the resultant chemical blocking. The important point was made that most of the existing antibiotics do not act on well-known energy-yielding pathways but on previously unstudied metabolic pathways specific to micro-organisms, especially those concerned with the formation of cell wall and membrane.

Neidhart (1963) gave special attention to mechanisms of metabolic inhibition of the synthesis of the constituents of the cell wall membranes and the protoplast, and of RNA and DNA. In this connection, the report of Shockman and co-workers (1958) that growth of *Streptococcus faecalis* can be halted by specific amino acid deficiencies has particular interest. Lysine is essential for synthesis of both wall and cellular proteins. Deprivation leads to autolysis. On the other hand, valine, histidine, and threonine are needed for cellular protein but not for wall protein. In culture media lacking these amino acids, protoplast synthesis is halted while the cell wall doubles in size.

Another recent review (Panos & Ajl, 1963) has related metabolic processes to pathogenicity. Virulent infectious agents are generally more active metabolically than are avirulent forms, although the relation may be reversed, as with the tubercle bacillus. Lost virulence in some strains of *S. typhi*, *S. typhimurium*, and *K. pneumoniae* is directly related to a developing metabolic dependence on purines and para-aminobenzoic acid, substances not normally available in animal hosts. A review by Seaman & Reifel (1963) considered metabolic pathways of protozoa as they relate to chemical structure and function.

The metabolic requirements of malaria parasites (McKee, 1951) are better known than most. Methionine deficiency retarded the development of *P. berghei* in mice and monkeys (Taylor, 1956) and of *P. knowlesi* in monkeys (Geiman & McKee, 1948). Ten days of starvation almost eliminated parasitemia with *P. berghei* in mice (Ramakrishnan et al., 1953; Ramakrishnan, 1954), and partial starvation inhibited *P. knowlesi* infections in monkeys (Geiman & McKee, 1948). A dramatic suppression of *P. knowlesi* infection was observed in vitamin-C-deficient monkeys; after ascorbic acid was administered, parasites increased overwhelmingly (McKee & Geiman, 1946).

The delicacy of balance between deleterious effects on host and on the parasite is indicated by the need for B vitamins in avian malaria. Moderate thiamine deficiency retarded *P. gallinaceum* parasitemia in chickens, but severe deficiency adversely affected the host and resulted synergistically in earlier death; high doses of thiamine accelerated parasitemia and hastened death, (Rama Rao & Sirsi, 1956). Pantothenate deficiency of chickens suppressed blood-induced *P. gallinaceum* infection, but not sporozoite-induced infections (Brackett et al., 1946).

More specific information on pantothenate requirements was obtained by *in vitro* culture of *P. lophurae* (Trager, 1954). Pantothenic acid was adequate for intracellular parasites, but extracellular forms required the more complex coenzyme A. Similarly, folic acid permitted *in vitro* growth of intracellular forms; infected erythrocytes actually contained more folic acid than normal cells. The more complex coenzyme form of folinic acid was required, however, by extracellular cultures of the parasite (Trager, 1958).

Of particular interest because of possible therapeutic application was the demonstration that a milk diet inhibited plasmodial infection in rats and dogs (Maegraith et al., 1952; Maegraith, 1953). The milk diet produced para-aminobenzoic acid deficiency, which, in turn, depressed the parasitemia of rats and monkeys. A dietary supplement of para-aminobenzoic acid abolished the milk diet effect (Hawking, 1953, 1954). This confirmed the increased parasitemia in partially starved monkeys (Geiman & McKee, 1948). Results from feeding trials in humans were negative. Trypanosomal infections have a similarly reduced severity in the presence of certain dietary deficiencies.

Tissue-culture studies have given precise and detailed information on the nutritional requirements of viruses and bedsoniae. Poliovirus requires methionine, as shown by metabolic blocking with the analogue ethionine (Ackermann, 1951; Brown & Ackermann, 1951). Influenza and mumps viruses require pyridoxine and thiamine, as indicated by blocking with deoxypyridoxine and oxythiamine (Cushing & Morgan, 1952). Thiamine is completely essential for the agent of psittacosis; and pantothenate, niacin, pyridoxine, and choline are needed for its maximal replication (Bader & Morgan, 1961).

In summary, antagonistic interactions between a nutritional deficiency and an infection are due in most instances to selective lack of one or more nutrients upon which the infectious agent is more dependent than the host. Only rarely does antagonism occur through such other mechanisms as physical interference with invasion or the readier spread of an infectious agent favored by altered tissue.

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## PRINCIPLES OF FIELD STUDY OF HUMAN POPULATIONS

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### Introduction

The evidence thus far introduced for an interaction of nutrition and infection has derived mainly from clinical investigation, from laboratory studies, and from animal experiments. This emphasis by investigators is a natural outgrowth of the development of experimental medicine during the past half century, the improved biochemical and biophysical methods of measuring life processes, and the fact that clinical research is now solidly based on scientific principles. The original concern with an interaction between nutrition and infection, however, arose out of observations in nature of the common association of war, famine, and pestilence. The community effect is still the dominant consideration if the knowledge gained, whatever its source, is to be applied to the benefit of the public health. The evaluation of community effect requires field investigation by epidemiologic methods.

Epidemiologic field study, too, has progressed in the course of years from a descriptive to an analytical discipline, and important advances have resulted from the application of modern methods of laboratory and clinical investigation to field observations. Formerly restricted to infectious diseases transmissible from person to person, epidemiologic investigation has now extended to practically all community diseases and injuries. In this transition, nutritional disorders have had a prominent part, especially through prevalence surveys of the nutritional state of representative groups of people. Nevertheless, several aspects of the epidemiologic field study of nutrition and its relationship to infectious and other diseases still remain largely untouched. Further progress depends heavily on good field studies.

As chronic disease processes came to be viewed from an epidemiologic standpoint, the technical methods of field study naturally developed along fresh lines. An outstanding feature was recognition that the procedures so well suited to sharply marked epidemics and to acute infectious diseases that run a rapid course did not wholly suffice for chronic diseases, of which mal-