Biochemical Determinants of Microbial Diseases

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Preface

It is common practice to refer to the diseases caused by the various groups of microorganisms as *infectious* diseases. This expression naturally puts emphasis on the propensity of microbial agents to spread from one individual to another. The event of infection, however, constitutes merely one phase of the problem of microbial diseases, for only in a very small percentage of infected individuals does the presence of microbial agents result in symptoms or pathological lesions.

The sciences of immunology, epidemiology, physiology, psychology, even of sociology, can all throw light on the influences which determine whether infection is abortive, self-limited or evolves into overt disease, but the problem will be considered here from a more limited point of view. The essays which follow deal exclusively with the biochemical factors that affect the ability of microbial agents to proliferate *in vivo* and to cause metabolic disturbances and alterations of tissues. These essays were presented in Boston on the occasion of the award of the Warren Triennal Prize of the Massachusetts General Hospital in November 1953. They constituted also part of the subject material of a series of lectures delivered in Berkeley and Los Angeles during my tenure of the Hitchcock Professorship at the University of California in the Spring of 1954.

To the Trustees of the Massachusetts General Hospital, and to the Board of Regents of the University of California, I wish to express my gratitude for having given me the opportunity to present in a friendly atmosphere these gropings toward a biochemical explanation of host-parasite relationships.

R.J.D.

CONTENTS

CHAPTER 1

Infection into disease

Ten years ago, I delivered under the auspices of The Lowell Institute in Boston a series of lectures entitled "The Bacterial Cell: In Its Relation to Problems of Virulence, Immunity and Chemotherapy" (Dubos, 1945). In the Lowell lectures, I dealt with the components, products, and properties of microorganisms which endow them with potential pathogenicity and immunogenic power, and which affect their susceptibility to immunity mechanisms and to chemotherapeutic agents. Little mention will be made of these problems in the present book. Instead of the infectious agent, I shall emphasize the properties of the infected host which determine the course and outcome of the infectious process. The study of the tissue factors which are responsible for arresting the progress of infection, or for allowing it to evolve into overt disease, is still in the most primitive state. Nevertheless, as it is today the center of my scientific interests, a discussion of it may serve better than a more classical, but less personal, topic, to express my gratitude for the invitation to deliver the first series of the Warren Triennal Lectures.

Before undertaking a detailed discussion of the various factors which determine the course of infectious processes, I shall attempt to outline the reasons which have oriented my interest toward this problem. This will be made easier by considering first an example taken from recent history. A group of young Danish physicians who were political prisoners in German concentration camps through part of the Second World War have written an engrossing account of the health problems which affected their

own group and other internees during captivity (Helweg *et al.*, 1953). Among infectious diseases, it was not the exotic, unusual epidemics like typhus, cholera, or even bacillary dysentery which proved the most troublesome in their experience, but rather ordinary skin ailments, colds, bronchopneumonias, staphylococcus infections, pulmonary tuberculosis — in other words, the type of diseases, minor or severe, caused by microorganisms *endemic* in the normal European communities. Increase in contact infections could hardly account for the aggravation of these endemic diseases. Far more important certainly was the loss of natural resistance caused by malnutrition and other forms of physiological misery. It was remarkable indeed that most of the internees overcame their microbial maladies shortly after their return to a normal environment and often without the help of specific therapy. Even in the case of tuberculosis, rapid recovery was the rule, though no antimicrobial agent was then available for its treatment.

This dependence of susceptibility to infection upon the physiological status of the host is of course a familiar observation. However commonplace, it focuses attention upon an aspect of infectious disease which is much neglected. Most human beings, indeed probably all living things, carry throughout life a variety of microbial agents potentially pathogenic for them. Under most conditions, these pathogens do not manifest their presence by either symptoms or lesions; only when something happens which upsets the equilibrium between host and parasite does infection evolve into disease. In other words, infection is in many cases the normal state; it is only disease which is abnormal. If space permitted, I could illustrate by many specific examples the relevance of these considerations to the problem of infection in man. It is certain that much of the burden of disease in our communities is caused by pathogenic agents which are maintained normally in "silent" infections and manifest their presence only under the stimulus of other factors of the environment. The happenings in the concentration camps are merely a magni-

fied manifestation of a state of affairs observed daily in medical practice.

Needless to say, there is an extensive literature dealing with the phenomenon of silent infection, often referred to also as carrier state, subclinical infection, or perhaps preferably latent infection (reviewed in Meyer, 1936; Andrewes, 1939, 1950, 1952; Koprowski, 1952; Smith, 1952). But latency, like resistance to, and recovery from infection, has been studied almost exclusively by immunological techniques. Humoral antibodies and cellular immunity are the forces which are most commonly invoked to define the state of equilibrium or of conflict between parasite and host. Yet the problem of the host-parasite relationship needs to be analyzed from many other points of view. Let us consider a few examples, taken almost at random from among the many where forces other than immunological ones determine the outcome of infection.

As is well known, the agent responsible for mammary carcinoma among breeding female mice has all the characteristics of a virus (Bittner, 1947). The Bittner virus can be present throughout the tissues of mice and yet cause no sign of disease and in particular no tumors until lactation begins. Male mice never develop cancer even if they carry or have ingested the virus, nor do female mice unless under the stimulus of continuous reproductive activity. Thus, the hormonal and other stimuli which accompany reproduction and lactation are necessary and sufficient to induce the virus to manifest its potential pathogenicity. In other words, these physiological stimuli are the factors which convert infection into neoplastic disease.

There exists a large body of clinical observations in man and of experiments in animals demonstrating beyond doubt that various specific hormones can modify the course of infection. Of particular interest is the fact that cortisone treatment can cause animals to develop a fatal disease owing to the multiplication of microorganisms which they normally carry in their tissues in the form of an inapparent infection. In experiments with white rats,

extensive necrotic lesions associated with the presence of *Corynebacterium pseudotuberculosis murium* were commonly found within a few weeks following the initiation of treatment with large amounts of the hormone. The corynebacteria were apparently present in the tissues of the normal animals, but in such small numbers that they could not be detected by either bacteriological or histological techniques until the physiological disturbance caused by the hormone had allowed their multiplication (Le Maistre and Tompsett, 1952). Pseudotuberculous lesions caused by corynebacteria have also been reported to occur spontaneously in albino rats maintained on deficient diets, and to regress when the deficiency was corrected by treatment with a crude ill-defined preparation designated as vitamin H (Gundel, Gyorgy, and Pagel, 1931). In very recent experiments with exactly defined diets it has been shown that pantothenic acid deficiency can evoke latent corynebacteria infections, and also render normal animals susceptible to experimental infection with these microorganisms (Zucker and Zucker, 1954; Seronde, 1954). Pseudotuberculosis caused by corynebacteria has also been observed after irradiation of mice (Schechmeister and Adler, 1953).

The number of latent virus infections which have been discovered in experimental animals and in plants is so large and their biological characteristics have been so minutely described that all aspects of the problem of latency in infectious diseases could be illustrated with examples taken from this field. Suffice it to mention here that almost every kind of nonspecific stress has been used to evoke these latent virus infections into activity: changes in temperature, nutritional upsets, introduction of foreign matter into different parts of the body, irradiation, heterologous infection, various forms of trauma and intoxication, poor husbandry, hormonal disturbances, and so on. It is of great significance that evocation of latent infections by nonspecific stimuli has been observed also with every other class of microbial agents — protozoa, fungi, bacteria, rickettsia — and in many species of experimental animals.

Situations where physiological-biochemical disturbances are far more important than immunological factors in accounting for the difference between infection and disease are common also in diseases of man. The problem of infectious disease in concentration camps during the War has already made us aware of this fact, which can be further illustrated by examples taken from ordinary circumstances of life. It is entertaining in this respect to recall the conditions under which the tubercle bacillus came to be regarded as *the* cause of tuberculosis. In the audience at the meeting of the Berlin Physiological Society where Koch presented his famous report in 1882, practically every person present was certainly infected with virulent bacilli — as almost every one in Europe was infected at that time. Indeed, Koch himself had had a touch of the disease, as shown by the violence of his reaction to tuberculin when he injected the substance into his own arm. Since the tubercle bacillus was then a ubiquitous component of the environment, the factors which converted mere infection into symptoms and destructive pathological changes were the real and practically important determinative causes of tuberculosis. Of these factors we know hardly more today than did Koch in 1882. Tuberculin tests reveal that, even in our most prosperous communities, a very large percentage of the adult population become at some time infected with tubercle bacilli. Yet the morbidity and mortality of tuberculosis have decreased ten- to twentyfold during the past century. It is obvious therefore that while the tubercle bacillus is the specific etiological agent of *infection*, we must look to other factors for a complete understanding of the etiology of tuberculous *disease*.

It may be helpful to illustrate by another example the contrast between infection and disease. In man, the herpes simplex virus is usually acquired during early years. Throughout most of the life of the infected individual, the virus lies latent in the body, without causing any symptom or obvious pathology until "provoked" into activity by some physiological disturbance. As is well known, fever (herpetic) blisters can be elicited by a variety

of nonspecific unrelated stimuli, as different one from the other as menstruation, colds and fevers of various origin, ultraviolet radiation, or eating cheese. Herpetic blisters thus provide a striking example of an infectious disease of man in which, contrary to the original tenets of the germ theory, the living agent of the disease (the germ) may be present all the time in the host, be intrinsic, so to speak, whereas the determinant of the pathological process is some physiological disturbance or some other extrinsic factor of the physicochemical environment (Burnet, 1945).

Toxoplasmosis is a protozoan infection now believed to be extremely common in an inapparent form (Weinman, 1952). Skin tests with toxoplasma antigen suggest that from 30 to 60 percent of the normal adult urban population is infected. Although the immunity mechanisms of the host are unable to bring about the complete eradication of the parasites from the tissues, at least for long periods of time, they are capable under usual circumstances of preventing their multiplication. In the brains of animals the pseudocyst membrane surrounding the parasites persists for several years and is probably responsible in part for the chronicity of the infection. Although severe and even fatal disease processes caused by toxoplasma have been observed in adults, they occur more frequently in the form of abortions, miscarriages, stillbirths, and various defects in the infants who survive. These infections are contracted from the female parent, who is almost invariably in good health during pregnancy and remains a healthy carrier (Weinman, 1952).

The normal microbial flora of the intestinal and respiratory tracts illustrates well the influence of various factors on the ability of tissues to restrain microbial multiplication. In normal animals and man, microorganisms normally present in these areas sporadically gain access to the blood stream, but the transient bacteremias which they cause are of consequence only when an associated abnormality, such as a previously damaged heart valve or a persistent wound, leads to the establishment of a focal in-

fection. As is well known, agents associated with subacute endocarditis are assumed to have often such an origin. In the absence of any abnormalities, the microorganisms are promptly filtered out of the circulation and usually destroyed by reticulo endothelial elements, in the spleen, liver, bone marrow, lung, etc. (Kerby and Martin, 1951). In dogs, clostridia which are potentially pathogenic are normally present in the intestinal tract but do not cause any detectable damage as long as they remain in the gut. Shortly after the application of tourniquet in any part of the body, however, they start multiplying in this area, where they soon reach enormous numbers and produce abundant toxin (Aub *et al.*, 1944).

The cellular and humoral defense mechanisms responsible for the clearing power of normal tissues can be inactivated by all sorts of influences, for example, by radiation, or by mustard gas. The effects of radiation are certainly multiple and complex. Thus, if the dose of x-ray is low enough, it has a fairly specific effect upon the epithelial lining, the crypts and covering of the villi of the small intestine, leaving empty crypts and naked villi exposed to swarms of bacteria. Yet the bacteremia produced under these circumstances rarely leads to an overwhelming invasion and it soon subsides. In contrast, a total impairment of antimicrobial defenses usually follows more intense and total body irradiation of mice. The microorganisms entering the blood stream from the intestinal and respiratory tracts multiply in unrestrained fashion, causing a bacteremia and toxemia which are responsible, at least in part, for the mortality following radiation; this is shown by the fact that many animals can be saved by treatment with antimicrobial drugs (Miller *et al.*, 1950, 1951; Kaplan, Speck, and Jawetz, 1952; Hammond *et al.*, 1954). Clearly, then, the barrier constituted by the epithelium is not the only protection against invasion by microorganisms of intestinal or other origin. There are in the tissues and body fluids multiple antimicrobial agencies probably far more important. We shall have much to say of them in subsequent chapters.

The widespread use of antimicrobial drugs has had unexpected effects which illustrate in a startling manner the complexity of the factors affecting the virulence of the parasites and susceptibility of the host (Finland and Weinstein, 1953). There are many drugs capable of causing almost complete sterilization of the intestinal contents. But whatever the drug used, this antimicrobial effect is usually followed within a few days by the appearance of a new microbial flora which replaces that normally present in the gut. The new microbial population does not necessarily consist of drug-resistant variants of the microorganisms present in the normal intestinal flora; rather, it is usually made up of different microbial species which apparently could not successfully compete with the original flora under normal conditions and had a chance to multiply only after the latter had been eliminated or depressed by antimicrobial therapy. As is well known, therapy with almost any type of antimicrobial drug results not infrequently in a secondary disease process caused by *Candida albicans*. This fungus is probably a constant contaminant of tissues but begins to multiply and manifests its pathogenicity only when the normal *in vivo* environment is disturbed by other debilitating pathological conditions or intensive therapy.

Examples of the disturbance by physiological or physicochemical forces of a state of equilibrium between two living things are not peculiar to the animal world. In nature many bacterial species, called "lysogenic," carry in an inactive form (prophage) one or several bacteriophages potentially capable of causing their lysis. Under ordinary conditions, the prophage is apparently reproduced with each bacterial division without causing any detectable disturbance in the cell. This equilibrium can be upset by a number of nonspecific procedures — for example, by irradiation or starvation of the lysogenic culture or by addition of certain substances to the culture medium — in such a manner that the prophage is converted into active bacteriophage, multiplies abundantly, and causes the destruction of its host cell. Thus, the prophage can become a pathogen for the bacterial cell

that carries it only when the proper kind of stimulus is applied. One might say that the prophage renders the bacterium sensitive to the radiation, or that the radiation renders it susceptible to the prophage, or that both agents are required for the causation of lysis. In fact, it has been shown that, in certain cases at least, the activating effect of radiation can take place only in media of certain composition, thus rendering even more complex the etiological determination of the disease lysis (Lwoff, 1953).

There are likewise many examples of plants which either live in harmony with fungi, bacteria, or viruses or are destroyed by them, depending upon the nature of the physicochemical environment in which the association takes place (Smith, 1952). The root nodule bacteria illustrate well this complex relationship. Under natural field conditions, leguminous plants establish with the nodule bacteria a spontaneous symbiosis which is of great advantage to both. Invasion of the plant by the nodule bacteria is facilitated by the production in the root of a specific exudation at a certain stage in the germination of the plant. Modification of both bacteria and host tissues occurs after invasion, the root nodule constituting in reality a modified root adjusted to the requirements of the bacterial symbionts. Since excessive production of nodules would deprive the plant of its functional root system and thus terminate its existence, the association is regulated by inhibitors produced in the meristems of the root and nodules. But this regulating system itself is under the control of environmental factors, general invasion of the plant by the bacteria taking place if boron is omitted from the soil or culture medium (Thornton, 1952). In this case, therefore, it takes both a nutritional deficiency of the plant (in boron), and the presence of the specific bacteria, to constitute the complete etiology of a change from the symbiotic relationship to the state of disease.

We shall briefly consider, as a last example, the problem of causation of the plant cancers known as crown galls, because the precise knowledge which has been gained of their physiological determinants and chemical basis illustrates in a striking manner

the conceptual difficulties involved in the determination of etiology. It is possible to induce at will characteristic tumors (crown galls) by inoculating certain plants with pure cultures of *Agrobacterium tumefaciens*. Since no other microorganism or substance is known to be capable of causing this pathological reaction, it seems fair to regard *A. tumefaciens* as the specific etiological agent. It has been established, however, that many of the secondary tumors developing on the same plant at sites removed from the initial infection are free of bacteria, and yet can be transferred in series to new plants, or propagated in tissue culture as self-reproducing structures. It is possible also to eliminate the bacterium from the tumor tissue by controlled heating, without affecting the power of autonomous growth of the tumor. Thus, propagation of the cancer can be made independent of *A. tumefaciens*, which was at first its essential etiological agent. It is known, furthermore, that extensive invasion of the plant by the bacterium may take place without resulting in tumor formation. Only plant cells which have been conditioned by certain stimuli associated with wound healing are rendered susceptible to transformation into tumor tissue by the bacterium. The physiological state of the host cells should therefore be considered also as etiological determinant of crown gall. Finally, it can be shown that whereas the normal plant tissue requires indole acetic acid and the cocoanut-milk factor for growth, the self-reproducing tumor tissue does not need these growth factors and indeed can synthesize them (as well, perhaps, as others); it is this biochemical characteristic which permits the cancer to grow profusely and in a completely uncoordinated manner. Thus, at the present state of analysis, the biochemical etiology of the disease appears to reside in an increased synthetic power, but, on the other hand, it takes *A. tumefaciens* to induce the change initially (Braun, A. C., 1952).

Depending upon the specialized interests of the investigator, and the techniques that he choses to use, the primary etiological determinant of crown gall can therefore be regarded as a specific