SCIENCE

VOL. 87 FRIDAY, JANUARY 14, 1938 NO. 2246

SCIENCE: A Weekly Journal devoted to the Advancement of Science, edited by J. MCKEEN CATTELL and published every Friday by

THE SCIENCE PRESS

New York City: Grand Central Terminal
Lancaster, Pa. Garrison, N. Y.

Annual Subscription, $6.00 Single Copies, 15 Cts.

SCIENCE is the official organ of the American Association for the Advancement of Science. Information regarding membership in the Association may be secured from the office of the permanent secretary, in the Smithsonian Institution Building, Washington, D. C.

TUBERCULOSIS, LEPROSY AND ALLIED MYCOBACTERIAL DISEASES[1]

By Dr. ESMOND R. LONG

HENRY PHIPPS INSTITUTE, UNIVERSITY OF PENNSYLVANIA

IN the earliest epochs of medical history diseases were classified empirically according to simple symptoms and obvious signs as fevers, tumors, declines and the like, and it was centuries, even, before a more exacting science added an organic incrimination so that physicians could speak more expertly of lung fever, renal dropsy, splenic anemia or pancreatic diabetes. To-day the trend is to set diseases apart on the seemingly rational basis of etiology. Thus lung fever has given way to pneumonia and brain fever to meningitis, with a definite causal association in the mind of speaker and listener with respect to the latter terms. To the ancient physician a febrile disease in which the patient remained in a stuporous or "typhoid" state was typhoid fever; the physician of a more enlightened age, recognizing its intestinal localization, called it enteric fever; physicians of to-day still use this term or the older one of symptomatic reference "typhoid fever," but instinctively think of the disease in terms of its cause, the "typhoid bacillus" (*Eberthella typhi*).

And just as the science of bacteriology took apart certain previous groups, like inflammations of the lungs, and set them in separate categories, so it also grouped together certain ailments once thought distinct. Formerly processes apparently as unrelated as chronic ulceration of the lungs, general swelling of the lymphatic glands, progressive destruction of the spine and cheesy degeneration of the kidneys, were separate clinical entities, although their frequent association led to some suspicion of a common underlying

[1] Address of the vice-president and chairman of the section on the Medical Sciences, American Association for the Advancement of Science, Indianapolis, December, 1937.

basis. With the discovery of the tubercle bacillus, at once they all become forms of one disease, tuberculosis.

With the advance of bacteriology, continued refinement in laboratory technique has become necessary in order to keep pace with clinical progress. The acumen of the clinician in distinguishing pneumonias of varying severity forced his colleague in the laboratory to separate the individual kinds of pneumococci. However, during the lag period, pending the requisite bacteriological progress, certain morbid processes were once set aside for the time being semianonymously as paratyphoid fever, parasyphilis, paratuberculosis and so forth.

But the course of progress in the scientific explanation of clinical facts has not always been smooth. Indeed the purpose of the present symposium of the Section on Medical Sciences of the American Association for the Advancement of Science has been to call attention to significant difficulties into which bacteriological progress has inevitably led in the effort to give full satisfaction to clinical medicine. There is a certain group of diseases easily distinguishable on the basis of symptoms and signs, and differentiable by refined technique in the laboratory, which yet have distinct bacteriological and histopathological aspects in common. The diseases are tuberculosis as it occurs in a variety of types in different animal species, leprosy, Johne's disease, or the so-called paratuberculosis of cattle, a curious bovine skin disease with as yet no limiting designation, and a motley collection of nodular ailments in birds, rodents and various cold-blooded animals, called for convenience rat "leprosy," fish "tuberculosis," etc., with little satisfaction to the namer and to the everlasting annoyance of the special experts in "true" leprosy and tuberculosis.

The significant factors in common in this group of diseases are two: (1) causation by a type of microorganism with the distinguishing tinctorial property of "acid-fastness," and (2) a host response characterized by the elaboration and local accumulation of monocytes, or large mononuclear phagocytic cells, which vary in appearance according to location and the work in hand, but seem to be of fundamentally identical origin. As a corollary of the first factor is to be mentioned a third characteristic, the common possession of certain chemical entities in the lipid, protein and carbohydrate fractions, which appear to be responsible for the distinctive staining character and certain serological reactions in common. As noted by White in the introduction, these fundamental similarities have been impressive enough to induce the National Tuberculosis Association to sponsor a long investigation in several laboratories on the fundamental facts concerned.

Were the similarities of the diseases limited to these common factors, the usual adjuncts to ordinary bacteriological and pathological technique, such as virulence tests in animals and absorption tests with serums, might prove sufficient to distinguish the causal agents accurately. The utterly confusing fact is, however, that the disease produced by one and the same microorganism changes its type in different animal species, and artificial modifications of a given microorganism cause it to induce different disease processes in the same species of animal. Indeed, the bacterial cause isolated from one clinical type of disease can, by manipulation of the organism or substitution of animal, produce a disease resembling one of the other clinical types of disease more closely than the type from which the germ was originally isolated.

With these facts in mind it seems logical to group these diseases caused by acid-fast bacteria for simultaneous discussion. The taxonomic designation *mycobacteria* for the "acid-fast" bacteria responsible for them has been adopted by the special committee on nomenclature in the Society of American Bacteriologists, and followed in the American Type Culture Collection. If we accept this it is perhaps in order to think of the whole group of diseases for the moment under the single appellation "mycobacteriosis."

Let me say at this point that I am not proposing the view that the naturally occurring ailments concerned have an overlapping bacterial etiology, or that in some past age the causal agent of one was derived from the causal agent of another by habitat and evolution. This has indeed often been suggested. I merely propose to analyze some of the known facts, and try to indicate a common pattern in these diseases, relying on future research to fill in the many gaps in our present knowledge and show what the larger significance of this common pattern may be.

The Spontaneous Mycobacterial Diseases

Such an analysis will probably have more meaning if brief pictures of the main diseases concerned are presented, or a short description is given of certain pathological processes as they occur in nature without the deliberate interference of the laboratory.

Tuberculosis has long been known as a disease afflicting man, domestic animals and birds. As Stiles has pointed out in a preceding paper of this series, it apparently affects a wider range of animal species than any other specific disease. In man it has been the great killer of the ages, in different times and places causing from one twentieth to as much as one third of the total continuing average mortality. In man it assumes many types, as indicated in the second paragraph of this paper, but in all of them either the so-called "human type" of tubercle bacillus, or the

closely related "bovine type" bacillus, is the causal agent.[2]

Of the domestic animals, cattle and swine are most prone to tuberculosis, while sheep, goats, horses, dogs and cats are considered relatively refractory. However, spontaneous tuberculosis may occur in any of them, and can be induced artificially in all. Wild animals in captivity acquire the disease and occasionally develop it when in their natural environment. Tuberculosis is a common disease of high mortality in primates in the zoological gardens, and has been found even in the lion. The economic importance of the disease is greatest in cattle, the incidence ranging from wide-spread infection involving the majority of herds of a country, where little attempt is made to combat it, to a fraction of one per cent. of the herds, where every effort is made to wipe it out, as in the United States and Canada. The disease may be transmitted from cattle to man through the medium of cow's milk, which is heavily infected in cows with tuberculous udders. According to accepted bacteriological criteria the causal agent of the cattle disease is the "bovine type" of tubercle bacillus.

Tuberculosis in swine is a disease of unknown extent. There is no general tuberculin testing of swine, comparable to the testing of cattle. It appears, however, to be sufficiently common to be of serious economic import. Its medical significance for man, however, is not great, as the organs affected are not ordinarily food products, and the flesh, which is eaten, is rarely affected, and in any event is cooked before consumption. Moreover, sow's milk, unlike cow's milk, is not an ordinary staple of diet. No special type of bacillus characteristic of swine tuberculosis has been recognized. Until relatively recently the bovine type of tubercle bacillus was accepted as the cause of swine tuberculosis, and it appears true that a decline in the latter is paralleling the drop in bovine tuberculosis. Recently, however, it has been startling to learn that as much as 90 per cent. of hog tuberculosis in some regions is due to a bacillus highly pathogenic for birds, and possessing other characteristics identifying it as the "avian" rather than the "bovine" type of bacillus. This foreshadows the main problem that I shall raise presently, but it is a fair question at the moment to ask if an initial concentration of bacteriological effort on hogs might not have given us a "swine type" of tubercle bacillus, to which it was later recognized that birds were susceptible. It is stated, however, that there is some difference in the effect of the two types of bacteria, the "avian" type causing a more or less generalized and the "bovine" type a localized glandular disease in swine.

Tuberculosis affects a wide variety of birds, as noted in Stiles's paper. Between 2 and 3 per cent. of the flocks in this country are affected, so that the economic loss is appreciable. The commercial solution of the problem seems to be slaughter of hens before they get to be old. The disease is believed to be of no medical importance as far as transmission to man is concerned, but there is every indication that the various phenomena of avian tuberculosis are of the utmost significance for our understanding of the fundamental mechanisms of tuberculosis. The disease in birds is due to a form of tubercle bacillus highly infectious for chickens on laboratory inoculation, and classified as "avian" in type. But the organism is also one of the most labile of the bacilli, as respects laboratory modification, and some strains, spontaneously occurring or artificially separated, are intensely infective for rabbits, producing rapidly fatal, generalized disease.

Tuberculosis in cold-blooded animals is a nodular disease, occurring in epidemics of more or less severity in zoological gardens, but of relatively rare occurrence in nature. Its general characteristics have been concisely presented by Aronson in a foregoing paper. Wide generalization of lesions throughout the viscera, prevalence of acid-fast bacilli in the lesions, epithelioid tubercles and caseation are characteristic. In general the disease can be transmitted from a cold-blooded animal of one species to another species of cold-blooded animal, while warm-blooded animals are not susceptible. The simplest explanation is the preference of the organism concerned for lower temperatures than that prevailing in the warm-blooded animals. However, differences in strains of this fourth type of tubercle bacilli, "the tubercle bacillus of cold-blooded animals," have been worked out by Aronson, and Gonzalez has recently found that an organism isolated from a frog, and readily affecting other frogs, is pathogenic for mice.

It will be apparent from the foregoing that a series of animal "bridges" can be used to link up the pathological processes caused by tubercle bacilli of the various types. The "human" type infects man, but not swine or fowls. The "bovine" type infects man and swine, but not fowls. The "avian" type infects swine and fowls, but not man. Guinea-pigs are readily susceptible to human and bovine, but not to avian infection, and rabbits succumb to bovine and avian, but not to human type bacillus infection. All three types of tubercle bacillus produce tuberculosis of some degree in some strains of mice, and if Gonzalez' claim is verified, the bacilli of cold-blooded animals, to which warm-blooded animals in general are refractory, may also cause tuberculosis in the mouse.

We now come to the disease of next greatest importance as far as man is concerned, *viz.*, leprosy. As

[2] Reports of tuberculosis in man caused by the "avian type" of bacillus are not generally credited in this country.

Burgess and Doull have pointed out, this disease is at present largely confined to tropical and subtropical countries, although once far more wide-spread, and still afflicts some three million people throughout the world. From the medical point of view it is a disease of many problems. The question of transmission has so far entirely escaped solution. As the several papers devoted to leprosy in this symposium have made clear, the disease has spontaneously decreased throughout recent history, and is obviously of a low order of contagion, while constitutional susceptibility apparently plays a rôle, a sexual difference being apparent, and only a fraction of those exposed contracting the disease.

The etiology has been one of the most baffling problems ever undertaken by bacteriologists. The involved tissues teem with acid-fast bacilli, and acid-fast bacilli have been repeatedly isolated, but they seem quite incapable of producing any progressive disease like leprosy. They vary greatly among themselves, but with the exception of the unusually interesting one described in this symposium by Soule and McKinley resemble the numerous common acid-fast saprophytes. Extreme views with respect to these various bacteria isolated from leprous tissues are (1) that no one of them is the cause of leprosy, the bacteria isolated being simply saprophytic acid-fast contaminants, and (2) that all of them are the cause of leprosy, attenuated and changed, one from another, as a result of artificial culture.

The notable histopathological characteristics of leprosy in its internal manifestations, described in the paper by Black, have much in common with some kinds of animal tuberculosis, such as generalized avian tuberculosis in fowls, the "Yersin" type of tuberculosis in rabbits, rat tuberculosis and various types of tuberculosis produced in laboratory animals through exaltation of virulence of some strains of tubercle bacilli. Yet leprosy is a disease of great chronicity. Immunologically it does not seem related to tuberculosis, unless the high mortality from pulmonary tuberculosis argues for a special susceptibility in the leper.

An equally puzzling, if much less important, disease is the so-called "rat leprosy," an ailment of rats characterized by skin nodules containing many acid-fast bacilli, which also appear to defy cultivation. Leprologists refuse to admit, however, any further resemblance, and certainly it is true that human leprosy tissue will not infect rats, nor will any of the numerous acid-fast cultures isolated from leprous tissues.

Another disease, with likeness to both tuberculosis and leprosy, is the unnamed "skin lesion" disease of cattle. A significant affiliation with tuberculosis lies in the fact that the pathological tissue contains numerous acid-fast bacilli and the animals themselves react to tuberculosis, with resultant serious confusion in diagnosis, leading to unwarranted slaughter, as indicated at length in the papers by Crawford and Daines. The resemblance to leprosy, previously stressed by Daines, rests chiefly on histological similarity of the skin lesions.

The unique condition called Johne's disease or paratuberculosis of cattle, described in detail in this symposium by Hagan, resembles all the other diseases of this series in the two respects of causation by acid-fast bacilli, and remarkable association of these bacilli with the large phagocytic cells, or modified monocytes, to which frequent reference has already been made. Another point of contact is the fact that cattle afflicted with Johne's disease react to tuberculin produced from avian type tubercle bacilli, apparently with even greater intensity than they react to the homologous "tuberculin" produced from cultures of Johne's bacillus. Moreover, a close relationship can be demonstrated serologically. A striking difference, however, is the virtual restriction of involvement of Johne's disease to the intestine. In this disease, interestingly enough, the causal organism, visible by millions in the intestinal mucosa, also long defied cultivation, but was grown at last when a needed accessory growth factor contained in acid-fast bacilli was added to the culture media.

Of other diseases caused by acid-fast bacteria nothing further need be said, as they are too ill-defined in present-day literature for good picturization. Reference should not be omitted, however, to the great group of acid-fast bacteria of no recognizable pathogenicity, the chief representatives of which are the smegma bacilli, which have a saprophytic existence on moist cutaneous surfaces, and the great group of "grass bacilli" saprophytic on various kinds of grass. To these are being constantly added newly discovered strains from soil and sewage, which are not pathogenic for the larger laboratory animals, but appear to have limited pathogenicity for cold-blooded animals. The occurrence in grass raises a suspicion of their relation to the skin nodules of cattle, and a possible relation of some strain to the causation of leprosy is at least not ignored in all comprehensive considerations of the latter subject.

The Mycobacterial Diseases Experimentally Produced

The foregoing review brings us to the more modern period of experimental pathology. It is the latter science that has exposed the puzzles described, and only through continued progress in experiment is there prospect of their solution. The fundamental problem

is the variation in type of mycobacterial disease in different animal species, the question raised by Corper in the closing discussion of the first group of papers presented in this symposium. The same problem has been stated in different forms by other speakers in this symposium, with hypotheses for explanation. The following remarks are composed largely from the suggestions they have offered.

In analyzing the material at hand on the subject of pathogenicity, it may be well to restate certain fundamental facts with illustrations. This will be done under five headings:

1. *There is variation in the disease caused by one strain of acid-fast bacilli in different animal species.* The most familiar example is the effect of the "human type" bacillus, isolated from human pulmonary tuberculosis, on guinea pigs and rabbits. Small doses produce progressive, fatal disease in the former, and restricted lesions with regressive tendency in the latter animal. A greater range of pathogenicity is observed if the selection of animals is widened. Some years ago Vorwald injected the same dose of human type tubercle bacilli, per unit of animal weight, intravenously in a series of monkeys, guinea pigs, rabbits, dogs, cats, chickens and turtles. The result was massive caseating tuberculosis in the monkey, only slightly less intense generalizing progressive disease in the guinea pig, initially progressive but soon essentially stationary lesions in the rabbit and the dog, very slight response with early regression in the cat and chicken, and no detectable lesion in the turtle.

2. *There is variation in the disease caused by different strains of acid-fast bacilli in one animal species.* A familiar example is the effect of bovine, avian and human type tubercle bacilli and other acid-fast bacteria, as Johne's bacillus, on the rabbit. The bovine microorganism produces progressive fatal disease; some strains of the avian bacillus cause rapid generalized disease with early fatality, while other strains lead only to a restricted pathological process; the human strain with infrequent exceptions induces localized disease with little tendency to progression; and Johne's organism causes no disease at all. The organisms isolated from skin lesions in cattle, the emulsified tissues of lepers, rich in acid-fast bacilli, and the large array of saprophytic acid-fast bacilli, also cause no disease, or at the most only foreign body tubercles. The same set of bacteria injected into other animals would lead to an equally wide range, but by no means the same serial order of pathogenicity.

3. *There is variation in the disease caused by one strain of acid-fast bacilli in one animal species modified by special treatment.* The most familiar example is infection of normal and immunized guinea pigs with the same dose of a given strain of tubercle bacilli. As noted previously, an ordinary virulent human type tubercle bacillus causes progressive fatal disease when injected into a normal guinea pig. However, attenuated tubercle bacilli of but slight virulence are available, which do not set up progressive disease in the guinea pig, but do induce a protective immunity. Well-known examples of such attenuated bacilli are the R_1 bacillus of Saranac Lake and the BCG of the Pasteur Institute. If virulent human type tubercle bacilli, in a dose fatal for a normal guinea pig, are injected into guinea pigs a month or two after a preliminary inoculation with a suitable dose of the attenuated organisms, the virulent bacilli do not set up a rapidly progressive tuberculosis, as in the normal guinea pigs, but a different type of disease, of much slower course. A similar variation in virulence can be demonstrated for other acid-fast bacilli in other animals, as, for example, bovine type tubercle bacilli in normal and immunized rabbits.

4. *There is variation in the disease caused by one strain of acid-fast bacilli in one animal species unmodified by special treatment.* All investigators in tuberculosis know that individual differences occur within a single animal species in respect to resistance to infection with tubercle bacilli. The well-known experiments of Lewis and Wright years ago proved that this variation was on a genetic basis. More recently Lurie, as he reported in this symposium, has shown that rabbits are similarly variable, breeding true as respects susceptibility through successive inbred generations. Indeed, as he pointed out, the disease in the most resistant strain of rabbit departs so far in character from disease in the susceptible strains as to remind one of infection of immunized rabbits, or even a different animal species entirely.

5. *There is variation in the disease caused by one strain of acid-fast bacilli, modified by special treatment, in one animal species.* This is the most recent discovery in the investigation of diseases caused by acid-fast bacteria, and in the minds of many investigators holds the key for the whole problem of variable pathogenicity. The pioneer studies of Petroff and the recent extensive, illuminating investigations of Smithburn and Sabin have furnished an entirely new approach to the problem. A few illustrations will suffice. A culture of tubercle bacilli isolated from a chicken suffering from avian tuberculosis and inoculated into another chicken causes tuberculosis in the second chicken, which may or may not be as severe and progressive as in the first chicken. If, however, the culture is planted on certain media, colonies of two different types develop, commonly designated as "rough" and "smooth," together with colonies of intermediate character, which apparently represent mixtures of the two individual types. The two new strains

thus "dissociated," when inoculated in equal dosage into chickens, produce very different effects, the one type being highly virulent and the other of relatively low virulence. Various media seem suitable for this differentiation of strains from an original single strain, but the results are inconstant. Apparently the loss of virulence that occurs with many cultures on repeated laboratory transfer is a manifestation of the same phenomenon. Smithburn has recently been able, however, to produce at will these dissociation modifications of strains of human and bovine origin merely by controlling the reaction of the culture medium. The organisms grown at pH 6.0 tend to be attenuated and those grown at pH 6.8 of high virulence within a single animal species. As Sabin has described clearly in her paper in this symposium, out of an original virulent bovine type culture producing rapid diffuse tuberculosis of the "soft" type in rabbits, an attenuated "dissociated" strain can be isolated by cultivation at an appropriate reaction, which will produce only discrete "hard" tubercles in rabbits. Sabin and Smithburn have called attention to the similarity of the "hard" tubercle produced by an attenuated dissociated strain of the avian type bacillus in a susceptible animal, like the rabbit, and the "hard" tubercle produced by the corresponding virulent dissociated strain of the same bacillus in a relatively insusceptible animal, like the dog. They compare the apparent reduction in virulence in the different animal species to the reduction of virulence directly induced artificially by cultivation on suitable media. Sabin concisely expressed it thus: "A species of animal which is highly resistant to a strain of acid-fast organisms virulent to some other species degrades the bacilli to the level of producing only hard tubercles." In support of this view is Smithburn's observation that bacilli isolated from a resistant animal after inoculation with a culture highly virulent for another species of animal show certain morphological changes from the original bacilli and from those isolated from a susceptible animal. Further work is needed, however, to prove whether or not these changes are associated with measurable and lasting loss of virulence for the susceptible animal.

It will be recalled, in this connection, that there is much evidence, such as that recently submitted by Jensen and Frimodt-Möller, indicating that animal passage may occasionally result in dissociation of the injected tubercle bacilli into strains of unchanged and increased virulence. The facts recorded, if finally confirmed, may explain in modern terms the well-known phenomenon of increased virulence with animal passage.

The Variables in Mycobacterial Disease

In reviewing the facts just outlined the reader must be struck by the lack of constants in the problem of pathogenicity. Both the infecting bacillus and the infected animal are variables. The resultant disease represents the interplay of two inconstant forces. Obviously neither the bacillus nor the animal controls the situation. Moreover, each modifies the other, if we accept all the results just outlined. The bacillus may change the character of the animal by "immunizing" it, and possibly the animal changes the character of the bacillus by "degrading" it, on the one hand, or separating out a strain of increased virulence, on the other.

A simple illustration raises a major problem. A bacillus isolated from a case of human pulmonary tuberculosis produces progressive disease in the guinea pig, and only little disease, and that of quite different character, in the rabbit. Why is the disease so restricted in the latter when the bacillus is so virulent for the former animal? It is not because the bacillus can not multiply in the rabbit. In fact Lurie has shown that at first it multiplies very rapidly there. But the initial multiplication itself seems a favorable factor for the rabbit, because rapidly the latter animal changes in character and becomes "immune" or at least of exalted resistance to further multiplication of the bacillus. On the other hand the bacillus itself may have suffered in the process, *i.e.*, been "degraded" in virulence, as suggested by Smithburn and Sabin. Have we, then, two explanations of immunity, one postulating a specific increase in resistance of the animal, and the other a specific decrease in virulence of the bacilli, or are these merely two forms of expression of the same fact?

In considering this we should return to one of the fundamentals in the pathology of the several diseases under consideration in this symposium, and recall that pathogenicity is a function of the monocyte and its derivative the epithelioid cell, whatever be the animal concerned. Within wide limits it appears to be true that the animal is as resistant as his mononuclear phagocytic cells. Animals whose monocytes are favorable to the growth of acid-fast bacilli, as White has repeatedly pointed out, are susceptible animals. Animals, on the contrary, whose monocytes do not support the growth of these bacilli, are resistant animals. The statements made for the resistance of animals in preceding paragraphs hold equally well for the resistance of the mononuclear phagocytes of those animals. As Sabin has written concisely in her paper, "in the lesions produced by avirulent or attenuated organisms it is probable that the bacilli are killed in the monocytes, while with the virulent strains, on the other hand, the monocytes are killed by the bacilli."

That the monocytes may change in a single animal infected with a single strain of bacilli, permitting rapid growth at first and little growth later, has been shown by Lurie. As he points out, the longer the dis-

ease produced by avian type tubercle bacilli lasts in the rabbit, the more it assumes the character of tuberculosis produced by bacilli of mammalian origin. At first it is of the "Yersin" type, a diffuse infiltration of mononuclear cells stuffed with acid-fast bacilli. Later the diffuse process disappears and nodular ("hard") tubercles with a necrotic center remain, formed essentially of mononuclear cells with few bacilli.

In the observations of Sabin and Lurie the nature of the epithelioid cell of tuberculosis and other diseases of mycobacterial origin seems apparent. Sabin has shown by experiment that through phagocytosis and dispersion of the lipid of these bacilli the monocyte becomes an epithelioid cell. Lurie has observed the epithelioid cell to be a phagocytic cell that has killed a large number of bacilli. In brief the epithelioid cell would seem to be one in which accumulation of bacilli first occurred, followed by their destruction and a characteristic dispersion of their ingredients.[3]

THE CONSTANTS IN MYCOBACTERIAL DISEASE

This discussion of the variables of tuberculosis and allied diseases brings us to our final consideration, the constants. Little enough is known about them, in spite of extended research. They are the chemical constituents of the reacting components in the disease, *i.e.,* the acid-fast bacilli and the cells accumulating about them. Of the essential distinctive chemistry of the cells, we know practically nothing. We must dismiss that subject from our consideration, not because of its lack of importance, but from almost complete lack of evidence upon it. On the chemistry of the acid-fast bacilli we are much better informed, although here, too, the gaps in our knowledge are immense. For years the National Tuberculosis Association has promoted research designed to discover chemical differences, accounting for variable pathogenicity, in the group of mycobacteria, and in spite of the gaps much valuable information has been obtained. It should be noted that the mycobacteria used in this big comparative study were all grown on the same medium and handled in the same way, in order to eliminate as much bacterial variability as possible.

One of the most distinctive features of the chemistry of the acid-fast bacteria is their common possession of unusual lipids, which apparently are not found in animals or other microorganisms thoroughly studied, like yeast. The high concentration of these substances in the bodies of the bacilli of the different types is also distinctive. It must also be significant that as a general rule the proportion of lipid to total body weight is highest in the pathogenic organisms.

The lipids of the acid-fast bacilli have been grouped by Anderson in three classes, phosphatides, fats and waxes. All are distinctive. Unusual features are their content of a liquid saturated fatty acid of high molecular weight, not encountered elsewhere, and the presence of distinctive carbohydrates. The fat-like substances do not contain glycerol, like true fats, but, instead, the disaccharide trehalose.

It is noteworthy that the phosphatides and certain fatty acids derived from them, as shown by the cooperative work of Sabin and Anderson, are active stimulants for monocytes and the development of epithelioid cells therefrom. Typical epithelioid tubercles, indistinguishable from those produced by dead tubercle bacilli, or early ones caused by living bacilli, can be induced at will in experimental animals by the lipid alone. Naturally one at once tries to link this fact with the pathogenicity of the organisms. The phosphatides seem to be characteristic of the group rather than any specific strain, and it may indeed be true that the chronic epithelioid cell reaction in all the diseases here considered is due to the mutual possession of large quantities of this phosphatide. It is interesting that Anderson has found that although quantitative differences in phosphatide content obtain among various strains of tubercle bacilli, qualitatively the latter are alike. The hydroxy acids combined within the wax-like phosphatides differ, however, with the different types of acid-fast bacteria, and so do the combined carbohydrates. The latter, as derived from the pathogenic human and avian types of bacilli and the non-pathogenic timothy grass bacilli, are entirely unlike.

Again in the great carbohydrate fraction uncombined with lipids, certain possessions in common are apparent, as brought out by Heidelberger and Menzel. Glycogen appears to be distributed through the group, as well as certain other serologically active and inactive polysaccharides. It was naturally hoped that serological distinctions, comparable to the well-known differences between types of pneumococci responsible for pneumonias of varying severity in man, might be discovered, but the most careful effort has failed to demonstrate any qualitative distinction between the polysaccharides of the bovine and human type of tubercle bacilli, although the disease produced by these two types in man is quite different. Thus the rôle of

[3] There are difficulties in the way of this view. In the epithelioid tubercles of the lymph nodes of man, no matter how young these tubercles are, acid-fast bacilli are very rare. It may be true, however, that a continuous process of accumulation and destruction goes on, so that but few bacilli are found at any given time. Moreover, we can not overlook the possibility that tubercle bacilli, such as those described by Kahn, may be present in epithelioid cells and never mature to the point where they give evidence of their presence by stains for acid-fastness. In this connection it is well to recall also that by far the largest accumulations of tubercle bacilli found in human tuberculosis are not in the epithelioid tubercles at all, but occur as free-growing masses in softened caseous debris.

carbohydrates in the disease process is uncertain, although they have definite toxicity for certain tissue and blood cells, and thus are accountable for some of the anatomical damage and probably for certain of the symptoms of disease.

In the protein fraction of the mycobacteria, as indicated by Seibert in this symposium, important differences are becoming increasingly evident. And just as the lipids appear to be responsible for certain of the more chronic anatomical features of the disease, so the proteins appear to be the cause of some of the acute responses. Indeed just as certain features of the disease can be reproduced by the lipid alone, so certain other elements of the pathological process can be constantly called forth by the protein. Experimental evidence would seem to indicate that the serous and much of the cellular elements of the exudations of tuberculosis are due to the protein, or are caused by derivatives of the bacillary proteins. The latter are intimately related to the whole phenomenon of hypersensitiveness. Of themselves they readily induce a type of hypersensitivity, and in hypersensitive animals, in exceedingly minute quantity, they cause severe reactions. It is notable that a great distinction holds among the mycobacterial diseases in respect to hypersensitiveness. The mammalian forms of tuberculosis are characterized by marked hypersensitiveness, and avian tuberculosis and Johne's disease by hypersensitiveness of lesser degree. Curiously, animals affected with Johne's disease react to the tuberculin, which in the final analysis means the protein, prepared from avian type tubercle bacilli more intensely than to tuberculin from Johne's bacillus itself.

In leprosy, up to the present time, hypersensitivity has not been demonstrated. In our present lack of certain information on the bacterial cause of leprosy the problem can not be investigated in the same way as tuberculosis. Proteins are easily obtained from the avirulent acid-fast bacilli so far isolated from leprous tissues, but they do not cause specific tuberculin-like reactions in lepers, as shown in a recent extensive study by McKinley and his colleagues in the Philippines. Henderson has compared these proteins with each other and with the proteins of other acid-fast bacilli by serological methods and found certain similarities and certain differences, the theoretical importance of which may possibly be great, although at present uncertain.

Cattle suffering from mycobacterial "skin lesions" are hypersensitive, but strangely enough appear more sensitive to tuberculin produced by human and bovine types of tubercle bacilli than to the tuberculin, or, in other words, protein of the skin lesion organisms themselves, an anomaly reminding one of the relation between Johne's disease and the avian type of tubercle bacillus.

Seibert, Heidelberger and Crawford, as noted in this symposium, as well as others, have discovered distinct and apparently constant differences in the proteins produced by different strains of acid-fast bacilli, using serological methods and the skin reaction in infected animals as criteria for identity or distinction. By these methods the proteins of the human and bovine type bacilli appear practically indistinguishable, while that of the avian bacillus is readily recognized as different. Other acid-fast bacilli, such as the timothy grass bacilli, the so-called leprosy bacilli isolated from leprous tissue, but avirulent, and the tubercle bacilli from cold-blooded animals, all show easily detectable differences. It is noteworthy, however, that they also reveal certain similarities. Indeed, there is a certain amount of similarity in the proteins of the entire mycobacterial family, as shown by cross precipitation within a certain range between the various proteins as antigens and the various antisera. Presumably, as emphasized by Seibert, Feldman and Crawford in this symposium, the overlapping sensitization, as demonstrated by the tuberculin type of test, is dependent on an underlying common possession of certain chemical groups in the antigens concerned. Indeed, Seibert has now sufficiently investigated the chemical composition of the proteins concerned to be certain of definite similarities and variations demonstrable by chemical analysis.

Thus in final analysis we seem concerned with certain biologically active chemical substances capable by themselves of inducing characteristic features of disease, differing from organism to organism, and yet with certain similarities withal. Studied intimately they are seen to exert specific effects on the great defensive cell of the body, the mononuclear phagocytic cell or monocyte. This can be shown not only in the intact animal, as described in this paper, but directly by interaction of both chemical and monocyte as described by Aronson, the one removed from its parent substance, the acid-fast bacillus, and the other explanted from its parent substance, the animal body.

SUMMARY

1. Tuberculosis, leprosy, the "skin lesion" disease of cattle, Johne's disease, rat "leprosy" and a series of ill-defined ailments of rodents, cold-blooded animals and birds, constitute a group of diseases of spontaneous natural occurrence, with two distinctive features in common: (1) causation by mycobacteria, *i.e.*, bacteria distinguished by the staining property of acid-fastness, due in turn apparently to mutual possession of certain chemical substances, and (2) a host response characterized by extensive proliferation and accumulation of large mononuclear phagocytes, or monocytes, and their development into "epithelioid" cells.

2. In addition to the naturally occurring mycobacterial diseases, just listed, a wide variety of disease processes caused by acid-fast bacteria can be induced experimentally by infecting different animals with the various bacilli of the group. Variations as follows can be produced at will:

(a) From one strain of mycobacteria in different animal species.

(b) From different strains of mycobacteria in one animal species.

(c) From one strain of mycobacteria in one animal species modified by immunization.

(d) From one strain of mycobacteria in one animal species with genetic variability in susceptibility.

(e) From the dissociated elements of one strain of mycobacteria in one animal species.

Thus a wide variety of mycobacterial diseases occurs as a result of animal and bacterial variability.

3. Although host and infecting agent are variables, constants occur in the chemical constituents of the two. Of the chemistry of the cells of the animal body which accumulate in the characteristic lesions of this group of diseases, little of significance is known. In contrast, much information is available on the chemistry of the artificially grown mycobacteria. Lipids, proteins and carbohydrates are distinctive, qualitative and quantitative differences being detectable within the group. The lipids act as stimulants for the large mononuclear phagocytic cells, and the proteins also call them forth in the acute processes of the diseases concerned. In general the inflammatory exudations and the toxic necroses seem to be the result of protein action, particularly after "hypersensitiveness" is induced in the course of the disease, while the chronic changes are due in large measure to the bacillary lipids. The ultimate "epithelioid" appearance of the characteristic cells making up the lesions of the several diseases concerned appears to be the result of destruction of acid-fast bacilli within them and cytoplasmal dispersion of their constituent lipids. Some of the carbohydrates as well as proteins appear to be toxic for animal cells.

4. With these facts at hand the great variability of mycobacterial disease is understandable as the result of interplay of variable bacteria and variable animal cells, each with its individually characteristic content of biologically active chemical constituents. This can be shown readily for the experimental disease in the laboratory, and the facts brought to light suggest plausible explanations for the great variability of spontaneous mycobacterial disease in nature.

OBITUARY

DAVID WILLIAM MAY

IT is with a deep sense of loss that his former colleagues record the death of David William May, retired director of the Puerto Rico Experiment Station of the United States Department of Agriculture. He died on December 12, 1937, at Mexico, Mo., and was buried in Lexington, Ky.

Director May was born in Platt County, Mo., on April 22, 1868. Educated in the schools of that state, he received his bachelor's and master's degrees from the University of Missouri in 1894 and 1896, respectively. He spent three years as assistant agriculturist at the Missouri Experiment Station and two years in Washington as assistant in agriculture in the United States Department of Agriculture. He was appointed animal husbandman at the Kentucky Experiment Station in 1901, and three years later became director of the Puerto Rico Experiment Station, in which position he continued for twenty-six years.

Director May came to Mayaguez only two years after the station had been established by act of Congress in 1902; at that time he was but thirty-six years old. He gave to the station the most productive years of his life, and his impress will remain for many years. He secured appropriations for and supervised the erection of the station laboratory and office buildings, developing an architecture which is constantly admired for its beauty as well as its adaptability to its tropical setting and climate. The lands operated by the station were extended to 420 acres. He and his staff assembled what has been stated to be the largest collection of tropical plants in the Western Hemisphere.

Director May did much to build up and diversify the agriculture of Puerto Rico and put it on a more self-sustaining basis. He also helped to devise and introduce improved methods, and he shared in the eventual development of the station as a tropical outpost for the United States Department of Agriculture. He served for many years as a trustee of the University of Puerto Rico, and it was primarily due to his initiative that the College of Agriculture and Mechanic Arts of the university was founded and located at Mayaguez contiguous to the station.

Mr. May's colleagues feel that no written word can be an adequate tribute to his accomplishments and personal qualities, but that the beautiful gardens and buildings of the experiment station itself will constitute a fitting memorial to him.

CARMELO ALEMAR
ATHERTON LEE