REPRINTED ARTICLE

[Editor's Note: Supplementing previous reminders of the interrelations of mycobacteria (The Journal **34** (1966) 57-60; and **34** (1966) 44-55) the following extracts from an essay by Allen K. Krause, Editor of the *American Review of Tuberculosis*, 1917-1940, are reprinted. In this essay Krause reviewed the discovery of certain pathogenic and non-pathogenic mycobacteria before 1900, principally by French and German investigators, and raised issues not yet settled, viz., the possibility of transmutation among mycobacteria and the logic in use of avirulent mycobacteria as immunizing agents against the pathogenic mycobacteria of human disease.]

The Non-pathogenic Acid-fast Bacilli

Their Discovery and Occurrence[1]

Allen K. Krause, M.D.

On the twenty-fourth of February, 1897, a scientist by the name of Dubard found a tumor in a carp that had been caught in a fish hatchery at Velars-sur-Ouche. He took the tumor for further study to Professor Bataillon; and, the following May eighth, Bataillon and Dubard, in association with a third investigator, M. Terre, told the Biological Society a story which initiated one of the most remarkable episodes in the history of tuberculosis.

They reported that the tumor, about the size of a pigeon's egg, had been situated in the fish's abdomen, and that it was not characteristically tuberculous, when viewed either with the naked eye or with the microscope. In one or two respects, however, it did suggest tubercle—for instance, it contained giant cells; and, when properly stained these giant cells exhibited numerous acid-fast bacteria that looked exactly like tubercle bacilli.

The authors had cultivated these bacilli and had found their biological characteristics to be somewhat different from those of real tubercle bacilli. The carp bacilli grew more rapidly than the latter on the standard media. They also grew at temperatures which were entirely unfavorable to the development of tubercle bacilli. At ordinary room temperature they flourished best. Nevertheless, with great care they could be trained to grow slowly at the temperature of the body; and, as this happened, they then took on certain properties that were more peculiar to human tubercle bacilli.

Such was the gist of the first communication on the carp bacillus; in diseased tissue in a fish, diseased tissue which may or may

[1]Reprinted with the permission of the National Tuberculosis Association from the *Journal of the Outdoor Life* **17** (1920) 222-225.

not have been tuberculous, though the authors inclined to the opinion that it was, there were bacteria which had all the appearances and peculiar staining properties of real tubercle bacilli; and, although these bacteria had habits of growth that tubercle bacilli do not possess, they could be made to take on the cultural characteristics of the latter.

Now this was not the first time that acid-fast bacilli had been seen in disease in lower animal forms than man, cattle, swine, hens, etc. In 1889 Sibley, working in Recklinghausen's laboratory in Strassburg, described having observed bacteria, which in every respect resembled human tubercle bacilli, in several tumors that he had found in a snake, a ringadder. Several years later Sibley reported similar observations which he had made on diseased tissue from a python. Meanwhile Shirley and Gibbs (1890) in a short contribution had mentioned detecting acid-fast bacilli in lesions in the livers of several pythons and boas. Sibley, as well as Shirley and Gibbs, were convinced that they had come across cases of tuberculosis in snakes and that the disease had been set up by tubercle bacilli. Shirley and Gibbs even hazarded the opinion that the snakes had become infected by feeding on material from tuberculous birds.

But Bataillon, Dubard and Terre were the first to *isolate* and *cultivate* acid-fast bacilli from diseased cold-blooded animals. And as soon as their work became known there opened up an unusually promising vista of new and fundamental studies to be made on the nature and prevention of tuberculosis.

More than one eminent authority had indulged in speculation concerning the genesis and evolution of tuberculosis throughout the world. By 1890 it was generally known that a variety of animal species became infected with tubercle, that the causative agent of infection was a very low form of vegetable parasite which had such unusual and peculiar properties as to put it in a class by itself, and that at least two types of this parasite—human tubercle bacilli and avian tubercle bacilli—were in existence. It was also taught that human tuberculosis, a disease as old as recorded history, was unknown among men who lived a nomadic life, and that we first become familiar with it after man has advanced a step in civilization—among pastoral peoples who live in close association with their herds and flocks. Though too late to prove the inference by direct scientific observation, was it wholly improbable that man first acquired tuberculosis by contact with his cattle?

But if cattle tuberculosis antedated that of man, from what source did cattle first receive the infection? It must have been from still lower forms of life or through the medium of microorganisms which existed free in nature—on plants, for instance, commonly used by cattle for food. Who could affirm that acid-fast bacilli did not, either at some far anterior time or perhaps even at the present, exist widely distributed throughout the vegetable world? And, even though they were incapable of bringing about progressive tuberculosis in animals, could it not be possible that, if such bacteria really had being, they might enter the bodies of animals, remain and perhaps slowly develop there; and by long-continued residence in the animal body, gradually undergo such changes that they would be converted from non-pathogenic to disease-producing forms? Once pathogenicity, parasitism, were acquired, the cycle of infection would take care of itself, as bacteria were passed on from animal to animal by contact.

And now Dubard's carp bacillus, apparently tubercle bacilli from what were apparently tuberculous masses in fish, had come; to forge one more link in the evolutionary chain of the most widespread of all infections and to give point and plausibility to speculations such as we have just set down.

But a still more remarkable discovery was hard at hand. Even before the existence of the carp bacillus had become common knowledge, the finding of acid-fast bacilli, free in nature and dissociated from animals, was announced.

Alfred Moeller is the man who had gone about the work of examining plants for tubercle bacilli or tubercle-like bacilli. He was a physician attached to the pioneer tuberculosis sanatorium, Brehmer's in Goer-

bersdorf; and in 1898 he reported having isolated acid-fast bacilli from several plants, but particularly from timothy grass. These acid-fast bacilli were not real and fully developed tubercle bacilli. They would grow much more rapidly than the latter. They would also develop well on media incubated at lower temperatures than that of the body. But, what was very suggestive and apparently ranked them closer than Dubard's bacilli to tubercle bacilli, was that like the latter they were also easily cultivated at body temperature, when they grew even more luxuriantly than tubercle bacilli.

But would they produce disease? Not in the sense that tubercle bacilli brought about lesion; that is, a very few timothy bacilli, inoculated into guinea pigs, would not originate a progressive cycle of lesions that led in time to an animal's death, a cycle which a few tubercle bacilli always initiated. Moeller had, however, tested the pathogenicity of the timothy bacilli, and reported that very large doses of the *first* culture from the grass, when inoculated into the abdomen of a guinea pig, brought about the typical lesions of tuberculosis which caused the death of the animal in from six to eight weeks. After the microorganisms had been cultivated for several generations on glycerin broth or glycerin agar, they were no longer pathogenic for laboratory animals. In Moeller's opinion, the timothy bacillus was to be looked upon as an acid-fast form, related to the tubercle bacillus, but of much less pathogenicity than the latter and, under natural conditions, harmless for man and the higher animals.

° ° ° ° °

Here, then, was the entire evolutionary chain completed. Acid-fast bacilli did commonly occur in nature, on plants, grasses and vegetables. They looked exactly like tubercle bacilli and stained like them. They did not produce disease in the same way: yet, lacking further evidence, nothing could be more plausible than to imagine that, taken in with food by cattle, in some instances and for some unknown reason, they gained a foothold in these animals. Prolonged residence in the animal body had gradually brought about certain changes in their habits. They slowly lost their ability to grow at ordinary temperatures, so much so that at last the temperature of the animal body was strictly necessary to their development. Finally, as they had become evolved or transmuted into what we know as tubercle bacilli, they acquired parasitism; and attachment to animal tissues became necessary to their characteristic development—to such an extent that, outside of the body, their cultivation had become a matter of great difficulty. After this had happened, tuberculosis of cattle was at last established: and with tuberculosis of cattle a fact, it was not difficult to assume how tuberculosis of man originated.

° ° ° ° °

A very few years later (1902-1903) medical annals recorded another startling discovery. F. F. Friedmann told of finding spontaneous tuberculosis in the lungs of two marine turtles that had died in the Berlin Aquarium. In one there was "tuberculosis of the entire right lung with large cavities"; in the other, "tuberculosis of both lungs with innumerable miliary tubercles and large caseous masses." In both were perfectly acid-fast bacilli which in size, form and arrangement could not be distinguished from human tubercle bacilli. The bacilli were not identical, in Friedmann's opinion, with Dubard's carp bacilli. He had cultivated them, and found them very close to human tubercle bacilli; yet, because of having resided in the turtles, changed in several important particulars from real bacilli. To use his words, they were "real, only wonderfully weakened tubercle bacilli" (echte, nur wundersam mitigierte Tuberkelbazillen). Whence did they originate? From an attendant who fed the turtles and who was suffering from a tuberculosis of both lungs. Here again the discoverer believed that he was in possession of acid-fast bacilli—of tubercle bacilli which, because of having resided in the cold-blooded animal body had undergone changes which amounted to partial reversion to original type.

In 1904 Rupprecht brought forward still another form of acid-fast bacillus, isolated from a cold-blooded animal. In a frog he found what he interpreted as real tubercles

and in these tubercles were acid-fast bacilli of much less marked acid-fastness than were Dubard's and Friedmann's bacilli. He, therefore, considered them a distinct variety.

There thus grew up a separate group of acid-fast bacilli, made up of Dubard's carp bacillus, Friedmann's turtle bacillus, Rupprecht's frog bacillus and the several snake bacilli which were never isolated; and this group was variously called the *cold-blooded tubercle bacilli* or the *pseudotubercle bacilli.* The enormous amount of work that has been done on these, the numerous speculations and theories this work has engendered, the high hopes that it has aroused and the bitter disappointments that later investigations brought in their train, are all part of the tuberculosis story of the last quarter century, and will be touched upon in later sections of this essay.

Meanwhile, acid-fast bacilli were being found in other materials and places. As soon as the tubercle bacillus was discovered and the methods of detecting it in infected material became common knowledge, there naturally arose tremendous eagerness to bring together all the etiological evidence that was possible. The first most obvious and easiest thing to discover was where tubercle bacilli commonly occurred; for, thirty years ago, where tubercle bacilli lurked spelled where disease originated. Hands, lips, nostrils, beef, pork, mutton, eggs, milk, butter, cheese and the greatest variety of articles, all came under bacteriological examination. And at that time bacteriological examination meant for the most part merely the microscopic inspection of a stained smear or section or swab of the suspected material; for, since, with one or two exceptions, tubercle bacilli were the only acid-fast bacilli known, the detection of the latter was practically proof positive evidence of the presence of the former.

It became so common to find acid-fast bacilli, interpreted usually as tubercle bacilli, in butter, that, by the advice of public health authorities, butter as an article of diet bade fair soon to be taboo. But in the fruitful year of 1897, two observers, working independently of each other, reported finding in butter acid-fast bacilli which were not tubercle bacilli.

Petri was the first to announce the occurrence of non-pathogenic acid-fast bacilli in butter—tubercle-like bacilli, growing rapidly at low temperatures and bringing about only minimal lesions when inoculated in large doses into guinea pigs; but the work of Lydia Rabinowitsch was the more complete and therefore the more convincing. Lydia Rabinowitsch had been a pupil of Koch and had also been connected with the Women's Medical College of Philadelphia. She examined 80 samples of butter obtained in the markets of Philadelphia and Berlin. In them she found no real tubercle bacilli, but in 28 per cent of the specimens she did discover acid-fast bacilli which were of questionable pathogenicity for guinea pigs and of none whatever for rabbits and white mice.

A number of other investigators reported similar observations later; and, besides the butter bacilli of Petri and of Rabinowitsch, the literature came to record those of Korn (two varieties) of Coggi, of Markl, of Binot, and of Maria Tobler (varieties I, II, III, IV and V of Tobler).

Moreover, in searching for acid-fast bacilli Alfred Moeller did not confine his attention to plants and grasses. In 1897 he isolated non-pathogenic forms from the dung of cattle and called this microorganism the *Mist* (dung) bacillus; and those familiar with the literature recalled that in 1892 Garth had seen similar acid-fast bacilli in cattle dung, while in 1895 Severn had observed the same forms in the dung of horses. Moeller also isolated nonpathogenic acid-fast bacilli from milk, a demonstration which pointed out that, before milk could be definitely proved to be tuberculous, something more than the mere detection of fuchsin-retaining rods in smears was necessary: animal inoculation must also be performed.

It thus became plain that, besides tubercle bacilli, we must also reckon with a number of practically harmless acid-fast bacilli which were widely distributed in nature. These fell roughly into three main groups: (1) those occurring free in nature, like the two types of timothy bacilli, isolated and described by Moeller; (2) those occurring in association with healthy animals, particularly cattle, like the dung ba-

cillus, the milk bacillus and the several butter bacilli; and (3) those of the cold-blooded animals.

Yet, from time to time, harmless acid-fast bacilli were being described as occurring in man, both in a state of health and in *non-tuberculous* disease. In 1884, Sigmund Lustgarten, then in Vienna and later to come to New York to take his place as an eminent syphilographer and dermatologist, startled the world with the announcement that he had discovered the bacillus of syphilis, that it was an acid-fast microorganism similar to the tubercle bacillus and that it occurred in the lesions of syphilis and under no other conditions. The next year, however, two Spanish investigators, Alvarez and Tavel, proved that Lustgarten's bacillus occurred in many healthy persons, altogether apart from syphilis. It has ever since been recognized as a definite nonpathogenic acid-fast form and has gone under the name of the *smegma bacillus.*

° ° ° ° °

The more significant discoveries of the nonpathogenic acid-fast bacilli were nearly all made in or about the year 1897—at a time when experimentation on active immunization against tuberculosis had become very active. The finding of these microorganisms gave a tremendous impulse to immunity work; for, as has already been mentioned, the ideal of immunization is protection by the use of a bacterium which is closely related to the causative agent of the disease to be guarded against, yet which is harmless for the animal on which it is to be employed. At the time it seemed as though several of these acid-fasts were about to fulfill both these requisites. It, therefore, became imperative to discover, if possible, how closely related they were to one another and to the tubercle bacillus, and to determine whether they were capable of producing disease in man and animals or were certain to remain innocuous under any and all circumstances. It was no less important to make certain whether transmutation of one form to another were possible, that is, whether, under any conditions timothy or fish or turtle bacilli could be transformed into human or avian bacilli, or whether human bacilli were really capable, as Dubard and Friedmann averred, of being changed into cold-blooded forms; and again, whether such a change would be permanent or whether a human bacillus, having become a cold-blooded bacillus, could be transmuted back again into a human bacillus. All these questions crowded fast upon one another as scientific problems to be answered; answered, that is, in only one way, and that by experiment—before one could be justified in using these various new bacilli as immunizing agents, even though it could be proved that they really did confer protection.