by a virus and bacterium, acting in conjunction, are increasing. All we can say is that it appears, with the evidence now available, to be highly improbable.

5. WHAT IS THE SIGNIFICANCE OF GRANULAR FORMS OF THE MYCOBACTERIUM? Hoffmann admits the possibility that some granular forms result from degeneration and disintegration caused

> in part by the defensive substances of the organism and in part by the action of our medicinal products and especially by chaulmoogra oil

so that their presence is to be interpreted as a favorable sign. Yet he concludes that

> The leprosy bacillus . . . produces in its evolutionary cycle great numbers of granular forms which are found both within the bacilli and as free-lying bodies. These granules constitute an essential phase in its evolution. Among the free-lying forms are those of all sizes down to the limits of visibility, so that it is probable that still smaller, perhaps invisible and filterable forms exist, which may be of special though as yet unknown importance in the pathology and epidemiology of the disease.

If the development of granular forms is in truth an essential phase in the life cycle of the bacillus, then killed bacilli injected into the body would be unlikely to form them. Similarly, if the formation of free-lying forms grading down in size to the limits of visibility is likewise a feature in the life cycle of the bacillus, then killed bacilli injected into the body would not be expected to show them. Killed bacilli have been injected on numerous occasions but nobody has taken the trouble to make detailed observations on granularity. Of course the answers might not be clear cut. The dead bacilli might break up into granules which only partly resemble those reported by Hoffmann, and a few rather indefinite particles grading down to the invisible might be encountered.

Like Hoffmann and many others, I have observed that in some biopsy specimens the bacilli are more granular than in others, but I have not had an opportunity to correlate this granularity with a favorable or unfavorable sequence of events in the particular nodule examined. Manalang thinks that under treatment, solid forms become segmented or granular. The difficulty is that we have so little accurate information to serve as a background for interpretation. Although in advanced cases of nodular cutaneous leprosy there are often many nodules, some are recent and active, others older and more mature and still others have regressed, flattened out and become quite fibrous. We do not know whether the degree of granularity is constant, whether it rises and wanes or whether it attains a peak in the oldest nodules. Consequently, though

a single biopsy specimen may exhibit marked granularity, this may or may not be a favorable sign.

According to Holt, who worked with tissue cultures, new bacilli are longer, thicker, more acid-fast, sharply outlined and possess definite bipolar granules, whereas the older ones are apparently fragmented; but how she reaches these conclusions is not clear.

It is probably significant that within a given nodule the degree of granularity of the bacilli is often fairly uniform. It may be rather more in evidence in the fatty lepra cells, but is also manifest in vascular endothelial cells, fibroblasts, the cigar packs of the phagocytes and even within giant globi, when such are present. Granularity may not be simply a stage in degenerative fatty metamorphosis, for fat is not accumulated in all of these locations. It is well demonstrated by staining frozen sections of formalin fixed tissues with Ziehl-Neelsen, hematoxylin and Sudan III. There are several possibilities. The fat may be produced at the expense of the bacilli and disappear very quickly by oxidation from some kinds of cells so that none is seen, and accumulate in others in which it is oxidized less rapidly or not at all. Until fat observed in the lepra cells is definitely identified as foreign to the human body and likely to be produced by the bacilli, there is a chance that it results from alterations in the mitochondria which I have detected in the lepra cells, or simply from failure of these particular cells to oxidize fat which they receive as nutriment in common with other cells of the body. Gross analyses of lesions would only indicate that the fat in the lepra cells is of bacillary origin if the nature of the fat is bacillary and its amount directly proportional to the frequency of lepra cells.

The main point is that the granularity may pervade the particular lesion, or be restricted to localities or even to types of cells. It calls insistently for an explanation. There is another type of granulation always of much rarer occurrence in which globules of distinctly greater diameter than the bacillus are formed. These may be evidences of degeneration, but again, we just don't know. Neither form of granulation is conditioned to any great extent by the kind of fixative used but, as Cowdry and Heimburger found, it is a little less marked after freezing in liquid air and dehydration *in vacuo* while still frozen, thus avoiding both fixation and alcohol.

6. WHAT IS THE SIGNIFICANCE OF LOSS IN ACID-FASTNESS? This question can best be considered by a parallel discussion of the tubercle bacillus. It is customary to stress similarities between leprosy and tuberculosis (Johansen 1937). Strong evidence has been discovered by Kahn

and Nonidez that the formation of granules is a stage in the developmental cycle of the tubercle bacillus. They look upon it as

> a type of segmentation rather than direct fission in which the rate of segmentation surpasses the ability of the elements thus formed to elongate. The bodies become increasingly numerous as they become smaller. The loss of acid-fastness, when the tubercle bacillus is in the granule phase and small-rod phase, may possibly be explained on a metabolic basis. As an example, during the division and subdivision of the various particles, the potential of elongation does not keep pace with the potential of division. Consequently, there is at certain stages seemingly insufficient time for the organisms to metabolize the substances essential for their acid-fastness.

But the granular forms of the leprosy organism are not always less acid-fast than the long rods. It is difficult, at best, to grade acid-fastness. The substance of a tiny particle may be as acid-fast as that of a long rod, but it may not retain the stain so strongly because, owing to its smaller size, stain may be more readily removed from it. And, further, it may look less intensely stained because, being of smaller volume, it absorbs fewer red rays of light than the large rod, though per unit of volume it may be equally stained. Leprosy organisms in the rod stage often lose their acid-fastness without granule formation, so that the phenomenon may not be due in leprosy to metabolic exhaustion caused by rapid multiplication. As Salle and Moser have demonstrated, cholesterol and some other substance must be supplied to provide the building material for acid-fastness. Loss of acid-fastness may be essentially a deprivation phenomenon but its relation to multiplication and granule formation remains obscure as far as the leprosy organism is concerned.

7. WHAT IS THE NATURE OF THE REACTING CELLS? There is a tendency to explain the tissue responses in such terms as are used in tuberculosis. But in doing so, we should make haste slowly. To say, as has been said for tuberculosis (Cunningham et al. 1925) that leprosy "is a disease of the monocytes," would be to make an unnecessarily exclusive statement. Also, to assert that susceptibility is conditioned by the possession of monocytes favorable to the multiplication of acid-fast bacilli, would be to blindly exclude other possible factors.

All those who have their minds focussed on a single cell type, do not call it a monocyte. Thus, Muir speaks of capillary endothelial cells, and Mallory, of endothelial leucocytes. The common designation of phagocytes, mononuclear phagocytes or macrophages is safer, because it is more inclusive and less definite. To speak of lepra cells, thus emphasizing the presence of lepra bacilli in them, is not objectionable except that some restrict this term to large, swollen, lipoid containing, foam or

Virchow cells, while others include the phagocytes before they reach this stage in the response.

A broader conception is that several kinds of cells may be involved. These include all varieties of reticulo-endothelial cells which have the habit of phagocytosing particulate matter, like carbon and vital dyes. The examination of any section through a typical nodule usually reveals numerous bacilli, not only in monocytes and in their derivatives, the closely packed together epithelioid cells and Virchow cells, but also in reticular cells and fibroblasts of the connective tissues, vascular and lymphatic endothelial cells, neutrophilic leucocytes, the sheath cells of nerve fibers, a variety of epithelial cells and perhaps in still others. It is indeed simpler to enumerate those cells which seldom, if ever, contain bacilli: Tissue mast cells and eosinophiles, small lymphocytes and plasma cells (Marschalkó 1895). To follow Unna's original definition of plasma cells is to be out of date. Since Bloom has shown that monocytes are formed from lymphocytes, we know that there comes a stage in the life cycle of some lymphocytes in which they do take in bacilli.

The neutrophiles, in particular, deserve more attention than they ordinarily receive. Instead of affording, like the R.E. cells, a nice place for the bacilli to grow, they destroy at least some of them. Their action may constitute a not inconsiderable factor in resistance to infection. Feldman, in his study of the tuberculin reaction in cattle, reports that at the third hour the polymorphonuclear leucocytes predominate and that after the thirtieth hour they decrease in number as "the mononuclear cells or histiocytes" (monocytes) increase. But Lurie refers to "the impotent polymorphonuclears" and Black states that "The neutrophiles play *no* part in a *pure* leprous lesion." (The italics are mine.) Does he include under the heading of the *pure* lesion all stages in its formation and later history, and has he in mind all of the possible functions of neutrophiles during this long period? To assert that the neutrophiles play *no* part in any particular cellular community in which they may happen to be, such as in this lesion, is to invite contradiction. Since neutrophiles are alive, they require food and give off waste. The products absorbed by other cells when they disintegrate, unless they are phagocytosed by macrophages, are not without influence. Carrel's theory of trephones is to be considered, especially when fibrosis occurs. In tuberculoid (reacting macular) leprosy from which, as we have noted, recovery is fairly frequent, Black admits the presence of leucocytes in "large focal accumulations." They evidently play a more conspicuous rôle in the lesions than they do in those of nodular leprosy (the leproma) from which recovery is exceptional. The energetic way in

which they phagocytose leprosy bacilli *in vitro* has been demonstrated by Denney and Eddy. In cutaneous nodular leprosy the monocytes (or macrophages) dominate the picture, but the possibility cannot be simply set aside that the neutrophiles may have attempted to overcome the invader during the incubation period and failed, and that in individuals, who though exposed did not contract leprosy, they succeeded, perhaps with the help of monocytes. Consequently, susceptibility to leprosy may not result merely from the possession of monocytes favorable to the growth of the acid-fast organisms. It may, on the contrary, be due to the exposure of such monocytes to bacilli not destroyed by neutrophiles. Yet I do not advocate this or any other explanation of susceptibility or resistance based wholly on the properties of any special kind of cell.

8. WHAT IS THE RELATION BETWEEN REACTING CELLS AND BACILLI? White, in describing the association between the tubercle bacillus and the monocyte, says

> It does not require a great stretch of the imagination to conclude that in the early life history of this symbiosis between bacillus and monocyte there is some chemical factor that is common to both through which they contend for the same food supply.

He states that the same conclusion is logical for leprosy and several other acid-fast bacterial diseases. He hazards no suggestion, however, as to what the chemical factor may be, but intimates that the reason why it has eluded us is "possibly because of the difficulties surrounding the study of the internal chemistry of the monocyte."

This conclusion is based on a series of assumptions. We do not know that the monocytes and the bacilli contend for the same food supply. The word "contend" implies a struggle between them for the food. In leprosy, other cells than monocytes become loaded with bacilli. Do they also compete with the bacilli for the hypothetical food? And why does the intracellular position of the bacilli suggest that they are trying to eat the same food as the cells harboring them, anyway? It would seem that if, by virtue of some common factor, the bacilli do strive for the same food as the monocytes, the very worst place to find it would be within the monocytes where effective competition by the monocytes would be greatest. In tuberculosis, Long reminds us that by far the largest accumulations of tubercle bacilli occur as free growing masses. Their location within monocytes is therefore not essential to secure an abundant food supply. In leprosy, multiplication is generally intracellular.

That entry into the monocytes is not necessarily an expression of

search by living bacilli for the same food as that required by the monocytes, is shown by the fact that killed bacilli are taken in by the monocytes in apparently the same way as live ones. The dead bacilli are certainly not contending for the same food supply. Moreover, the monocytes which phagocytose the non-competitive dead bacilli and go on to form epithelioid cells, or fuse to form giant cells, exhibit no differences in structure or behavior suggestive of release from the handicap of possessing live organisms which do contend with them for their food supply.

While we cannot accept the idea that bacilli enter the monocytes and live in them in consequence of the possession by both of a common chemical factor by virtue of which they are enabled to fight for the same food supply, *some* of the bacilli evidently go on living as guests of the monocytes. We say *some,* because the number of bacilli increases and the cells swell. The increase is probably due to multiplication of organisms already intracellular, but the taking in of additional organisms from the tissue fluid about the cells is a possibility, perhaps less certain in the case of leprosy than in tuberculosis, since there are less extracellular bacilli in leprosy than in tuberculosis.

As yet we are unable to tell from their appearance which of the intracellular leprosy bacilli are alive and which are dead. Denney is of the same opinion. It would be interesting to follow the fate of bacilli removed from a leper, killed, marked by staining with fuchsin and injected into an area containing many monocytes charged with bacilli presumably alive. What proportion, if any, of the bacilli in a given lesion are dead is at present purely a matter of speculation. In lesions which are not developing, or are even regressing, the majority may be dead, and this may explain the difficulty sometimes experienced in cultivating them on artificial media.

9. HOW ARE THE CELLS INFLUENCED BY THE BACILLI? Perhaps they are controlled by their own personal properties, by differences in the number or quality of the bacilli and by the special local tissue fluid environments which temper their actions. And there is also the factor of time. The monocytes beginning to respond will look very different from others involved for different lengths of time.

The lesions progress, not by the multiplication of infested cells, but by the addition of more and more of them. As Black remarks

> It is to be supposed . . . that slow disintegration of the phagocytized organisms occurs, as well as death of the phagocytes, but this is not apparent from histological study.

It is remarkable what little evidence there is of cellular degeneration. The bacilli-laden cells seem to enter a kind of living death. That, when immobilized in this way, they go on living is indicated by the appearance of their nuclei and the normal number and shape of their mitochondria. Demonstration of mitochondria and bacilli side by side in the same cells distinctively colored can best be accomplished by fixation in Regaud's fluid and staining with anilin fuchsin and methyl green.

For how many days or years monocytes distended with bacilli continue to live is one of the many unanswered questions. That they lose their functional abilities and become ineffective is clear. Their capacity to phagocytose trypan blue is decreased in rats and probably also in man. They have been partly blocked by the bacilli. They are also unresponsive to supravital stains like neutral red and janus green, whereas their original monocytic state was characterized by the ease of demonstration of neutral red granules and mitochondria. My attempts to reveal Golgi networks and centrosomes in them have not been successful, but this is insufficient evidence of the absence of these components.

Microincineration by the method of Scott shows that a change has been brought about in their cytoplasmic mineral constituents. They exhibit, after application of this technique, more finely granular bluish white ash than they possessed in the first place, before they were choked by bacilli. This ash, viewed in the dark field, is of the bluish white type which is said to contain sodium. That it is probably not the mineral residue of the bacilli is indicated by the fact that it is evenly spread through the cytoplasm and is not heaped up in masses corresponding to masses of bacilli. In respect to the mineral residue of their cytoplasm these thickly packed macrophages resemble closely the cells of sarcomatous tumors as described by Scott and Horning.

Flat white ash suggestive of calcium is inconspicuous, and phosphorus volatilizes off during incineration. There is very little red ash indicative of iron.

Application of the Feulgen reaction for thymonucleic acid, as detailed by Cowdry, yields interesting results. No trace of thymonucleic acid is revealed in the cytoplasm and the nuclei contain comparatively little, less than the nuclei of lymphocytes, monocytes, plasma cells, fibroblasts and polymorphonuclear leucocytes, and about the same as the nuclei of macrophages. Since the volume of cytoplasm is larger in proportion to that of the nucleus (or nuclei) in lepra cells than in most macrophages, it follows that the former possess relatively less thymonucleic acid. In general, those cells with much of it, like lymphocytes which are of all cells the richest, are more radiosensitive than cells poorly supplied with

thymonucleic acid. Consequently this study affords a possible explanation as to why X-ray therapy of leprous nodules is not helpful. The infrequency of mitosis is also noteworthy in this connection.

But the study of the influence of bacilli on the cells has hardly been commenced. Of the many available techniques, few have been employed. Some useful ones will be found in cytological papers on cancer.

10. HOW ARE THE GLOBI FORMED? Globi are clumps of bacilli, rather firmly packed together, and are seen in neither human nor animal tuberculosis. They vary in diameter from, say 4 or 5 to 100 or more microns.

Denney summarizes the opinions expressed about globi as follows

> Some observers have considered them to be intracellular colonies; others have considered them to be clumps formed within lymph spaces, mechanically compressed into spherical or spheroidal form; still others have expressed the opinion that the masses represent colonies of individual rods bound one to another as zooglea. A fourth view is that they may be characteristic colonies growing within an as yet unidentified restraining membrane.

To this last view Denney himself subscribes.

In the absence of a close sequence of transitions from the smallest to the largest globi, the possibility has to be entertained that they do not constitute a homogeneous series and that they may be formed in more than one way, as macrophages may have several origins (reticular cells, endothelial cells, monocytes). To handicap any theory of development of globi by making it apply to all globi is not the part of wisdom, at least for the present.

It is convenient to classify globi under two headings, small and large. The first, in terms of our arbitrary definition, occur only in the cytoplasm of uninucleated cells. The typical cigar packs of bacilli in the macrophages, and the more rounded clumps often seen in endothelial cells, fibroblasts and others, fall into this category. Sometimes the areas of cytoplasm which they occupy stain less intensely and look so clear as to suggest the beginning of liquefaction.

The large globi occur within giant multinucleated cells, or else the space which they occupy is limited by an investment made up of many cells. Some may have the retaining wall constituted in one way and some in the other. To trace their precise origin is not possible with the data available. I have been trying to do so for years, but my studies lack continuity because the required cases of leprosy are far distant from St. Louis.

I shall mention my work on the acid-fast bacterial disease of rats, first, for it gives a background which helps in the interpretation of

human globi. We owe our strains of rat leprosy to the kindness of Dr. E. B. McKinley and Dr. E. L. Walker. There are ordinarily in rat leprosy no globi comparable to the cigar packs in human leprosy. But occasionally we find in the centers of nodules, distant from the peripheral blood supply, masses of bacilli packed in clumps side by side in the cytoplasm. The cells containing them are definitely smaller than those laden with bacilli nearer the periphery. Among them are a few polymorphonuclear leucocytes. Perhaps with reduction in cytoplasmic area and depression in cytoplasmic activity the bacilli are mechanically pressed into packs. However this may be, it is unlikely that these small packs are indicative of multiplication of bacilli—a prevalent notion regarding such packs in human beings.

Lowe states in his review that the bacilli of rat leprosy do not form globi. We have encountered large clumps of bacilli quite frequently in well established rat lesions. They attain a diameter of 25 to 40 microns —never in my experience as large as the maximum in human leprosy. They differ from the giant globi sometimes found in the outer human reticularis about which the cellular infiltration is less dense. Within the past few weeks, Dr. Ravold and I have clearly traced their origin. Some certainly appear in the multinucleated giant cells produced by the fusion of monocytes or macrophages. But we cannot say that their beginnings are not present in single cells. In some lesions most of these multinucleated giant cells exhibit early clumping of bacilli, while in others few if any of them do. Those involved are found chiefly at the periphery of extending nodules, or parts of nodules, near the blood supply, rather than in the more central areas. The bacilli within them are at first distributed without order, but quite evenly throughout the cytoplasm. The clump begins by a localized concentration or multiplication of bacilli and by their orientation in a characteristic radial fashion in all directions from a central point—a "rosette." This arrangement is not to be regarded as a degenerative intracellular agglutination. The increase in number of bacilli is so great, after the first signs of radiation, that multiplication of the bacilli can be safely assumed to take place. This is important because in these stages of evolution of the rosettes the bacilli are typically not granular. On the contrary, their outlines are remarkably smooth and even. In only about 1 in 40 or 50 radiate forms in well developed lesions were the bacilli very granular. In this case the cells containing them showed signs of beginning degeneration.

We have discovered nothing comparable to these radiate bodies in human lesions. The stellate structures which have been reported by several investigators in human leprosy, and which have been lately studied

by Mitsuda, are wholly different formations. They occur in giant cells in conditions other than leprosy. Their nature should be explained, but it has no direct bearing on our problem. It is interesting to note, nevertheless, that I have identified two lepers in Puerto Rico from whom one can conveniently obtain biopsy specimens which always show these stellate bodies in giant cells.

In the further evolution of the large rosette in rats the background of the cytoplasm loses its affinity for stains and appears clear. The radial arrangement of bacilli is lost. They become packed more closely together and a large clear space develops between them and the cytoplasm of the distended multinucleated giant cell. I have as yet no first hand information on the incidence of these rosettes, their place in the disease reaction and their fate.

Some of the large human globi, especially in very cellular lesions, develop likewise in multinucleated cells; but, as I have said, they do not exhibit this radiate multiplicative phase. And there are other differences. They appear, at first sight, when in sections, to be independent globular masses, and Denney has excised fresh tissues and examined them as individuals. But their reconstruction from thin serial sections demonstrates unmistakably that many of them are connected with their neighbors.

In active, densely cellular lesions, the globi are contained in spaces which remind one of a mass of bubbles of unequal size separated to some extent by tissue fluid and by cells and fibers within the spaces. Where the "bubbles" come in contact with one another, the walls generally collapse, and the space is continuous from one cavity to another. Such connections are usually short and wide and their diameter less than the maximum diameter of either of the two confluent spaces involved. One large globus may connect in this way with seven or more others. Passing from one globus to another the sudden sharp inward projections of the walls are faintly suggestive of the valves in lymphatics. Longer and narrower connections are rare. The walls are quite thin. When a large globus is cut through the center, most of its surrounding wall is not nucleated. The few nuclei which are present are situated on one side in a segment of the wall noticeably thicker than the rest. They may be a little more flattened but are otherwise indistinguishable from the nuclei of individual macrophages possessed of small globi. Denney and Eddy have investigated the relation of leucocytes, apparently of the polymorphonuclear variety, to globus formation; but it is certainly not these cells which are involved.

The contents of these large globi are interesting and worthy of more than the haphazard study which has been accorded them. The bacilli

are frequently granular. Many of them lose their acid-fastness and degenerate, but curiously enough there is little if any coincident fat formation or accumulation. Vacuoles comparable to those in the foamy lepra cells are usually lacking. Among the degenerating bacilli is a substance which stains faintly with eosin and brightly with fuchsin in Mallory's combination. This often occurs in discrete masses about the size of a red blood cell and has been observed by many workers. I have not as yet met with this material (gloea) in the large rosettes of rat leprosy.

The materials observed within a globus in stained sections never fill its entire lumen. They generally occupy a central position, leaving a clear area intervening between them and the membrane, but they may be plastered on the inner surface of the membrane. In fresh globi, which I have not studied as thoroughly as Denney, for lack of opportunity, they are probably more evenly spread in the contained fluid. The central accumulation, or the peripheral plastering and intermediate conditions, may be the result of the coagulative action of fixatives.

Nearer the epidermis, in less active lesions which may be older, there are found in some cases still larger globi, the exact relation of which to those just described, is not clear. The cellular infiltration about them is less dense. There are fewer macrophages with small globi and plasma cells, but foamy lepra cells may be fairly numerous.

These globi generally correspond in position to the outer lymphatic plexus in the reticular and papillary layers of the dermis. In some specimens a single globus occupies almost all the area of a dermal papilla, while in others a few are found deeply situated in the fatty subcutaneous tissue or between strands of fibrous tissue.

Viewed in cross sections such globi resemble those just described in the more cellular lesions, insofar as in most instances the wall is of uneven thickness. The thin segment is of greatest extent and devoid of nuclei, and the thick one of less extent and possessed of nuclei—more of them than in the globi of the cellular active lesions. Some sections pass through the nucleated side of the structure and, when this happens, no lumen containing a globus is evident. The appearance is that of a multinucleated cell in which the nuclei form a kind of crescent partly surrounding an area of cytoplasm free from nuclei.

Reconstructions of some of these globi from serial sections show one or more delicate channels leading off the lumen through the side of the globus possessed of the thinnest wall. I have not been able to trace them very far, neither have I been able to find them connected with all globi. The openings are narrow, of the same caliber as the channel and not funnel-shaped. They are rather like lymphatic capillaries. Whether they

are lymphatics, and these globi are housed in local roughly spherical dilatations of lymphatics, will have to be held over for decision until satisfactory injections of the lymphatic plexus have been made with india ink by the method of Hudack and McMaster, before the tissue is biopsied. If the globi are in truth sharply localized spherical enlargements of the closed beginnings of lymphatic channels, like grapes on a bunch, then the dilating force must be due to growth of the bacilli or be occasioned by osmotic factors connected therewith. It would not be possible to explain the dilatation in terms of Pullinger and Florey's conception of the normally operating mechanism.

At present it is only feasible to assert that these large globi may be contained either in multinucleated cells, like those mentioned earlier in describing the more active lesions, in support of which is the abundance of nuclei in one side of the retaining wall, or in local enlargements of the lymphatic plexus, as indicated by the delicate vessels alluded to. It is entirely possible, of course, that some are formed in the one, and others in the other situation.

11. WHAT ARE THE SEQUENCES IN THE DISEASE REACTIONS? Many cells ingest the bacilli, and in some types of leprosy, globi form, but these are only two of the obvious microscopic changes. Cells which do not contain bacilli are also more or less involved; likewise nerves, blood vessels, lymphatics and tissue fluids.

It is no exaggeration to say that less is known about the pathogenesis of leprosy than of any other important disease. The special difficulties are clear to any person who will give them a moment's thought.

In the first place leprosy manifests itself in so many ways that clinicians are not agreed as to the classification of types and the terms to be used for each. Yet to reach a logical classification is a prerequisite to further work.

In the second place, in the absence of susceptible animals, studies on pathogenesis must be limited to human beings.

Furthermore, the long incubation period is practically a closed book because, until a positive diagnosis is reached or the disease suspected, biopsy tissues are never collected for examination. We would not know where to look for our material, and only by systematic cytological examinations of children up to and including the age of puberty, selected from classes of people and from regions most subjected to infection and who have died from all causes, are the first symptomless stages in pathogenesis likely to be encountered.

Even after leprosy has been recognized, the path of investigation is

beset with obstacles. Except in the cases of accidental death, or death from some condition other than leprosy, the early lesions can only be studied in biopsied tissues. The skin lesions, and in some cases, nerve lesions, yield limited but valuable information. Such specimens have, however, been used mainly to establish the diagnosis or to test the effects of treatment.

The clinical and pathological correlations may not be as close as they should be. An investigator, on an extended tour, sees many cases, removes specimens and studies them at home, months later. Even less satisfactory is the habit of some investigators away from the tropics of examining and reporting on specimens sent to them from cases which they have never seen. A detailed clinical history, not only of the condition before biopsy, but also of the subsequent progress of the disease is a requisite. For instance, in cutaneous nodular leprosy, the lesions should be mapped at regular intervals so that information as to the age and other features that can be determined by macroscopic observation will be available for each. Unless this is done, misleading conclusions may be reached by examination of a single specimen which may be of an early, mature, or partly healed nodule. All too frequently clinical interest begins to fade when it becomes evident that nothing really helpful can be done for the patient. Those in charge may not remain so alert as to secure either biopsy specimens from late stages, or autopsy specimens when death occurs from leprosy or other conditions.

To determine the sequence of events in a given case from the recognized beginning to recovery or to death is rarely feasible, because often the disease extends over so many years. Consequently, the sequence described is a kind of patch-work established by the judicious selection of specimens from several cases. Much has been learned in this way, but the results are not all that we could wish.

There is also the question of adequacy of examination. While methods for the demonstration of bacilli and for routine analysis of lesions are expertly used by trained pathologists and public health officers, the results are not quantitative. We read that plasma cells are *rare* or *numerous,* and the literature is full of equally vague statements regarding neutrophile leucocytes, foamy Virchow cells, and so on. Few investigators are of one mind as to what *rare* and *numerous* mean. And these are examples which could be multiplied.

It is desirable to grade selected lesions, which have been carefully studied clinically before biopsy, by counting cells in sections and by reducing them to percentages of the total per unit volume of tissue. In the counts, a column would have to be devoted to unclassifiable cells.

because no reliable cytologist claims to be able to identify all cells seen in any section. Correlated with this would be the more difficult and necessarily crude grading of bacilli with respect to estimated number, acid-fastness and granularity. The value of any sequences established by these means would be greater than that of information conditioned by the personal opinions of several observers.

Since leprosaria are generally situated far from centers of medical research, the techniques familiar to workers in the fundamental preclinical sciences of cytology, biochemistry and physiology have seldom been properly utilized. Young workers in these sciences hear of leprosy, but may never see a case and so their energies are directed elsewhere. To place the problem before them, as the Leonard Wood Memorial is doing, is a strategic move, and funds should be supplied to enable a few of them to work under favorable conditions in leprosaria.

Evidently, many reasons can be cited as to why we have so little accurate information on the pathogenesis of leprosy. Not the least is the fact that pathogenesis is at best a laborious study, demanding team work, which is forgotten in the natural eagerness of people to prevent or cure the disease.

12. ARE THERE ANY PROSPECTS OF TREATMENT WHICH ARE NOT BEING EXPLORED? Numerous haphazard experiments have been conducted. Some have been based on the idea that to block the reticuloendothelial system might be helpful. Others have been based on one symptom or another: since the lesions in cutaneous nodular leprosy are cool, owing to reduced blood supply, heat is applied; because chaulmoogra oil derivatives were administered under the best of living conditions, attention was directed to improvements in diet and general hygiene and these were demonstrated to be helpful.

We need to act less blindly, to provide a logical basis for experimental therapy through searching study of pathogenesis of the principal types of leprosy. This must include data on how the physiological processes both systemic and local are modified in order that help can be given with knowledge of what the body itself is doing or is trying to do.

Two suggestions are here offered, although with hesitancy, since it is easy and tempting to theorize when so little is definitely known. The earliest lesions are often small and sharply localized. Attention is directed to a certain small lesion because it is chronic and persists. I have in mind the barely noticeable enlargement and loss of sensation of the ear lobules of a small boy. Biopsy showed tremendous numbers of macrophages packed with bacilli, reduced blood supply and injury of

nerve terminals. This suggests that changing the lesion from chronic to acute might be worthwhile. By the injection of starch, Chambers and Grand have slowed growth and in many cases brought about complete regression of sarcoma No. 180 in mice. Sugar produced from the starch attracted legions of polymorphonuclear leucocytes (corresponding to human neutrophiles) which marched through the closely packed cells and promoted aseptic necrosis and subsequent resorption. The lepra cells are of the same lineage as the sarcoma cells. Tuberculoid leprous lesions, which are by contrast relatively acute, do exhibit many neutrophiles and quite frequently do heal. If the netrophiles are a defense mechanism this would be one way to get them into the lesion.

The second suggestion is based on the fact that spectrographic studies show that in cutaneous nodular lesions there is an increase in the phosphorus-calcium ratio (Cowdry, Heimburger and Williams 1936). Attempts might be made to combat this change. In addition, the spectograph could be employed to ascertain the fate of very small quantities of substances likely to influence the growth of the bacilli. These could be injected locally and intravenously. Some might accumulate in the lesions and retard or even accelerate the metabolic activity of the organisms or the cells containing them. Clues as to what substances to use might be obtained by noting their influence, or lack of it, on acid-fast bacteria, which can be readily cultivated *in vitro*.

13. SHOULD OTHER ACID-FAST BACTERIAL DISEASES OF ANIMALS BE SYSTEMATICALLY STUDIED? This is indicated because it would broaden our base of operations by including several diseases on which we could experiment freely. The field is wide open, because of the fact that several of them are of no economic importance and their study has been neglected. Only with respect to those which have been labelled tuberculosis, has the economic or human motive impelled research.

The selection should depend on a survey in which the lesions would be compared with those of human leprosy. Only in the disease of water buffaloes have giant globi been discovered comparable with those in man. We have mentioned the rosettes in rats, which are less like the human globi. Whether or not they occur in mice remains to be seen. Attention is directed to Johne's disease of cattle by Hagan's statement that the incubation period is never less than a year. I am indebted to Dr. A. Zeissig, associate of Dr. W. H. Hagan, for opportunity to study a series of preparations, the examination of which has supplemented his account. The "skin lesions" in non-tuberculous cattle will bear close study (Daines 1938). A concise statement of "Tuberculosis in cold-

blooded animals" has been contributed by Aronson. The relation of this disease to leprosy is not very close. It gives rise to tubercles, frequently associated with caseation. The organisms can be cultured and are capable of reproducing the disease in susceptible animals. The only lesions of this category I have had the privilege of studying were kindly sent to me by Dr. J. F. Nonidez.

How our conception of human leprosy would thereby be advanced, cannot be predicted. Here, also, to experiment wisely, data on the morphological, biochemical and physiological sequences in the several disease reactions should be first secured. The behavior of bacilli and of cells during the incubation period could at least be discovered. It would be interesting to find out whether the first line of defense is polymorphonuclear or monocytic and whether the nerve involvement is primary or secondary. The significance of granularity and of loss of acid-fastness of the bacilli could probably be clarified. Certain reactions between host and parasite would be outstanding in some, and absent or so inconspicuous as to escape notice in others, except when intentionally sought. It would be a comprehensive study of Nature's methods of adjustment, of various ways she has developed through the ages to combat acid-fast bacteria in hosts which themselves differ radically in nutrition, manner of life and in many other important particulars.

Whatever the facts observed, it is safe to say that they would pave the way for experiments designed to assist Nature in these animal diseases, which we hope will give illuminating side lights on human leprosy and tuberculosis. At present, not only leprosy, but also tuberculosis is in the doldrums. Cunningham has truly said of the latter "that we have made but little progress with regard to any specific medical treatment since the days of Laennec."

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