EXCITATION OF SPECIFIC IMMUNE RESPONSE IN LEPROSY PATIENTS

JOHN H. HANKS, Ph.D.
Leonard Wood Memorial Laboratory
Department of Bacteriology and Immunology
Harvard Medical School, Boston, Mass.

The possibility of enhancing immune response during chronic, nonfatal infectious disease tends to be overlooked. In the case of leprosy, attention too frequently is focused upon the disaster of lepromatous leprosy and also upon the apparent feeble antigenicity of *Mycobacterium leprae*. Well-established facts concerning leprosy, the nature of antigens, and the focal character of the secondary immune response, challenge these pessimistic views.

In the first place, many early cases of leprosy and the majority of older cases are nonlepromatous. Within any recognized lesion there are significant amounts of specific antigen. The character of any lesion is in fact a measure of the concentration of bacilli required to elicit a tissue response (and doubtless an immune response) within that particular individual. It is possible that, in many indeterminate or "intermediate" cases, the lepromatous condition might be averted by early application of pertinent immunologic principles.

In the second place, present knowledge of the nature of antigens permits concern over "weak" antigens to be replaced by more constructive concepts. For example, it is the *specificity* of antigens which is determined by structural groups in proteins and polysaccharides. The *degree* of response is influenced by two totally different factors: (*a*) The innate, inheritable capacity of the host to respond to determinants in the antigen ([39]). (*b*) The type and amount of lipid components, i.e., "adjuvants," which are introduced in conjunction with these specific determinants and which influence the degree of response. These stimulators of response have been shown to exert their remarkable influences in at least two ways: (1) by dissemination and conservation of antigen in non-wettable oils, and/or (2) through modification of cell metabolism by lipopolysaccharides ([20]) and by similar compounds in waxes and esters containing mycolic acid ([31, 32]).

Given an animal or human which can be modified by the determinants of specificity in an antigen or infectious agent, magnification of response can be induced at will. In short, there are no "weak" antigens. Some antigen complexes naturally contain sufficient adjuvant to elicit the desired elevations of immune response. Others can be made more effective by combination with added adjuvant or by improving the adjuvant situation within host tissues. There is, therefore, no reason to suppose that poor antigenicity is an inalterable defect in leprosy bacilli.

Recognition that *M. leprae* possesses the one essential quality of an antigen, namely, individuality of specific determinants, encourages re-examination of the general impression that it contains antigens which are less potent than those in *M. tuberculosis* or *M. leprae murium*. This impression is supported and explained by the following facts: (*a*) only tissue-grown microorganisms are available, and (*b*) not infrequently many of them appear pale or "decrepit" ([41]). Segal and Bloch ([36]) have shown that mouse lung-grown suspensions of H37Rv are less immunogenic than comparable weights of *in vitro*-grown H37Rv. The lung-grown bacilli form stable suspensions in aqueous washing fluids. This wettability and lower immunogenicity have been explained by a relative deficit in chloroform-soluble waxes. It has also been shown that *in vivo*-grown antigen from *M. leprae murium*, when combined with BCG as adjuvant, protects against rat leprosy much more decisively than either homologous antigen or BCG administered separately ([18]).

In view of the chemical nature of lipids associated with acid-fastness and adjuvant action, Wade's analysis of the "restorative" action of mineral oil on carbol-fuchsin staining ([41]) and the observations by Chaussinand and Viette ([2]) on the failure of *M. leprae* to stain with Sudan black provide an additional evidence that this bacillus is poorly endowed with the kinds of lipids which afford maximal enhancement of immune response. If *in vivo*-grown antigens from tubercle bacilli and murine leprosy bacilli can be made more useful by the addition of adjuvants, there would seem to be little doubt that such measures are more needful in the case of the leprosy bacillus.

In the third place, from the point of view of host response, it may be incorrect to assume that prophylactic immunization is the most effective way of inducing immunologic excitation in sites where an infectious agent may be introduced. The prompt, highly efficient secondary phase of immune response occurs focally in pre-existing depots of antigen or infectious agent ([44]). A reconsideration of the usual approach to such problems may be in order.

It seems useful, therefore, to call attention to the many ways in which antigens such as those of *M. leprae* may be made more effective by creating adjuvant mixtures *in vitro* and adjuvant situations *in vivo*; also to examine means whereby full advantage may be taken of the mechanisms of host response. As will be seen, existing knowledge of leprosy makes important contributions in two ways: (*a*) it affords assurance that similar immune modifications have been or can be induced in leprosy patients; (*b*) it sometimes provides evidence which should be known more widely to immunologists.

ENHANCEMENT OF IMMUNE RESPONSE BY MEANS OF ADJUVANTS

The development of knowledge concerning the adjuvant properties of butter, vegetable oils, paraffin, and paraffin (mineral) oils, and of mycobacteria has been reviewed by Freund ([9, 10]). The results of many investiga-

tions illustrate applications of the adjuvant principle in every field of immunology, including the study of allergic aspermatogenesis, encephalomyelitis, neuritis and uveitis ([9, 10]).[1]

Mechanisms of adjuvant action.—While emphasizing in the introduction that the effectiveness of antigens is increased by certain lipids, the two most clearly recognized types of adjuvant action were stated. Freund has described the preparation of "incomplete adjuvants," i.e., antigens in a water-in-mineral oil emulsion, and also of "complete adjuvants," which are comprised of antigen, mineral oil and mycobacteria. Antibody production is enhanced by antigens in oil. Addition of mycobacteria, or mycobacterial waxes or lipopolysaccharides, is necessary to produce tuberculin-type sensitivity to proteins. Whether the tuberculin-type sensitivity induced by bacterial lipopolysaccharides is due to metabolic modifications which cannot be caused by antigen in oil, or merely to much greater accentuation of all aspects of immune response, remains an unsettled issue.

White *et al.* ([45]) have described the interrelationships between antibody response and the cytologic character of the local and disseminated tissue responses to adjuvant mixtures containing antigen, mineral oil and mycobacterial waxes. More recent views concerning the enhancement of immune response by bacterial lipopolysaccharides, and the activation of genetically controlled templates, have been summarized by Johnson *et al.* ([20]).

METHODS OF STUDY

The methods employed in studies on adjuvant action, and typical results, will be illustrated in appropriate sections to follow.

Applications pertinent to the study of leprosy.—It has seemed convenient to organize the topics in this section in accordance with the different substances or agents which have been shown to produce adjuvant effects and to indicate their application in experimental models or in an infectious disease. In this way it will become evident that each approach is applicable to a problem or event which occurs in leprosy; also that adjuvant effects may have been produced in leprosy patients under many circumstances without recognition or exploitation of the principles involved.

Adjuvant effect of oils.—Freund and associates must be credited with the first adequate elucidation of the enhanced effectiveness of antigens incorporated in oil ([9, 10]). It was Freund, Casals and Hosmer ([11]) who demonstrated that a remarkable excitation of both antibody production and tuberculin-type sensitivity was induced by killed tubercle bacilli in mineral oil as readily as by live bacilli.

A view of the adjuvant effects of antigen in oil alone can be gained from the work of later investigators. Salk and Laurent ([35]) observed an 80x increase in antibody production when formalinized suspension of influenza

[1] This area of experimental physiology has important implications in regard to the clinical manifestations and pathology of leprosy.

virus were incorporated in mineral oil. Their most significant finding, however, resulted from contrasting the minimal amounts of antigen required to produce antibody when vaccines were suspended in saline and in mineral oil. Suspensions diluted beyond 1:10 in saline were ineffective; at dilutions of 1:100,000 in oil the limits of effectiveness had not been reached, i.e., all animals were protected. They concluded that the stimulation afforded by very small amounts of this antigen in oil was greater than that produced by more than 10,000 times as much antigen in aqueous systems.

An example which illustrates one of the reasons for superior antigenicity in oil is provided by the data of Talmage and Dixon ([40]) on the retention of protein antigens at the sites of injection. Soluble antigens were eliminated rapidly; those in alum were retained only slightly better; antigens emulsified in oil were removed from the site much more slowly, the half-life being 14 days. The antibody production to antigens in oil was relatively constant for at least 300 days. The response incited by aqueous antigen diminished rapidly after 10 days.

The use of antigens in oil has been shown to enhance protection and/or immune response in three mycobacterial diseases caused by agents which resist cultivation by bacteriological methods. Sigurdsson and Tryggvadöttir ([38]) have shown that unusual levels of skin sensitivity occurred in sheep receiving small amounts of killed *M. paratuberculosis* (Johne's bacillus) in mineral oil; also that the levels of complement-fixing antibody were 32-128x those induced by natural infection. Sigurdsson ([37]) later reported a 3½ year study of protection against paratuberculosis of sheep[2] by means of "vaccine" prepared from cultivable strains suspended in mineral oil. Of 266 unvaccinated lambs, 16 died, while 21 of the survivors showed definite lesions at autopsy. Among 289 vaccinated lambs there were no deaths; only 4 presented lesions at autopsy.

Though mineral oils are required to improve the antigenicity of tubercle bacilli ([9]), the lesser adjuvant effect of vegetable oils suffices to enhance the immunogenicity of two types of *in vivo*-grown mycobacteria. Hanks and Fernandez ([18]) have shown that a single injection of killed *M. leprae murium* in olive oil is the equivalent of BCG for protecting rats against challenge with murine leprosy bacilli. The more specific response incited by this antigen promoted eventual healing of a significant proportion of lepromatous lesions, while BCG did not.

Fernandez and Mercau ([7]) have described the particularly intense 48-72 hour reactions produced by killed *M. leprae* in mineral and olive oils and in the benzylic esters of chaulmoogra oil. The causes of the toxicity of these concentrated preparations and the consequent apparent broadening of specificity have been elucidated by the recent work of Weiss and Dubos ([42, 43]). In spite of employing dried leprosy bacilli which had been extracted three

[2] A particularly interesting form of Johne's disease, since "ovine strains in general cannot yet be cultivated outside the host."

times in chloroform and washed twice in ether, Fernandez and Mercau reported that the bacilli in mineral oil produced in several Mitsuda-negative, lepromatous patients, after three to five months, reactivity to the usual lepromin in saline. Floch ([8]) recently has employed mixtures of glycerol and mineral oil in order to convert his 1:750 dilution of lepromin into a more sensitive test reagent. The preparation of more economical test reagents and more immunogenic products obviously deserves careful study.[3]

Adjuvant effects in vivo.—Though enhancement of immune response by incorporating antigens in oil is common practice, the results achieved by these procedures are presented chiefly as background for a more pertinent examination of the enhancement of antigenicity *in vivo*. The first evidence that such enhancements can be excited without simultaneous introduction of antigen and adjuvant is to be found in the experiments of Lewis and Loomis, Dienes, Hanks, and others, to be reviewed below. It seems of interest, however, that the injection of oils and esters into leprosy lesions probably constitutes the first clinical application of the adjuvant principle to an immunologic problem.

Retrospection indicates clearly that an oily adjuvant system was established in leprosy lesions by injections of hydhocarpus oils or esters.[4] The partition of mycobacteria from aqueous systems to those comprised of oils, fatty acids and esters is known from the classical studies of Mudd and Mudd ([26]) and of Reed and Rice ([33]). The intracutaneous or "plancha" method ([21]) today would be adopted as the optimal means of introducing adjuvant into the sites of antigen deposit. Nolasco ([29]) has shown that the bacilli in hydnocarpus-infiltrated leprosy lesions became involved in small pools of oil. Furthermore, the most efficient results probably were obtained by the use of oils or esters which were more stable than ordinary vegetable oils.

Since optimal conditions for adjuvant situations in tissues were approxi-

[3] Henderson ([19]) already has used oils to separate *M. leprae* from spleens, etc., obtained at autopsy. Data in this laboratory indicate that *in vivo*-grown mycobacteria are coated with tissue components which cause the isoelectric flocculation to occur at pH 4.7. Enzymatically-cleaned bacilli, like those grown *in vitro*, flocculate at pH 1.5. Since Mudd and Mudd ([27]) found serum-coated mycobacteria to be less readily wetted by oil, it might be desirable to learn whether purified leprosy bacilli are more readily adapted to adjuvant modification.

[4] There are several reasons for questioning whether the effects of chaulmoogra or hydnocarpus derivatives were due to the unsaturation or the peculiar structure of their fatty acids. 1. Iodization titrates out unsaturation. Nevertheless, iodized esters were regarded as superior to natural oils. 2. If special structure were an adequate explanation, administration of oils and esters by oral or intramuscular routes should have compared more favorably with the "plancha" method. Fatty acids are unique among foodstuffs. They are stored in tissues primarily in the forms in which they are ingested. Hence, the fatty acid composition of tissues can be modified almost at will, and by dietary methods. 3. Although the tubercle bacilli lead a more extracellular existence, and have been shown to be highly sensitive to unsaturated fatty acids, the oils and esters utilized in leprosy did not appear to be efficacious in tuberculosis.

mated during the "chaulmoogra era," the disappointments might be explained as follows.

A. The adjuvant principle had not been elucidated. The probably immunologic mode of action could not have been suspected.

B. Distinctions could not be made between patients who were truly lepromatous and those whose borderline state afforded a potential for more effective response. The selective action of an immunologic principle within these heterogeneous populations would permit discharge of those possessing potential for enhanced response and leave a residuum which justified the eventual discouragement.

Adjuvant effects of cultivated mycobacteria.—Although the lipids in *M. leprae* or *M. leprae murium* might exert an adjuvant effect upon less stimulating materials (e.g., purified proteins), such organisms are here regarded as antigens which often fail to excite the high degree of response required to protect susceptibles against mycobacterial disease ([16]). Major interest, therefore, centers around means by which other mycobacteria can be employed to enhance response to specificity determinants in the two types of leprosy bacilli. It will again be seen that adjuvant combinations can be prepared either *in vitro* or *in vivo*.

Freund's use of tubercle bacilli in mineral oil to enhance immunologic response to unrelated antigens is now a classical procedure, and has been applied to all types of antigenic materials. Although the tubercle bacillus has been employed most frequently, it must be emphasized that comparable excitation is caused by *M. butyricum* and by nocardia ([12]). It follows that no saprophytic strain of acid-fast organism can be mixed with *M. leprae* either *in vitro* or *in vivo*, without considering the probability that any significant immunologic modification is due to enhanced response to the specific antigens of *M. leprae*.

Raffel and associates found the crude, chloroform-soluble waxes ([30]) and the esters of mycolic acid ([31, 32]) from the tubercle bacillus to be the major components which modify and enhance immune response. As indicated, the action of these materials and of lipopolysaccharides from Gram-negative bacteria seem to depend not only on oily properties but also on modification of cell metabolism.

Evidence that tubercle bacilli in tissues exert an adjuvant effect for unrelated antigens originated with the observations of Lewis and Loomis ([23]) concerning the exaggerated production of antibody to sheep cells, etc., in tuberculous guinea-pigs. Later experiments by Dienes ([5, 6]), Hanks ([14]), and others revealed that these effects are maximal when unrelated antigens are injected into established tuberculous lesions ([5]) or associated lymphatic pathways ([14]).

The evidence of a profound modification and enhancement of immune response in such tissues was the production of a tuberculin-type hypersensi-

tiveness which was specific for the extraneous antigens. The exact type or degree of enhancement required to incite tuberculin-type hypersensitiveness to ordinary protein antigens cannot be stated. Nevertheless, some measure of the improved response caused by nonsimultaneous introduction of antigen and mycobacterial adjuvant into tissues is indicated by the fact that all guinea-pigs developing this exaggerated sensitivity later produced from 10-100x more antibody than nonprepared controls which received the same antigen ([17]).

Hanks and Fernandez ([18]) demonstrated that the adjuvant principle is applicable to immunization against a lepromatous disease in rats; also that heightened specific response led to significant protection and/or to healing of established lesions. Modest amounts of *M. leprae murium* antigen combined with living BCG as adjuvant conferred greater protection than 14x this amount of *M. leprae murium* antigen given alone; also greater protection than BCG alone. The degree of enhanced resistance has been calculated in terms of the challenge doses which should be required to bring about comparable incubation periods in the immunized and non-immunized animals. For the conditions analyzed, the average animals immunized by the adjuvant combination of *M. leprae murium* antigen in BCG would tolerate $x10^6$ greater challenge dosage than those immunized with *M. leprae murium* or BCG alone, and $x10^{11}$ greater dosage than the nonimmunized controls ([16]).

In respect to human leprosy, early clinical studies with various mycobacterial antigens or derivatives were evaluated by Muir ([28]) as follows: "There seems to be no doubt that favorable results have been obtained with many of these substances *in certain patients*" (italics mine). Other reports in the literature culminate in the recent intradermal injections into lepromatous patients of *M. marianum* "vaccine" by Blanc *et al.* ([1]). Though the procedures represent a reversal of those employed by Dienes, Hanks, and others, it will be recognized that adjuvant effects on host response do not depend on the order of introduction. It may also be noted that improvement of response to specific determinants in *M. leprae* may be more significant than any supposed closeness of antigenic relationship.

Adjuvant effects of killed suspensions of other microorganisms.—In view of evidence mentioned in the introduction and in the foregoing section, it already is apparent that adjuvant factors are not confined to acid-fast microorganisms. During investigations of combined antigens for immunization of school children or soldiers against diphtheria and tetanus toxins, it was found that elevated titers were produced by toxoids containing pertussis or typhoid "vaccines" ([13, 22]). Other examples, including bacterial toxins, streptococci, cholera vibrio, Gram-negatives, etc., have been reviewed by Freund and by Johnson *et al.* ([20]).

The reported improvements in leprosy patients after administration of various vaccines, or following cutaneous ulcers and intercurrent infections ([28])

would be difficult to explain on the basis of cross-immunization with antigens from related organisms. They can be explained more readily by adjuvant effects which enhance response to the specific antigens of *M. leprae.*

Adjuvant effects produced by viruses.—Among agents of viral diseases, the one which produces allergic phenomena comparable to those incited by tubercle bacilli is the vaccinia virus. Dienes (4), in fact, employed vaccinia virus as an alternative to tubercle bacilli in his studies on enhancing immune response and tuberculin-type sensitization to the antigen of horse serum and egg-white.

In respect to leprosy, the report of Denney and Hopkins (3) may qualify as a straightforward example of an adjuvant effect on *M. leprae* antigen caused by a benign, but highly stimulating virus infection. After describing the abnormally severe vaccinal lesions and the erythematous macules in certain patients, these authors summarize their impressions of the "specific leprous reactions" as follows: "In some instances the reactions were simple recurrences of old symptoms; in others, the reactions were typically those of leprosy, but entirely new to the individual affected." The most pertinent section of their discussion speaks for itself.

"It is not without interest that in no instance did a case present more pronounced symptoms of chronic leprosy at the subsidence of the acute symptoms provoked by smallpox vaccination, but, on the contrary, some showed actual improvement. It is not improbable that during the acute leprous exacerbation, long acquiescence of the host to bacterial invasion was disturbed, and immunizing substances were produced which were not elaborated during the quiescent periods. The production of such substances in the acute leprous lesions would readily explain the completeness with which such lesions disappeared and also such improvement as has been noted in chronic symptoms."

THE MECHANICS OF HOST RESPONSE

Discussion thus far has concerned the means by which adjuvant effects can be used to enhance immune response to *M. leprae.* It is equally important to realize that the pre-existence of antigen depots or infection makes it possible to take advantage of one of the most sufficient features of the immune response.

In order to investigate or discuss the immunological significance of tissue reactions which occur in mycobacterial disease, it is necessary to distinguish two types of response:

A. Those due to pre-existing hypersensitivity.

B. Those associated with the generation of new immunologic capacity: B, antibody production; B′, altered cellular response.

The Type B response is characterized by the growth and maturation of plasma cells (45). Following a primary subcutaneous injection of antigen, this response does not occur *in situ*, but in adjacent lymph nodes. Here, over a period of 30-60 days, there develop small foci of plasma cells; antibody production is meagre. A second injection of antigen which can drain through the stimulated nodes causes phenomenal proliferation of plasma cells and a

correspondingly rapid production of large amounts of antibody (i.e., the second response). With a given amount of antigen, optimal efficiency results from multiple primary injections which stimulate a greater number of lymph nodes, and from re-stimulation of the corresponding tracts.

Additional loci of secondary response.—As has been emphasized, mycobacterial disease, or compounds producing an adjuvant effect, increase greatly the dissemination of antigen and the number of foci in which both Type B and B′ activations occur. Since the cytologic character of cells generating enhanced capacity for allergic response has not been delineated, it can only be stated that Freund ([10]) and others ([45]) regard the cells which accumulate around mycobacterial derivatives as accessory "lymphoid" tissue. These new tissues represent additional "organs" in which there transpire events associated with the secondary response. The role of the tubercle and of associated lymphatic pathways in the generation of tuberculin type hypersensitivity have been shown by several modifications ([14]) of the Dienes experiment.

Westwater ([44]) seems to have been the first to demonstrate that, following a second introduction of mycobacterial antigen in oil, the events responsible for the remarkable secondary response do not occur promptly in the new site of antigen injection. Such sites develop cellular response and local formation of antibody as though they are primary sites. Each new injection triggers the secondary response in primary or pre-existing sites.

Before examining means of taking advantage of the Type B responses during mycobacterial disease, it is well to recognize that Type A and Type B responses seldom, if ever, are seen in pure form. Nevertheless, it can be deduced that in certain instances Type A or Type B′ preponderate. For example, although tuberculin has a "booster" effect in sensitive individuals, both the local and the focal reactions to this substance are relatively pure Type A responses. The Fernandez (48-hour) reaction to lepromin appears to be of this type, while the Mitsuda reaction is thought to depend in part upon capacity to enhance the Type B′ response. The activations seen in recognized or unperceived lesions in leprosy probably depend on both types of response.

A further example in which Type B′ response may preponderate has been known to leprologists for several years under the designation of "remote positivization." Primary injections of lepromin in children frequently fail to produce Mitsuda reactions. Rosemberg and associates ([34]) have observed that these lepromin sites sometimes become activated following BCG oral vaccination, even after intervals of three years. Their suggestion that the phenomenon is explained by local retention of *M. leprae* antigen is consonant with data afforded by more analytical immunologic study. "Remote positivization" affords strong support for the proposition that, if properly stimulated, the apparently indifferent animal or human can make focal response to very small amounts of antigen.

Realization that the efficient secondary response occurs focally in depots of previous antigen or infection has interesting implications for further experimentation on immunologic modifications in leprosy. In the first place, it suggests that a new approach might be made by reversing the usual procedures employed in immunization studies in experimental models. Such work hitherto, as in the experiments of Hanks and Fernandez ([18]), has required the preparation of a fairly large supply of *M. leprae murium* antigen. It would be more economical to permit a small inoculum of viable bacilli to synthesize the necessary antigen within the experimental animals. Furthermore, the order of introduction of antigen stimuli may not have been ideal. The challenge sites, being the seat of a more primary type of local response ([44]), may not have permitted the most effective immunologic resources of the animals to be exerted against the infecting agent during the critical, early period ([25]) while infection was becoming established. It is equally likely that the prompt secondary response to the challenge dose exerted its sharpest effects in sites containing the immunizing antigens from *M. leprae murium* and/or BCG. Such action may have hastened the destruction of the viable immunizing agent, BCG.

Evidence is already at hand that immunologic study in experimental models may be directed toward suppression or treatment of established infections as profitably as toward prophylaxis. In earlier days, the healing of lepromas in rats was unknown and, by the author, regarded as impossible. Nevertheless, 100 per cent of rat leprosy lesions caused by bacilli subjected to an accompanying handicap have been observed to heal, and they did not recur ([15]). This phenomenon also was observed in 40 per cent of animals which had been immunized with specific antigen plus BCG, but challenged after the prophylactic response had been allowed to subside for seven months ([18]).

The focal activation of lesions in human leprosy, followed by subsequent improvement, is such a classical observation that it deserves but one comment. Explanations not infrequently are based on the supposition that these episodes are due to heightened activity of the bacilli when, in fact, it is more probable that previously quiescent immunologic mechanisms have become activated.

DISCUSSION

The major points emphasized in the foregoing remarks are now to be summarized and synthesized. Points Nos. 1 and 2 below outline the theoretical basis of prophylactic immunization, while Nos. 1 and 3 concern more directly the enhancement of specific response in persons already infected.

1. The initiation of infection in young individuals and the subsequent transformation of progressive disease into lepromatous leprosy in some of these persons may be attributed to several causes: (*a*) lack of immunologic preparation; (*b*) in certain individuals, genetic traits which limit response to

specific determinants in the proteins and/or polysaccharides of *M. leprae;* and (*c*) the low adjuvant content of tissue-grown mycobacteria and of *M. leprae* in particular. Such deficiencies in the individual and the bacillus are expressed by failure to restrain the bacillus population, by histologic response which shifts from indeterminate to lepromatous, and by conversion of weak to negative Mitsuda reactions.

2. Although the genetic basis of susceptibility cannot be altered, prior administration of specific antigen accompanied by adequate adjuvant offers a means of minimizing these liabilities. Methods of collecting *M. leprae* from lepromas are available; the preparation of injectable antigen-adjuvant mixtures has been reviewed here and elsewhere ([10]). Students of leprosy recognize that the most favorable immunologic balances exist in the absence of large numbers of bacilli. Hazards which may arise from introduction of large amounts of antigen have been emphasized by Weiss and Dubos ([42, 43]), and by Hanks and Fernandez ([18]). The reasons why frequent injection of mycobacterial antigens is unnecessary and undesirable have already been stated ([18]); they have been re-emphasized by describing in foregoing pages the slowness with which a primary immunologic response develops.

3. A major purpose of the present paper, however, has been to state why genetic limitations, limited supplies of antigen, and "poor" antigenicity do not justify a pessimistic approach to the management of persistent, nonfatal infections. In a disease such as leprosy, even though the causative agent has not been cultivated, there are four reasons to consider such problems with confidence:

A. Genetic limitations express themselves in part by slowness of response. Even when existing levels or rates of response have not averted disease, time permits the pursuit of immunization until maximal capacity has been attained.

B. Examination of the mechanics of the immune response reveals that although it may be slow (of primary character) in sites where antigen is newly introduced, it is much more prompt and effective in sites where antigen (or infectious agent) was previously deposited. Prophylactic immunization, therefore, is not the only procedure applicable to immunologic problems in leprosy. There are, in fact, reasons for suspecting that to reverse the usual sequence of steps may be a more efficient means of bringing immune processes to bear on the infectious agent.

C. Because of infection, specific antigens exist in the tissues, probably in amounts proportional the antigen requirements of the individual concerned. In geographic areas or among races where the problem is most serious, additional antigen for this or other purposes can be procured from lepromatous lesions. A major lesson derived from immunologic studies of the type reviewed, and also from observations in leprosy, is that the basic problems in this disease do not arise from lack of antigen but from lack of effective response.

D. Whether one proposes to utilize the antigen in lesions or to obtain it from lepromas, immunogenicity can be enhanced by use of appropriate adjuvants. Adjuvant effects have been produced *in vivo* by oils and esters, by mycobacteria (whether alive or dead, pathogenic or saprophytic), by other microorganisms, by lipid components of the foregoing bacteria, and by viruses. Aside from the necessity of introducing these stimulators directly into lesions or associated lymphatic pathways, there appears to be no sharp restriction of maneuver in developing the art of exciting more effective response to *M. leprae* antigen in patients.

Many students of leprosy might consider it logical to consider alternatives in somewhat the following order:

(*a*) In tuberculin negative persons, to use BCG.

(*b*) In tuberculin positives, to employ *M. butyricum, M. marianum, Nocardia asteroides,* pertussis vaccine, etc., as might be permitted by existing or induced sensitivity.

(*c*) If such avenues seem blocked or undesirable, to employ vaccinia or other viral agents, in accordance with knowledge of their tolerance by leprosy patients and further development of information concerning their effectiveness as adjuvants.

(*d*) Finally, to employ wetting agents with oils and esters which are not quite so completely indestructible as mineral oil, but are less wettable, saponifiable and digestible than the usual vegetable oils. Adjuvant properties already have been noted in synthetic esters of oleic acid (e.g., in Arlacel) and in longer chain synthetic esters ([31]).

Further study will be required to ascertain whether agents which accentuate the allergic aspects of response (e.g., *a, b,* and *c* above) create effects which are less desirable than those caused by oils. This and other interesting questions challenge those engaged in investigating the immunology of leprosy.

The views which arise from re-examination of the nature of antigens and adjuvants and of the immune response bring into more realistic focus the considerations which already have impressed many students of the biology of leprosy. Lie ([24]), for example, summarized a life-time of thought and study as follows:

> "This reactional power, which is the most important factor in deciding the fate of a leprosy patient, is by far the greater in the maculo-anesthetic (i.e., tuberculoid) patient. However, nodular (i.e., lepromatous) patients who appear incapable of overcoming the bacilli may be completely cured when, for whatever reason, they are made to react. Here lies the most important problem regarding the therapy of leprosy, namely, to bring about a reaction at as early a stage as possible." "Medical science, therefore, must never cease striving to arrive at that which nature has shown to be obtainable. It would seem . . . that the way or at least one of the ways to attain this goal is to arouse and strengthen the reaction-power of the human organism against the intruding leprosy bacillus."

REFERENCES

1. BLANC, M. and PROST, M. Clinical and therapeutic study of an antigen prepared with *Mycobacterium marianum,* applied to 457 leprosy patients. Internat. J. Leprosy **23** (1955) 23-31.
2. CHAUSSINAND, R. and VIETTE, M. Étude de la coloration des bacilles acido-alcoolo-résistants par le Noir Soudan. Ann. Inst. Pasteur **89** (1955) 280-289.
3. DENNEY, O. E. and HOPKINS, R. Specific leprous reactions and abnormal vaccinia induced in lepers by smallpox vaccination. Publ. Health Rep. **37** (1922) 3141-3149.
4. DIENES, L. Local hypersensitiveness; hypersensitiveness of guinea pigs treated with eggwhite during smallpox vaccination. J. Immunol. **14** (1927) 53-59.
5. DIENES, L. Technic of producing tuberculin type of sensitization with eggwhite in tuberculous guinea pigs. J. Immunol. **17** (1929) 531-538.
6. DIENES, L. and SCHOENHEIT, E. W. Reproduction of tuberculin hypersensitiveness in guinea pigs with various protein substances. American Rev. Tuberc. **20** (1929) 92-105.
7. FERNANDEZ, J. M. M. and MERCAU, R. Immunology of leprosy. Antigens derived from *Mycobacterium leprae* in oily suspension. Internat. J. Leprosy **15** (1947) 389-405.
8. FLOCH, H. Enhancement of positivity of the Mitsuda reaction obtained with diluted antigen. Internat. J. Leprosy **23** (1955) 295-300.
9. FREUND, J. The effect of paraffin oil and mycobacteria on antibody formation and sensitization. A review. American J. Clin. Path. **21** (1951) 645-656.
10. FREUND, J. The mode of action of immunologic adjuvants. Bibl. Tuberc. (Basel); *in supplement* Adv. Tuberc. Res. **7** (1956) 130-148.
11. FREUND, J., CASALS, J. and HOSMER, E. P. Sensitization and antibody formation after injection of tubercle bacilli and paraffin oil. Proc. Soc. Exper. Biol. & Med. **37** (1937) 509-513.
12. FREUND, J. and LIPTON, M. M. Potentiating effect of *Nocardia asteriodes* on sensitization to picryl chloride and on production of isoallergic encephalomyelitis. Proc. Soc. Exper. Biol. & Med. **68** (1948) 373-377.
13. GREENBERG, L. and FLEMING, D. S. Increased efficiency of diphtheria toxoid when combined with pertussis vaccine; preliminary note. Canadian J. Publ. Health **38** (1947) 279-282.
14. HANKS, J. H. Mechanism of tuberculin hypersensitiveness; role of tubercle and of inflammatory reaction of early tuberculous lesion in production of tuberculin hypersensitiveness as determined by inducing "tuberculin type" of hypersensitiveness with egg-white or horse serum. J. Immunol. **28** (1935) 105-121.
15. HANKS, J. H. Three factors which may influence the experimental transmission of leprosy. Internat. J. Leprosy **18** (1950) 33-37.
16. HANKS, J. H. The magnitude of the immune response incited by killed and attenuated mycobacteria. J. Immunol. **81** (1958) (in press).
17. HANKS, J. H. Unpublished data.
18. HANKS, J. H. and FERNANDEZ, J. M. M. Enhancement of resistance to murine leprosy by BCG plus specific antigen. Internat. J. Leprosy **24** (1956) 65-73.
19. HENDERSON, H. A method for separating intact leprosy bacilli from leprous tissue. Proc. Soc. Exper. Biol. & Med. **50** (1942) 92-94.
20. JOHNSON, A. G., GAINES, S. and LANDY, M. Studies on the 0 antigen of *Salmonella*

typhosa. V. Enhancement of antibody response to protein antigens by the purified lipopolysaccharide. J. Exper. Med. **103** (1956) 225-246.

21. LARA, C. B. Plancha, or infiltration, method of treating leprosy. J. Philippine Islands Med. Assoc. **9** (1929) 336-343.
22. LEVINE, L. and STONE, J. L. Factors affecting the efficiency of combined prophylactics; effect of diphtheria toxoid and pertussis vaccine on tetanus toxoid. J. Immunol. **72** (1954) 258-262.
23. LEWIS, P. A. and LOOMIS, D. Allergic irritability. The formation of anti-sheep hemolytic amboceptor in the normal and tuberculous guinea pig. J. Exper. Med. **40** (1924) 503-515.
24. LIE, H. P. The curability of leprosy. Internat. J. Leprosy **3** (1935) 1-22.
25. MILES, A. A. Early tissue reactions to tubercle bacilli and their products. *In* Ciba Foundation Symposium on Experimental Tuberculosis. With an addendum on leprosy, pp. 87-101. G. E. Wolstenholm, Ed., London: J. & A. Churchill Ltd., 1955, 396 pp.
26. MUDD, S. and MUDD, E. B. H. The surface composition of the tubercle bacillus and other acid-fast bacteria. J. Exper. Med. **46** (1927) 167-172.
27. MUDD, S. and MUDD, E. B. H. On the mechanism of the serum sensitization of acid-fast bacteria. J. Exper. Med. **46** (1927) 173-195.
28. MUIR, E. Treatment of leprosy. A review. Internat. J. Leprosy **1** (1933) 407-458.
29. NOLASCO, J. O. Histologic studies on the plancha or infiltration method of leprosy treatment. Internat. J. Leprosy **2** (1934) 159-174.
30. RAFFEL, S. The components of the tubercle bacillus responsible for the delayed type of "infectious" allergy. J. Infect. Dis. **82** (1947) 267-293.
31. RAFFEL, S., ASSELINEAU, J. and LEDERER, E. The chemical nature of the lipoidal factor of the tubercle bacillus responsible for the induction of tuberculous hypersensibility. *In* Ciba Foundation Symposium on Experimental Tuberculosis. With an addendum on leprosy, pp. 174-187. G. E. Wolstenholm, Ed., London: J. & A. Churchill Ltd., 1955, 396 pp.
32. RAFFEL, S., LEDERER, E. and ASSILINEAU, J. Lipoidal factors in induction of tuberculous hypersensitiveness. Federation Proc. **13**, I (1954) 509.
33. REED, G. B. and RICE, C. E. Behavior of acid-fast bacteria in oil and water systems. J. Bact. **22** (1931) 239-247.
34. ROSEMBERG, J., SOUZA CAMPOS, N. and AUN, J. N. Da relação imunobiológica entre tuberculose e lepra. VII. Positivação remota do Mitsuda por efeito da vacinação BCG oral. Rev. brasileira Leprol. **20** (1952) 84-96.
35. SALK, J. E. and LAURENT, A. M. Use of adjuvants in studies on influenza immunization. I. Measurements in monkeys of the dimensions of antigenicity of virus-mineral oil emulsions. J. Exper. Med. **95** (1952) 429-447.
36. SEGAL, W. and BLOCH, H. Pathogenic and immunologic differentiation of *Mycobacterium tuberculosis* grown *in vitro* and *in vivo*. American Rev. Tuberc. & Pulm. Dis. **75** (1957) 495-500.
37. SIGURDSSON, B. Vaccination against paratuberculosis (Johne's disease). J. Immunol. **68** (1952) 559-565.
38. SIGURDSSON, B. and TRYGGVADÖTTIR, A. G. Immunization with heat-killed *Mycobacterium paratuberculosis* in mineral oil. II. J. Bact. **59** (1950) 541-543.
39. STALLYBRASS, C. O. Mechanism of antibody production. Proc. Roy. Soc. Med. **43** (1950) 137-144.
40. TALMAGE, D. W. and DIXON, F. J. The influence of adjuvants on the elimination

of soluble protein antigens and the associated antibody responses. J. Infect. Dis. **93** (1953) 176-180.

41. WADE, H. W. Demonstration of acid-fast bacilli in tissue sections. American J. Path. **28** (1952) 157-170.
42. WEISS, D. W. and DUBOS, R. J. Antituberculous immunity induced in mice by vaccination with killed tubercle bacilli or with a soluble bacillary extract. J. Exper. Med. **101** (1955) 313-330.
43. WEISS, D. W. and DUBOS, R. J. Antituberculous immunity induced by methanol extracts of tubercle bacilli; its enhancement by adjuvants. J. Exper. Med. **103** (1955) 73-85.
44. WESTWATER, J. O. Antibody formation in a lesion produced by tubercle bacilli suspended in paraffin oil; excision of the antigenic depot. J. Immunol. **38** (1940) 267-272.
45. WHITE, R. G., COONS, A. H. and CONNOLLY, J. M. Studies on antibody production. IV. The role of a wax fraction of *Mycobacterium tuberculosis* in adjuvant emulsions on the production of antibody to egg albumin. J. Exper. Med. **102** (1955) 83-104.