CONTRIBUTIONS FROM ANIMAL EXPERIMENTS TO THE UNDERSTANDING OF SENSITIVITY TO *M. LEPRAE*[1, 2]

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Certain features of the immunology of leprosy can be studied in animals without waiting for the solution of the frustrating problems of experimental transmission. By improving the technique of eliciting the Fernandez and Mitsuda reactions and learning how to interpret these skin responses, it may be possible to develop a potent tool for epidemiologic and clinical investigation. Animal experimentation has already produced valuable information in the following three general areas of knowledge.

1. *Transmission experiments.*—The inability to transmit human leprosy to animals, in itself, tells us much about *Mycobacterium leprae* and the disease it causes. The high degree of host specificity is indicative of a fastidious organism. The need for standardized doses of inoculum and accurate estimates of growth have led to techniques for counting bacilli.

2. *The meaning of cutaneous reactions to leprosy bacilli.*—In turning to research dealing with skin sensitivity, it is necessary to draw on pertinent information acquired by our colleagues who have been wise enough to tackle the problem of tuberculosis. Drs. Palmer and Edwards (present at the meeting) have contributed especially to our understanding of mycobacterial sensitization.

(*a*) Host specificity: It is commonly said that the most definite feature of reactions to lepromin is the negativity of lepromatous cases. It was shown by several early workers that this is specific to the extent that lepromatous patients may retain their reactivity to antigens prepared from other mycobacteria.

It was shown by Rodriguez ([43]), and especially by Wade ([48, 49]), and confirmed by Feldman *et al.* ([16]), de Faria ([13, 14, 15]) and Olmos Castro *et al.* ([36]), that dogs respond to the Mitsuda-Hayashi lepromin in much the same way as human populations. The positive Mitsuda rate on the first test was practically 100 per cent in the Philippine and South American dogs and 37 per cent in Minnesota dogs. With retesting, Fernandez reactions appeared and the Mitsuda reactions became larger and developed earlier. The positive Mitsuda reactions in Minnesota

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dogs increased from 37 per cent to 55 per cent when the interval between injections was 3-5 months, but when the second injection was given 22 days after the first, all dogs were positive and the responses developed in 3 days rather than the 14 days typical of the first injection.

Guinea-pigs are more readily available for laboratory work than dogs and, because of their demonstrated usefulness in tuberculosis research, several studies have been reported testing their sensitization to leprosy bacilli. Early reports by Hadler ([19]) and Yanagisawa *et al.* ([52]) indicated that guinea-pigs were not sensitized to lepromin even when adjuvants were used. These negative results were partly due to the use of single sensitizing doses, retesting after inappropriate intervals, and using weak antigens such as the Dharmendra antigen. Yanagisawa ([53]) was able later to get good sensitization by using leprosy bacilli which had been partially purified by 3 days' digestion in trypsin at 30° C and adding a waxy extract from tubercle bacilli as an adjuvant. Convit *et al.* ([8]) reported that of 25 guinea-pigs given a second injection of lepromin, 52 per cent became Fernandez-positive and 68 per cent Mitsuda-positive.

In a series of guinea-pig experiments by our group which have now continued over the past three years, we have been able to produce skin sensitivity with great consistency under the following conditions ([44]). Intracutaneous injections of various leprosy-bacillus preparations are given at intervals of one month. On the first injection of Mitsuda lepromin prepared by Wade, almost all the animals showed small and probably nonspecific early reactions and after 2 weeks about one-half showed persistent reactions more than 2 mm. in diameter, which may or may not be equivalent to a Mitsuda response. Using the purified bacillus suspensions prepared by methods which I shall describe shortly, marked accentuation of response occurred. With both heated and fresh suspensions the mean response at 2 days was almost 10 mm., and at 2 weeks the mean response was 3 mm. with autoclaved bacilli and 5 mm. with unheated suspensions. When heated BCG was injected, all guinea-pigs showed both early and late responses, almost one-half of them being distinctly bimodal.

In response to monthly injections of lepromin, the reaction size reached a maximum with the third injection, beyond which there was no further enhancement. Standard deviations of mean reactions also spread after the third injection as individual differences in guinea-pigs became more evident. Our cross testing is now routinely done at the third injection, and we find that our standard deviations are running below 1 mm. and usually less than 0.5 mm. with groups of around 20 guinea-pigs. In sensitized guinea-pigs, responses are maximal at 24-48 hours and then slope downward at varying rates with a plateau typically occurring between 1 and 2 weeks. Histologic examinations have shown that the typical transition from monocytic inflammatory to epithelioid response occurred during this interval.

In monkeys ([6]) also, lepromin positivity can be induced by injecting either viable or heat-killed bacilli. In one monkey which had a lepromatous nodule implanted in the splenic stump, a series of daily injections of lepromin led to the production of hemorrhagic nodules. Rats ([25]) have been reported to remain negative after repeated lepromin injections, but they did show positive lepromin reactions after injection with BCG or *M. leprae murium*. Hadler ([20]) reported that rat macrophages tended to store *M. leprae* for up to 90 days without destroying them. Histologically, lepra cells were formed which were quite different from the epithelioid cells found in guinea-pigs. Rabbits frequently show sensitivity to single lepromin tests for preexisting sensitivity ([18, 43]), just as do dogs. The following animals are reported to have been negative on single tests: cat, monkey, fowl, pig, rat, turtle (how you test a turtle I am not sure), goat, mouse, and hamster ([18, 43]).

(*b*) The meaning of the Fernandez reaction: Most leprologists agree that the Fernandez response probably signifies established tissue allergy to mycobacterial antigen and is, therefore, equivalent to the tuberculin reaction. To what extent this agreement has been due to the obvious and logical analogy with tuberculosis, or how much is based on clinical findings and how much on animal experiments, it is impossible to guess. There are many reports of sensitization of normal persons by repeated injections of lepromin. Fernandez ([17]) also reported desensitization of children by repeated injections of a protein extract of bacilli, with the Fernandez response being eliminated while the Mitsuda reaction was retained.

In animal experiments the major difficulty in interpretation comes from the rarity of the classical bimodal curve distinguishing the Fernandez and Mitsuda responses. The only animals showing the late Mitsuda bimodal response on first injection have been dogs. Wade's dogs ([48]) tended to show a bimodal response on the first injection with an interval of minimum reaction from 2-7 days. With reinoculation the bimodal character of the curve disappeared, and lesions evolved in 2 days and sloped down gradually. In 37 per cent of Feldman's Minnesota dogs the typical response on first injection developed gradually over 12-21 days and was presumably equivalent to a Mitsuda reaction. With repeated injections the response was accelerated and tended to become strongly positive on the second day, thereafter progressing to necrosis and ulceration.

(*c*) The meaning of the Mitsuda reaction: Ever since its discovery, leprologists have enjoyed speculating about the meaning of the Mitsuda response. That it is related to gradual breakdown of bacilli seems clear. Supporting this are observations such as the correlation between the appearance of the reaction and microscopic evidence of bacillary destruction on histologic examination. Tubercle bacilli have been shown by Hadler ([21]) to be destroyed more rapidly in guinea-pigs than leprosy

bacilli, which coincides with the observation that the palpable secondary responses to heated BCG reach a peak at 2 weeks rather than the 3 weeks which is typical with leprosy cases. This may, however, be due merely to differences in lipid coating because the leprosy bacilli come from infected tissues and the tubercle bacilli from relatively old *in vitro* cultures. Tubercle bacilli extracted from tissues should be tested to give a true comparison.

Apparent disagreement has developed in the literature because some authorities have presented the hypothesis that a positive Mitsuda response is indicative of "capacity to react" ([47, 50]) while others say it is merely an extension of, and in fact a more clear-cut demonstration of, the same sensitization which produces the Fernandez response and probably represents the degree of resistance ([17]). These two points of view are not necessarily exclusive. Data from animal experiments permit an overall hypothesis incorporating both points of view as applying to different stages of sensitization. This schema can also be related to the specific types of human leprosy.

A conceptual framework incorporating evidence from both animal and human sources is presented in Table 1. Animal observations help us mostly with the earlier stages of the process. In the unsensitized host the Fernandez response is negative, while the positive Mitsuda reaction appears only in hosts with high "capacity to react." According to this hypothesis, as sensitization proceeds the development of the positive Fernandez response and accentuation of the Mitsuda reaction continues to depend on reactive capacity. I have tried to separate our guinea-pigs into two groups on the basis of the size of their initial reactions, in order to see if eventual sensitization correlated with the results of the first injections. Although the differences are in the right direction, they are too small to be conclusive because our guinea-pigs are too much alike. We need genetically-selected experimental groups of different susceptibility like Lurie's rabbits ([30, 31]). In the absence of such evidence I have had to use the less valid comparisons between species that is presented.

In Figure 1 the dynamic relationships of local antigen level, local tissue sensitivity, and palpable reaction are shown as they appear to occur at various stages of sensitization. As said, unsensitized guinea-pigs and dogs vary in the speed with which they respond to initial injections. As they become uniformly sensitized, the booster effect of locally-released antigen is added to prior sensitization to speed up the inflammatory response. Evidently there is free or easily accessible antigen in lepromin which is available to produce the Fernandez response in already-sensitive hosts, as shown by experiments with filtrates of that product. When both antigen and sensitivity curves are above a postulated threshold line, the aggregation of inflammatory cells becomes sufficient to produce a palpable nodule. Burnet's theory ([4]) of

TABLE 1.—*Interpretation of Fernandez and Mitsuda reactions according to sensitization of host and "capacity to react" to mycobacteria.*

Status of sensitization (capacity to react)	Fernandez reaction	Mitsuda reaction	Animal host	Human leprosy
Unsensitized host				
a. Low capacity	Negative	Negative	Rats, *M. leprae murium*	Susceptible
b. Moderate capacity	Negative	Occasional and weak	Guinea-pigs and dogs, first injection of *M. leprae*	Moderately susceptible
c. High capacity	Negative	Positive	Guinea-pigs with BCG	Slightly or nonsusceptible
Early sensitization				
a. Low capacity	Weakly pos. or negative	Weakly pos. or negative	Rats, *M. leprae murium* Rabbits with *M. avian*	Prelepromatous & indeterminate
b. Moderate capacity	Positive	Positive	Guinea-pigs and dogs with repeated injections of *M. leprae*	Tuberculoid
c. High capacity	Strongly positive	Strongly positive	Guinea-pigs with repeated injections of BCG	Healed or no clinical infection
Late infection				
a. Low capacity	Negative	Negative		Lepromatous
b. Moderate capacity	Strongly positive	Strongly positive		Tuberculoid
c. High capacity	Positive	Positive		Healed or subclinical

specifically sensitized clones of phagocytes fits well with the type of sensitization seen.

In guinea-pigs injected with lepromin for the first time, a small reaction appears and lasts for a few days (Fig. 1A), apparently representing a nonspecific inflammatory response. With BCG and *M. leprae murium* on first inoculation, the secondary rise diagrammed in Figure 1B appears. As shown in Fig. 1C, bimodal curves in an already-sensitive host depend as much on the speed of breakdown of bacilli as on the sensitivity level. After sensitization, all of our guinea-pigs showed a nonbimodal curve similar to Figure 1D, with variations in the persisting profile of the nodular infiltration. In sensitized humans, also, it is my impression that the bimodality of curves is more a matter of tradition than actual observation. When repeated measurements are done on patients rather than reading only at 2 and 21 days, the reaction curve tends to slope down with a varying plateau at 2 to 4 weeks as observed in our guinea-pigs ([38]).

To return to the apparent disagreement in the literature, according

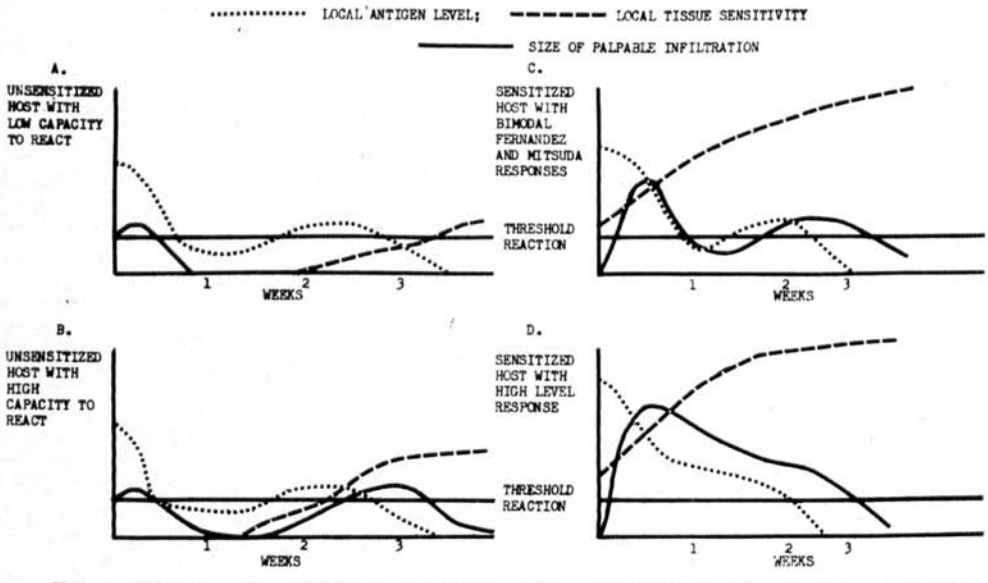

FIG. 1. Patterns of sensitization to mycobacterial injections, showing hypothetical levels of local antigen, local tissue immunity and size of reaction.

to this interpretation both points of view are correct. In nonsensitized hosts the Mitsuda response probably does show "capacity to react." In sensitized hosts the Mitsuda response is primarily a continuation of the same manifestations of sensitivity which lead to the Fernandez reaction with an added booster effect. This booster effect may be strong enough to produce the apparently anomalous finding that tuberculoid patients may be Fernandez negative but Mitsuda positive.

In trying to clarify the question of a possible relationship between mycobacterial sensitivity and resistance to infection, an experimental model using *M. leprae murium* has been reported by Sushido and Yamada ([45]). Vaccines were prepared both with and without oil adjuvant. A protective effect was demonstrated with the two adjuvant vaccines using olive oil and mineral oil, but none with heated bacilli alone. Hanks and Fernandez ([26]) demonstrated enhanced resistance to rat leprosy following vaccination with *M. leprae murium* plus tubercle bacilli as an adjuvant.

(*d*) Reactivity to tissue elements: We turn now to the matter of whether or not the tissue elements in lepromin may affect the reactions observed clinically. There is definite evidence from our guinea-pig studies that tissue elements can produce nonspecific sensitivity ([44]). Control preparations of normal human skin, liver or spleen antigen produced good sensitization of guinea-pigs. No great difference in reaction to human spleen antigen was observed when guinea-pigs sensitized to tissue were compared with others sensitized to Mitsuda lepromin. Another indication of important reactivity to tissue antigen was

the observation that lepromin tests in guinea-pigs sensitized to BCG-plus-spleen antigen were significantly larger than in animals sensitized to BCG alone. A significant degree of specific mycobacterial sensitization was, however, indicated by the greater reaction to lepromin in animals sensitized to lepromin than in groups sensitized to spleen antigen. The Dharmendra antigen, also, was demonstrated to retain much of its tissue reactivity, although a slight reduction in tissue-sensitizing capacity was observed.

Homologous tissue antigens also can produce sensitization in the species of origin. We have used heated guinea-pig serum and a guinea-pig spleen antigen prepared in the same way as Mitsuda lepromin. It is well known immunologically that denaturing of protein by heating produces alteration in its antigenicity with broadening of its antigenic range ([46]).

It seems to be basic for further research on the immunology of leprosy to obtain purified antigens. Since our source at this time must be human tissue, we have tried various modifications of the techniques developed by Henderson ([27]), Dharmendra ([11]) and others to remove tissue elements from bacillary suspensions. We now use the following steps: (1) grinding lepromatous material mechanically in a glass mill and shaking in a Mickle's vibrator with carborundum or glass beads to break up tissue cells; (2) shaking with olive oil and then centrifuging to separate the majority of the bacilli into a layer at the interface, and then washing and centrifuging; (3) digesting the suspension for one-half hour with pancreatin; (4) repeated washing and centrifuging at 2000-3000 g.

Both *M. leprae* and *M. leprae murium* obtained from tissues vary considerably in their specific gravity or surface tension. The best-looking bacilli with maximum acid-fastness float to the surface even in distilled water. Bacilli which sediment easily tend to be poorly-staining and granular. The purer our suspension, the more trouble we have in keeping bacilli from flocculating and sticking to the glassware. Tween 80, 1:1000, makes it possible to maintain a reasonably good suspension. With these methods we have been able to get preparations which produce no demonstrable cross reactivity with tissue antigens.

Our present efforts are focused on trying to obtain a leprolin from tissue-free bacillary suspensions which we expect to use as a specific skin-testing antigen. Using the urea extraction procedure developed for preparing the equivalent of PPD-S from tubercle bacilli by Baldwin *et al.* ([3]), we have obtained preparations which show the ultra-violet absorption spectra shown in Figure 2. The bulge at 260 mμ is similar, but less, than that obtained by Baldwin for his dialyzed urea-extract tuberculin. One further comment: Our observations support the possibility which has been mentioned that autoimmunization may be important in the pathogenesis of leprosy. Circumstantial evidence sug-

gests that reactions in lepromatous leprosy may be autoimmune crises brought on by abundant discharge of tissue antigens. Similarly, the whole tuberculoid phenomenon could well have a major autoimmune component.

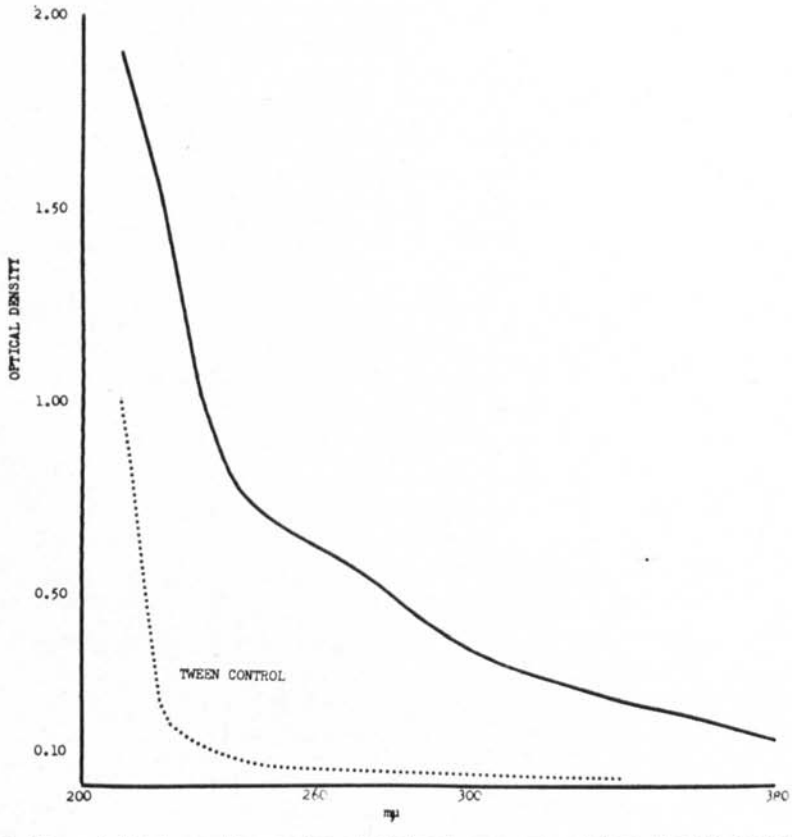

FIG. 2. Ultra-violet absorption spectra of dialyzed urea extract from live *M. leprae murium*.

3. *Cross reactions with other mycobacteria.*—(*a*) Preventive implications: Present-day enthusiasm for the possibility of using BCG immunization as a measure for leprosy control is predicated largely on the hope that cross reaction between the two will be sufficiently great to influence resistance as was originally demonstrated by Fernandez ([17]). Without going into the question of the relation between sensitivity and resistance, it is true that in mycobacterial infections the two usually rise and fall together. That some degree of cross reaction does exist is indicated by the production of the capacity for positive lepromin reactions by BCG vaccination in humans. Demonstration of whether or not there is an accompanying increase in resistance will have to await follow-up studies of vaccinated populations.

Skin reactions in specifically sensitized animals may permit better definition of cross sensitivity between various acid-fast organisms.

Early advances in delineating the antigenic grouping of mycobacteria came out of the work of veterinarians, who distinguished on the basis of the size of tuberculin reactions in cattle between human and bovine tuberculosis infections and a more general group including avian tuberculosis, Johne's bacillus and various saprophytes. Mycobacteria infecting various cold-blooded animals fell into a third group ([9]).

More recently, investigations have been carried forward with a great deal of precision by Palmer and Edwards and their colleagues ([39, 40, 41]). In well-controlled guinea-pig studies they have defined the group reactivity of various atypical acid-fast organisms from human pulmonary lesions and other sources. They have demonstrated that, by comparing the size of tuberculin reactions, homologous sensitivity can be identified with fair statistical accuracy even when a considerable degree of cross reactivity exists.

Equivalent research with leprosy will require more specific antigens than we now have. Using standard lepromin, the following studies have been made of lepromin positivity induced by BCG in animals. Azulay ([2]) reported that 45 days after BCG inoculation, 54 per cent of 45 guinea-pigs showed positive Fernandez reactions and 94 per cent gave positive Mitsuda responses. Hadler ([22]) reported that BCG-immunized guinea-pigs had an average reaction of 5 mm. to lepromin with peaking on the 6th day, while the earlier tuberculin reaction reached a peak of 12 mm. Yanagisawa *et al.* ([51, 52]) lepromin-tested groups of 6 guinea-pigs 2 months after vaccination with BCG and with heat-killed virulent tubercle bacilli plus mineral oil. They obtained good reactions which lasted 2 weeks, the early reactions being particularly intense in the group injected with Freund's adjuvant. Olmos Castro ([34, 35]), using very small numbers of guinea-pigs, showed that the time of maximum reaction was influenced by the interval between the injections of BCG and lepromin. His data suggest that maximum Fernandez responses were obtained when the interval after immunization was 2 to 3 weeks, while with longer intervals reactions were delayed to resemble Mitsuda reactions. He also reported that dogs ([36, 37]) specifically immunized with either BCG or lepromin showed cross sensitivity to the other antigen. When testing was done 9 months after sensitization, heterologous cross reactions were no longer elicited but specific sensitivity remained. Again the number of animals used was so small that the results are not convincing. We, too, have demonstrated in guinea-pigs moderate cross reactions between BCG and lepromin ([44]).

Of particular value in indicating a high degree of cross reactivity between BCG and leprosy were the studies of Pereira *et al.* ([42]) on rhesus monkeys. In one series 11 out of 12 monkeys became lepromin-positive after BCG, with sensitivity lasting 12 months. Controls which received a heterogenous mixture of acid-fast bacilli, including *M. avian*, and *M. smegmatis*, did not develop lepromin sensitivity. Chaussinand

([5]) reported that BCG produced lepromin positivity in guinea-pigs while *M. marianum* and *M. phlei* did not.

So far there is no good evidence that BCG protects from murine leprosy infection, except for two inconclusive reports in which *M. leprae murium* was used. Azulay ([1]) challenged 57 rats with that microorganism 4 months after they had been vaccinated with BCG. When they were sacrificed 10 months later, pathologic grading suggested that infections were less severe in the BCG-immunized group than in controls. Hadler and Zitti ([24]), on the other hand, could demonstrate no protective effect of BCG vaccination in rats, but reported more rapid destruction of bacilli in guinea-pigs vaccinated with BCG.

Also pertinent to the question of cross sensitization are examples in which tuberculin sensitivity was induced by injection of human and rat leprosy bacilli. One clear-cut demonstration was Melsom's reports ([32, 33]) that when guinea-pigs were injected intradermally with fresh lepromatous material, they became tuberculin-positive and remained sensitive for more than one year. Suggestive of a particular antigenic association between *M. leprae murium* and BCG was Hadler and Zitti's report ([23]) that guinea-pigs given *M. leprae murium* intraperitoneally showed as strong sensitivity to tuberculin as BCG-vaccinated animals, while those given *M. leprae* developed only a weakly positive tuberculin reaction.

One of our still-incomplete experiments is shown in Table 2. Groups of 18 guinea-pigs were immunized to various mycobacterial antigens. Tuberculin cross reactions were considerably larger with purified, unheated *M. leprae murium* than with either of two purified *M. leprae* preparations, but not as large as specific reactions in BCG-immunized guinea-pigs. Kawaguchi ([28]) challenged mice with tubercle bacilli after they had been sensitized with *M. leprae murium*. There was some delay in the appearance of clinical infection, but no difference in eventual survival time.

No laboratory data now available contribute to understanding of the "natural reactivity" to lepromin reported by Davey *et al.* ([10]) and Doull and Guinto ([12]). Kuper ([29]) reported that a battery of mycobacterial and fungal antigens tested in East Africans showed more cross

TABLE 2.—*Reactions to BCG and PPD-S in guinea-pigs previously sensitized to mycobacterial antigens. Average 48-hour reactions (in mm.) in groups of 18 guinea-pigs.*

Sensitization groups	Cross-test antigens	
	BCG	PPD-S
Heated, purified *M. leprae*	7.2	2.0
Unheated, purified *M. leprae*	6.8	1.8
Unheated, purified *M. leprae murium*	9.3	5.5
Unheated BCG	11.8	12.0
Tween 1:1000	7.8	1.8

reaction between human and avian tuberculins than between these antigens and lepromin.

(*b*) Possible epidemiologic usefulness of leprolin: The principal objective of my own research is, as said, to attempt to develop an epidemiologically useful leprolin, i.e., a preparation of the soluble antigenic elements of the leprosy bacillus ([7]). Much of our knowledge of the epidemiology of tuberculosis has come from judicious use of the tuberculin test. Any reactions to leprolin which are stronger than those produced by tuberculin would be of interest in perhaps indicating prior infection with the leprosy bacillus. Such a tool is needed because of two characteristics of leprosy: (1) the long incubation period makes for difficulty in tracing spread; (2) the probability that, because of high natural resistance, most infected individuals do not manifest clinically recognizable disease. It is important, however, to be able to identify all infected individuals in order to understand the biologic gradient of the disease and factors influencing community spread. Additional values of a good leprolin would be its use in diagnosis, and the possibility of demonstrating cross reactivity with BCG and other mycobacterial infections. Purified bacillary suspensions producing the Mitsuda response may perhaps be used in unsensitized individuals as an index of resistance.

CONCLUSIONS

Animal experiments have provided the following information pertinent to an understanding of the cutaneous sensitization to *M. leprae*.

1. The tissue elements in present lepromins are antigenic.
2. The Fernandez reaction is clinically and histologically similar to the tuberculin response, and presumably has similar significance in indicating tissue sensitivity.
3. The Mitsuda response to lepromin appears to be a more complex phenomenon, and probably depends on an allergic interaction between locally-available antigen released by the disintegration of the bacilli and local tissue sensitivity which may develop during the period of the reaction in an unsensitized host, or undergo a booster effect in previously sensitive animals.
4. Leprosy bacilli are not broken down in tissue as rapidly as tubercle bacilli, and the skin reactions are also relatively slower. In sensitized hosts, dissolution of both leprosy and tubercle bacilli occurs more rapidly than on initial injection, and reaction curves tend to slope down to plateaus rather than to exhibit bimodality.
5. Although there is evidence of considerable cross reactivity between tubercle and leprosy bacilli, definitive demonstrations of the strength of this antigenic relationship are not yet available. It is not known where *M. leprae* fits into the three or more antigenic groups of mycobacteria which have been tentatively postulated on the basis of skin tests. The need for better antigens is evident.

CONCLUSIONES

Los experimentos en animales han suministrado la siguiente información pertinente a una compresión de la sensibilización cutánea al *M. leprae.*

1. Los elementos histológicos en la lepromina actual son antigénicos.
2. La reacción de Fernández es clínica e histológiamente semejante a la reacción a la tuberculina, y presuntamente posee una importancia similar en lo tocante a indicar sensibilidad histológica.
3. La reacción a la lepromina de Mitsuda parece ser un fenómeno más complejo, y probablemente está basada en una interacción alérgica entre antígeno disponible localmente y liberado por la desintegración de los bacilos y la sensibilidad local del tejido que puede formarse durante el periodo de la reacción en un huésped insensibilizado o experimentando un efecto realzador en animales previamente sensibles.
4. Los bacilos leprosos no se desintegran en el tejido con tanta rapidez como los tuberculosos y las cutirreacciones son también relativamente más lentas. En los huéspedes sensibilizados, la disolución de los bacilos tanto leprosos como tuberculosos ocurre más rápidamente que en la inyección inicial y las curvas de las reacciones tienden a declinar y formar mesetas más bien que a manifestar bimodalidad.
5. Aunque hay signos de considerable reactividad cruzada entre los bacilos tuberculosos y los leprosos, no existen todavía demostraciones definitivas de la intensidad de esta relación antigénica. No se sabe donde encaja el *M. leprae* en los tres o más grupos antigénicos de micobacterias que se han postulado tentativamente a base de las cutirreacciones. Es evidente que se necesitan mejores antígenos.

RESUMÉ

L'expérimentation chez l'animal a fourni les informations suivantes en ce qui concerne la compréhension de la sensibilisation cutanée au *M. leprae.*

1. Les éléments tissulaires trouvés dans les lépromines actuellement utilisées sont antigéniques;
2. La réaction de Fernandez est similaire à la réponse à la tuberculine, tant d'un point de vue clinique que d'un point de vue histologique; il est à présumer qu'elle a une signification analogue en ce qui concerne la sensibilité cutanée.
3. La réponse de Mitsuda après lépromine paraît un phénomène plus complexe; elle dépend probablement d'une interaction allergique entre l'antigène libéré sur place par la désintégration des bacilles et la sensibilité tissulaire local, celle-ci pouvant soit se développer durant la période de réaction chez un hôte non sensibilisé préalablement, soit subir un coup de fouet chez des animaux préalablement sensibilisés.
4. Les bacilles de la lèpre ne sont pas aussi rapidement désintégrés dans les tissus que les bacilles de la tuberculose, et les réactions cutanées sont aussi relativement plus lentes. Chez les hôtes sensibilisés, la dissolution des bacilles de la lèpre comme des bacilles de la tuberculose survient plus rapidement que lors d'une première injection, et les courbes de réaction tendent vers un ralentissement et un plateau plutôt que vers un caractère bimodal.
5. Malgré l'évidence d'une réactivité croisée considérable entre les bacilles de la tuberculose et de la lèpre, la démonstration définitive de la force de cette relation antigénique n'est pas encore disponible. On ne sait pas quelle est la place de *M. leprae* parmi les trois groupes antigéniques, ou plus, de Mycobactéries qui ont été suggérés à titre provisoire sur la base des tests cutanés. La nécessité d'un meilleur antigène demeure évidente.

REFERENCES

1. Azulay, R. D. The protective role of BCG in murine leprosy. Internat. J. Leprosy **22** (1954) 61-64.

2. Azulay, R. D. Our experience with the lepromin test from 1944 to 1955; new experiments with guinea pigs. Arq. mineiros Leprol. **16** (1956) 200-211; *also* **17** (1957) 324-334.
3. Baldwin, R. W., Gilbert, G. A. and Jones, A. S. A specific tubercle antigen, extracted from living *Myco. tuberculosis*. Biochem. & Biophys. Acta **10** (1953) 402-409.
4. Burnet, F. M. The new approach to immunology. New England J. Med. **264** (1961) 24-34.
5. Chaussinand, R. and Viette, M. Peut-on utiliser la vaccination par le *Mycobacterium marianum* dans la prophylaxie et la thérapeutique de la lèpre? Bull. Acad. nation. Méd. **139** (1955) 165-169; *reprinted,* Internat. J. Leprosy **23** (1955) 435-440.
6. Cochrane, R. G. Leprosy in Theory and Practice. Bristol: John Wright & Sons, Ltd., 1959, p. 15.
7. [Congress, Tokyo] Technical resolutions. Immunology: The lepromin reaction. Trans. VII Internat. Congr. Leprol., Tokyo, 1958; Tokyo (Tofu Kyokai), 1959, pp. 463-466; Internat. J. Leprosy **26** (1958) 385-389.
8. Convit, J., Lapenta, P. and Jórgensen, J. The Mantoux and Mitsuda reactions in hamsters and guinea-pigs before and after vaccination with BCG. Internat. J. Leprosy **23** (1955) 162-166.
9. Crawford, A. B. The specificity of the tuberculin reaction in cattle and in laboratory animals. *In* Tuberculosis and Leprosy—The Mycobacterial Diseases. Symposium Series, vol. 1, American Assoc. Adv. Sci., Lancaster, Pennsylvania, 1938, pp. 59-65.
10. Davey, T. F., Drewett, S. E. and Stone, C. Tuberculin and lepromin sensitivity in E. Nigeria. Leprosy Rev. **29** (1958) 81-101.
11. Dharmendra. The immunological skin tests in leprosy. Indian J. Med. Res. **30** (1942) 1-22.
12. Doull, J. A., Guinto, R. S. and Mabalay, M. C. The origin of natural reactivity to lepromin. The association between the Mitsuda reaction and reactions to graded doses of tuberculin. Internat. J. Leprosy **27** (1959) 31-42.
13. de Faria, J. L. Contribuição ao Conhecimento de Natureza de Reação de Mitsuda. Rio de Janeiro: Dept. Imprensa Nacional, 1953.
14. de Faria, J. L. Der heutige Stand der Forschung über das Wesen der Leprominreaktion mit besonderer Berücksichtigung der histopathologischen Befunde. Arch. Dermat. u. Syph. **198** (1954) 37-50.
15. de Faria, J. L. Revisão dos estudos experimentais sobre a reação a lepromina relacionados com a natureza desta reação. Rev. brasileira Leprol. **22** (1954) 145-156.
16. Feldman, W. H., Karlson, A. G. and Grindlay, J. H. Lepromin: Mitsuda's reaction with experimental observations in dogs. Ann. New York Acad. Sci. **54** (1951) 53-72.
17. Fernandez, J. M. M. Estudio de los factores que intervienen en la leprominoreacción. Internat. J. Leprosy **21** (1953) 584 (abstract).
18. Fielding, J. W. The lepromin test in laboratory animals. Med. J. Australia **1** (1944) 439-441.
19. Hadler, W. A. Estudo comparado das lesões provocadas pela injeção intradérmica de suspensoes de *M. leprae* e *M. tuberculosis* en cobaios normais. Rev. brasileira Leprol. **21** (1953) 315-340.
20. Hadler, W. A. Comportamento do cobaio e do rato, normais, injetados com "lepromina" por via intradérmica. Rev. brasileira Leprol. **21** (1953) 165-194.
21. Hadler, W. A. Estudo comparado das lesões provocadas por suspensões de "M. leprae" e de "M. tuberculosis" injetadas por via intradérmica. Rev. brasileira Leprol. **22** (1954) 109-123.

22. HADLER, W. A. Influencia de inoculação prévia de BCG sôbre os resultados da reação da lepromina em cobaios. Bol. Serv. Nac. Lepra **15** (1956) 5-62.
23. HADLER, W. A. and ZITTI, L. M. Estado da sensibilidade tuberculínica em cobaios normais inoculados experimentalmente com *M. leprae, M. lepraemurium* e *M. tuberculosis*. Rev. brasileira Leprol. **21** (1953) 341-364.
24. HADLER, W. A. and ZITTI, L. M. Efeito da vacinação pelo BCG sôbre a evolução da lepra murina; observação em ratos inoculados por vía peritoneal com pequena dose de "M. lepraemurium." Rev. brasileira Leprol. **22** (1954) 124-134.
25. HADLER, W. A. and ZITTI, L. M. Estudo da reação da lepromina no rato previamente inoculado com *M. lepraemurium* e com *M. tuberculosis* (BCG). Rev. brasileira Leprol. **23** (1955) 53-68.
26. 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.
27. HENDERSON, H. J. A method for separating intact leprosy bacilli from leprous tissue. Proc. Soc. Exper. Biol. & Med. **50** (1942) 92-94.
28. KAWAGUCHI, Y. The immunological correlation of tuberculosis to leprosy. Part 2. La Lepro **24** (1955) 288-294 (in Japanese; English abstract).
29. KUPER, S. W. A. Hypersensitivity in tuberculosis and leprosy; skin reactions; summary of preliminary report. East African Med. J. **34** (1957) 349-350.
30. LURIE, M. B. Heredity, constitution and tuberculosis; experimental study. American Rev. Tuberc. **44** (1941) 1-125 (supplement).
31. LURIE, M. B. Experimental epidemiology of tuberculosis; in hereditary resistance to attack by tuberculosis and to the ensuing disease and effect of concentration of tubercle bacilli upon these 2 phases of resistance. J. Exper. Med. **79** (1944) 573-589.
32. MELSOM, R. M. Tuberculin hypersensitiveness in guinea-pigs inoculated intradermally with human leprous nodules. Internat. J. Leprosy **6** (1938) 462 (abstract).
33. MELSOM, R. Experiments in inoculating guinea-pigs with leprous material. Acta dermato-venereol. **20** (1939) 451-462.
34. OLMOS CASTRO, N. Sensitization to lepromin induced experimentally with BCG. Internat. J. Leprosy **20** (1952) 221-228.
35. OLMOS CASTRO, N. Reacción a la lepromina en cobayos calmetizados; la reacción precoz de Fernandez inducida experimentalmente por calmetización. Arch. Farm. y. Bioquim. del Tucumán **6** (1953) 255-260.
36. OLMOS CASTRO, N. and ARCURI, P. B. Sensitization of the dog with lepromin and BCG, and evidence of cross sensitization. Internat. J. Leprosy **25** (1957) 231-237.
37. OLMOS CASTRO, N. and ARCURI, P. B. Persistence of sensitization and cross-sensitization to lepromin and BCG in the dog. Internat. J. Leprosy **25** (1957) 238-242.
38. OLMOS CASTRO, N., ARCURI, P. B., USANDIVARAS, R. L., BONATTI, A. A., LEBRÓN, E., TORANZOS, L. B. and CONEJOS, M. Hipersensibilidad de vacunación y de infección por el "Mycobacterium leprae." (Comunicación previa.) Arch. argentinos Derm. **8** (1958) 221-229; *reprinted,* Internat. J. Leprosy **27** (1959) 148-156.
39. PALMER, C. E., EDWARDS, P. Q., FEREBEE, S. H., MEYER, S. N. and BLOCH, H. Experimental studies of vaccination, allergy, and immunity in tuberculosis. 1. Design of a research program. Bull. Wld. Hlth. Org. **12** (1955) 13-29.
40. PALMER, C. E., EDWARDS, P. Q., FEREBEE, S. H., MEYER, S. N. and BLOCH, H. Experimental studies of vaccination, allergy and immunity in tuberculosis. 2. Effect of varying the dose of B.C.G. Bull. Wld. Hlth. Org. **12** (1955) 31-45.
41. PALMER, C. E., EDWARDS, P. Q., FEREBEE, S. H., MEYER, S. N. and BLOCH, H. Experimental studies of vaccination, allergy and immunity in tuberculosis. 3. Effect of killed B.C.G. vaccine. Bull. Wld. Hlth. Org. **12** (1955) 47-62.
42. PEREIRA, M. J., JR. and GUIMARAES, F. N. Mitsuda's reactions induced by BCG in normal rhesus (*Macacca mulatta*). Mem. Inset. Oswaldo Cruz **53** (1955) 609-619.

43. Rodriguez, J. N. Observations on the leprolin (Mitsuda) reaction. Internat. J. Leprosy **6** (1938) 11-32.
44. Taylor, C. E., Hanks, J. H., Moses, H., Mittal, M. C. and Kant, L. The antigenic components of lepromin as assayed in guinea-pigs. Internat. J. Leprosy **28** (1960) 284-299.
45. Sushida, K. and Yamada, N. Immunological studies on murine leprosy. II. Relation between resistance to infection of mice immunized with killed murine leprosy bacilli and the serum protein fraction pattern. La Lepro **27** (1958) 263-270 (in Japanese; English abstract).
46. Uwazumi, S. Prazipitin und komplement-bindsversuche. Arb. Med. Fakultat. (Okayama) **4** (1934) 53-104.
47. Wade, H. W. The immunology problem. Internat. J. Leprosy **6** (1938) 95-101 (editorial).
48. Wade, H. W. The lepromin reaction in normal dogs; preliminary report. Internat. J. Leprosy **9** (1941) 39-56.
49. Wade, H. W. Sensitization of dogs by the lepromin reaction; a preliminary report. Mem. V Congr. Internac. Lepra, Havana, 1948; Havana, 1949, pp. 617-620.
50. Wade, H. W. Agammaglobulinemia and the lepromin reaction. Internat. J. Leprosy **25** (1957) 395-398 (editorial).
51. Yanagisawa, K., Asami, N. *et al.* Studies on the lepromin test. Parts I, II and III. La Lepro **24** (1955) (supplement) pp. 29-34, 35-46, 47-59.
52. Yanagisawa, K., Asami, N. and Ishihara, S. Studies on the lepromin test. V. Potency test of lepromin antigen using guinea pigs sensitized with tubercle bacilli. La Lepro **25** (1956) 198-202.
53. Yanagisawa, K. and Asami, N. Studies on the lepromin test. VIII. Sensitization experiments with trypsin isolated bacilli. La Lepro **27** (1958) 107-113.