AN APPRAISAL OF GENETIC STUDIES ON LEPROSY

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SUMMARY

The present paper reviews the research lines which have been explored to evaluate to what extent genetic factors are intervening on the mechanism of resistance and susceptibility to leprosy.

It presents a critical discussion of the investigations on the familial association of leprosy, familial association of leprosy types, intrafamilial contagion of leprosy, concordance of leprosy in twin-pairs, racial differences on leprosy prevalence and lepromatous rate, pedigree studies, association of leprosy to genetic markers, Australia antigen, and dermatoglyphic patterns. Space was also allotted to review family and twin-pair studies on the Mitsuda reaction, as well as to the investigation on the in vitro behaviour of blood macrophages against killed *M. leprae*.

Some areas in which further research on leprosy and genetics may be considered as prioritary are outlined with some detail.

INTRODUCTION

Leprosy is a chronic infectious disease caused by the acid-fast and Gram-positive bacillus classified as *Mycobacterium leprae*. Skin and mucosae are thought to be the most common portals of entry of leprosy bacilli, but the possibilities of infection through the circulatory, digestive and respiratory systems cannot be ruled out.

Before Hansen recognized *M. leprae* as the pathogenic agent of leprosy, the hypothesis of hereditary transmission of this disease was supported by eminent authorities (Danielssen and Boeck 1848). While this idea became rapidly forsaken, it is clear that at no moment was it put into doubt that leprosy manifestation depends upon the degree of susceptibility or resistance of the host to the development of the bacilli. This principle of general pathology is applied to any chronic or acute infection which, as it is known, are considered to depend upon three crucial factors: pathogenic agent, environmental conditions, and degree of resistance of the host.

Therefore, it is surprising that so much emphasis was put, in the late thirties, on speculations on the existence of a genetic component of the host responsible for leprosy manifestation (Rotberg 1937, Tolentino 1938, Aycock and McKinley 1938, Aycock 1940), once no phenotypic manifestation can be supposed without the commitment of some genetic entity.

1 This study was supported by the World Health Organization.
In the last decade, after a gap of about twenty years, interest on genetic research became renewed on different basis. Instead of speculations on the existence of genetic influence of the host for susceptibility to leprosy, systematic research was developed to evaluate to what extent hereditary factors are intervening on the host’s response to leprosy infection. Otherwise stated, research became focused on investigating whether the genetic component of the host should or not play an important role on leprosy manifestation.

Several lines of investigation up to 1966 have been reviewed elsewhere (Beiguelman 1967). In the present paper a wider approach has been tried by commenting on other research lines or by reviewing the investigations performed from 1966 onwards.

THE LEPROSY FORMS

From the physiopathological point of view, leprosy is not a monomorphic disease. Under the name of leprosy two polar forms are included, that is to say, the lepromatous and the tuberculoid types, usually stable, as well as two unstable forms, the indeterminate and the borderline (dimorphous) groups. While the lepromatous and borderline patients usually offer risk of contagion, the tuberculoid and indeterminate groups consist, as a general rule, of nonbacillipherous patients. As a consequence, they are considered as benign forms from the epidemiological point of view. Nevertheless, many indeterminate and tuberculoid patients may, in fact, be considered as benignantly affected, also from the clinical viewpoint, since they may heal without treatment and, sometimes, without sequelae. Indeterminate and borderline cases are considered unstable because they may evolve either to the lepromatous or to the tuberculoid type of leprosy.

The histological examination of lepromatous lesions reveal that they are infiltrates in which the Virchow cells predominate. These cells are full of acid-fast bacilli which may become granulated or disappear if the lesion is regressive. In opposition, the tuberculoid lesions are infiltrates of sarcoid or follicular type, which are usually bacteriologically negative.

Concerning the histological picture of the unstable group, it may be said that the indeterminate lesions are simple inflammatory infiltrates composed chiefly of lymphocytes, where bacilli are rare or absent. The borderline lesions are more difficult to characterize because they show patterns of both the tuberculoid-in-reaction and the lepromatous lesions, with bacteriological positivity.

The antithetical differences in tissular resistance to M. leprae presented by lepromatous and tuberculoid cases explain the contagiousness and progressive relapse of the former in absence of appropriate therapy, as well as the possibility of spontaneous regression of the tuberculoid lesions.

When it is said that the polar forms of leprosy are stable, or that the lepromatous cases are bacteriologically positive while the tuberculoid patients are bacilloscopically negative, it is not meant that under special circumstances some changes might
not occur among them. As a matter of fact, some tuberculoid patients may become bacillipherous and, at least theoretically, infectious cases. In such situations, the tuberculoid structure of their lesions may be changed, the epithelioid cells becoming dissociated as a consequence of hyperemia and oedema of the exudative process. The bacteriological index of reactional tuberculoid cases is sometimes very high, but, according to Bechelli and Guinto (1970), they may become bacteriologically negative in less than six to twelve months, even without sulphone treatment.

With respect to lepromatous cases, some of them, when under sulphone therapy, may develop the so-called pseudoexacerbation, first described in great detail by Souza-Lima and Cerqueira (1946), Souza-Lima and De Souza (1949), and Souza-Lima (1953). The same phenomenon was recently described by Ridley (1969) under the name of reversal reaction.

This acute reaction episode, whether recurrent or sporadic, may induce lepromatous patients to manifest tuberculoid-in-reaction-like lesions in variable degrees. These lesions may coexist with the previous ones and be transient or of long duration. At any rate, the changes introduced by pseudoexacerbation do not modify the negative late lepromin reaction exhibited by lepromatous cases.

The classification of leprosy patients in two polar types and two interpolar groups, also known as the South-American system, has been widely followed after its official recognition by the VI International Congress of Leprosy held in Madrid in 1953. The fact that comparisons of clinical diagnoses to histopathological results have shown high concordance rates contributed to its success. Such results were obtained both when the clinical diagnoses were made by wide experienced clinicians, as, for instance, Quagliato (1959) on 250 cases, and by several leprologists with variable clinical experience on 1,130 cases (Azulay 1953). In spite of this, in recent years, some leprologists have reopened the question of leprosy classification (Ridley and Jopling 1962 and 1966, Leiker 1966, Pfaltzgraff 1966).

Taking into account the remarkable importance of this matter, as well as the risk of misunderstanding caused by the introduction of different and peculiar terminologies on leprosy classification before a general agreement among leprologists, Leiker (1966) proposed a long term study by a committee of experienced workers from different countries, to analyse this problem. This study would be based on a large number of cases presented in full detail and would include cases from many endemic areas. Meanwhile, the adoption of modified systems with their particular terminology does not seem to be advisable, since this attitude may contribute to difficult rather than to clarify the understanding of leprologic studies.

Actually, the World Health Organization (WHO) has designated an International Reference Center for histological diagnosis and classification of leprosy at the Instituto Nacional de Dermatologia, Caracas, Venezuela. A cooperative study with other centers located in several areas of the world will be carried out under the direction of WHO in an attempt to obtain standardized criteria for histological diagnosis and classification of leprosy.
FAMILIAL RECURRENCE OF LEPROSY

Leprosy has always been considered as a familial disease. Families with high concentration of leprosy cases have been observed since ancient times and were reported in ancient literature (Danielssen and Boeck 1848). Nevertheless, a clear demonstration of the nonrandom recurrence of leprosy in families living in an endemic area has only recently been provided (Beiguelman et al. 1968a).

In order to avoid the pitfalls underlying the two generation studies, that demonstration was made by analysing all sibships (961) with at least one case of leprosy, registered in Campinas, S.P., Brazil. The sizes of the sibships varied from 2 to 15 members.

Since the sibships have been obtained by truncate selection, their expected number, with one and with more than one case, has been calculated according to the formulae:

\[ \sum N \left( n p^n - 1 - q \right) / \left( 1 - p^n \right) \]  
\[ \sum N \left[ 1 - (p^n + 1 - q) \right] / \left( 1 - p^n \right) \]

(Beiguelman 1968a). In these formulae, \( N \) = number of sibships of size \( n \); \( q \) = probability that an individual will be affected; \( p \) = probability that an individual will not be affected (\( p = 1 - q \)).

If the familial recurrence of leprosy were at random, the expected and observed number of sibships with single and with multiple cases of leprosy would not differ significantly when \( q \) values are similar to the prevalence of leprosy in the population.

By attributing different values to \( q \) it was observed that the hypothesis of random recurrence of leprosy could only be fitted for values between 80 to 100 per 1,000. Since it is known that the prevalence of leprosy in the studied area might, at most, be estimated as 3 per 1,000, it becomes clear that the hypothesis of random familial recurrence of leprosy must be rejected. Otherwise stated, it is accepted that leprosy shows a familial association.

Taking into account that the nonrandom familial recurrence of a communicable disease may not be demonstrated when the prevalence values are high, it seems of great interest to verify whether the family association of leprosy may also be confirmed in other endemic areas with prevalence values higher than those found in Brazil.

Obviously, the demonstration that leprosy is a familial disease does not mean too much, since differential exposure conditions cannot be ruled out even in highly endemic areas. However, familial association, while not sufficient, is a necessary condition for supposing that some important genetic component of the host is involved in leprosy manifestation.

FAMILIAL ASSOCIATION OF LEPROSY FORMS

Information on familial association of leprosy forms may be drawn from investigation of concordance of polar types of leprosy among sib pairs. Exclusion of pairs with at least one indeterminate or one borderline case derives from the difficulty
arised in ascribing concordance or discordance to a pair when the unstable forms are included. Otherwise, the choice of sib pairs is a consequence of the fact that, excluding twins, they constitute the most natural pairs. On the average, samples of sib pairs will disclose greater genetic and environmental similarities, as well as smaller age differences than any other pairs of consanguineous relatives.

This methodological approach was applied by Beiguelman et al. (1968b) to a random sample of 111 sib pairs belonging to different sibships. The observed distribution (78 lepromatous pairs, 11 tuberculoid pairs, and 22 pairs exhibiting different polar types) deviated significantly from the expected binomial distribution ($\chi^2 = 15.234; 1 DF; P<0.001$) because of an excess of pairs concordant for leprosy type.

Discordant results published on this matter are not comparable because of methodological differences. Thus, Horton and Povey (1966) have analysed 84 multiple-case families, but they do not distinguish the borderline from the indeterminate cases that were included in the same class. Moreover, they have considered all first-grade relatives for pair comparisons, that is to say, parent-child and sib pairs.

More recently, Rao et al. (1969) have also made pair comparisons. However, as they have stressed in page 94 of their paper, the concept of family they adopted is divorced from genetic significance, since they defined a family as "a group of individuals partaking food from a common kitchen."

Beiguelman et al.'s data (1968b), besides fitting the hypothesis of family association of polar forms of leprosy, may also suggest that this association depends upon a genetic variation of the host rather than of $M. leprae$. Accepting as true the epidemiological principle that affected individuals derived from the same focus are a consequence of infection by the same strain of bacilli, that suggestion would be favoured by the fact that a lepromatous focus has been recognized for all but one sib pair. Moreover, 31.8% of the discordant pairs have shown signs of leprosy practically at the same time.

**INTRAFAMILIAL CONTAGION OF LEPROSY**

A large number of unsuccessful leprosy infection trials made in anima nobile in the last century by Danielssen, Profeta, and Mouritz, are mentioned in the classical literature (Alonso 1966). They have been used both to emphasize that most of the human beings offer tissular resistance to $M. leprae$ and to support the hypothesis that susceptibility to leprosy depends upon an important genetic factor of the host.

Manifestation of tuberculoid leprosy after accidental inoculation of leprosy bacilli has also been used for the same purposes. The cases most frequently mentioned are those described by Porrit and Olsen (1947) of two American soldiers who exhibited tuberculoid lesions in their tatoos, which had been made three years before, at the same day, by the same professional living in Melbourne (Australia). More recently, Aguas (1967) described the occurrence of tuberculoid leprosy in a pair of twins aged 3 years, who received three transfusions of blood from a lepromatous donor at the age of 20 months.
Papers on the frequency of leprosy contagion among spouses of affected individuals have also been published to point out low contagiousness of leprosy (see references in Quagliato 1957a and Mohamed-Ali 1965a). However, some of them refer to the proportion of spouses who became affected, among the affected relatives of an index case, instead of to the proportion of affected individuals among those exposed to an open case of leprosy. Others have not taken into account either the form of leprosy exhibited by the spouses or the time of cohabitation among them. The latter information is very important, since, according to Quagliato’s data (1957a), 44% among the spouses who became affected manifested signs of leprosy during the first three years of cohabitation, while during the first five years this rate reached 95%. Obviously, a better argument to support the hypothesis that susceptibility to leprosy is under the influence of an important genetic component of the host would be provided by the demonstration that the attack-rate of leprosy is proportional to the coefficient of relationship of the individuals to the index case. Since time of cohabitation may also be associated to the coefficient of relationship, it seems clear that, in order to obtain conclusive results, a well controlled experiment should be planned.

Spickett (1962a) gave great importance to Páteo and Pereira’s data (1936), which he quoted as being Duarte and Lima’s. However, their data referred to the relationship of 456 affected contacts to presumed foci, no references having been made to the leprosy form of either the affected contacts or the index cases. Identical criticism is applied to the figures published by Mohamed-Ali (1965b).

A partial analysis of the problem has been published by the author (Beiguelman 1971) and is summarized in Table I. Several conclusions may be drawn from

### Table I. Contagion Rate and Cohabitation

<table>
<thead>
<tr>
<th>L focus</th>
<th>Contacts a examined</th>
<th>Contagion rate (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>L</td>
</tr>
<tr>
<td>Father (167)</td>
<td>Son (346)</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>Daughter (334)</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>Both (680)</td>
<td>9.3</td>
</tr>
<tr>
<td>Mother (92)</td>
<td>Son (180)</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Daughter (176)</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Both (356)</td>
<td>9.3</td>
</tr>
<tr>
<td>Both parents (30)</td>
<td>Son (74)</td>
<td>25.7</td>
</tr>
<tr>
<td></td>
<td>Daughter (55)</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Both (129)</td>
<td>23.2</td>
</tr>
<tr>
<td>Husband (271)</td>
<td>Wife (271)</td>
<td>2.9</td>
</tr>
<tr>
<td>Wife (159)</td>
<td>Husband (159)</td>
<td>5.7</td>
</tr>
<tr>
<td>Spouse (430)</td>
<td>Spouse (430)</td>
<td>4.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> With at least five years of cohabitation with a familial lepromatous focus.

<sup>b</sup> Respectively for the lepromatous (L), tuberculoid (T), indeterminate (I), and borderline (B) forms of leprosy.
the figures on that table. The first is that, in such type of work, different leprosy forms must be distinguished. Failure in observing this situation may be responsible for the controversial results of some authors (Mohamed-Ali 1965b, Rao et al. 1969). As a matter of fact, if leprosy forms were not discriminated, it could be concluded from Table I that the attack rate among spouses with a long and intimate contact with a lepromatous partner is only a little lower than that found in the offspring of couples including one lepromatous partner.

In opposition, when the forms of leprosy are considered, a quite different picture emerges, and it becomes evident that the consanguineous relatives of lepromatous cases are proner to exhibit the same polar form of leprosy than nonconsanguineous relatives (spouses), in spite of the intimate contact among the latter.

The higher frequency of lepromatous cases in the offspring of families with both parents affected does not exclude environmental influences. However, some figures may be used to suggest the importance of a genetic component of the host for leprosy manifestation. Thus, besides the lower proportion of lepromatous cases among nonconsanguineous relatives (spouses), it has been observed that the frequency of lepromatous leprosy in the offspring of couples including one affected parent was independent of the sex of the parent.

**TWIN PAIR STUDIES**

Twin pair studies could afford a powerful test for proving or disproving the theory that an important genetic component of the host intervenes on leprosy manifestation. Nevertheless, taking into account that leprosy may manifest itself under different clinical forms, it seems clear that these studies cannot consider simple comparisons of the proportions of concordance for leprosy exhibited by MZ and DZ pairs. Further criteria must be added to avoid inconclusive or biased results.

Since leprosy is an infectious disease, it seems obvious that twin pairs should be ascertained starting from infectious cases, tuberculoid and indeterminate patients not being included as index cases, as a consequence of their usual negative bacteriological index.

Although tuberculoid-in-reaction patients may have a high bacteriological index, it is safer not to include them as index cases for twin pair studies, because they may become bacteriologically negative even without treatment (Bechelli and Guinto 1970). The same criterion should be applied to borderline cases. Besides their instability and low frequency in most endemic areas, it should also be considered that their bacteriological index may have a rate of decrease three times faster than that observed among lepromatous cases, when under dapsone therapy (Bechelli and Guinto 1970). In short, it may be said that it is safer, in a first approach, to begin twin pair studies starting from lepromatous index cases.

Concordant cases will be represented by cotwins who are both lepromatous, while discordant pairs will include either a healthy or a tuberculoid cotwin of a lepromatous patient. At the present state of knowledge, a twin pair composed by a
lepromatous and a tuberculoid case cannot be considered as concordant because of the tissular resistance to growth and development to *M. leprae* exhibited by the latter. Concerning the indeterminate and borderline cotwins of lepromatous index cases, it seems reasonable to exclude them from the sample, because the instability of these forms will not allow to attribute concordance or discordance to the pairs in which they are included.

Since leprosy is thought to have a long incubation period, it seems advisable that the healthy individuals cohabiting for less than five years with their lepromatous cotwins are not included in the sample. It also seems advisable for analytical purposes, that MZ male and female pairs, as well as DZ like-sexed pairs should be considered separately. Since it is known that leprosy shows higher incidence among males, at least in age groups over 14 years (Doull et al. 1942, Bechelli et al. 1966 and 1970, Beiguelman et al. 1968c), it seems advisable to disregard unlike-sexed DZ twins.

Other important data for leprosy studies on twin pairs should refer to age, therapeutics used, evolution of the disease, as well as to the topographic-anatomical distribution of the lesions.

Twin pair studies are scarce and have not taken into account the above mentioned items. Moreover, the absence of zygosity investigation by means of objective evaluation may be severely criticized in some of them.

Since the twin zygosity investigation is a fundamental step in twin pair studies, it is clear that the comparisons made by Spickett (1962b) on 13 pairs gathered among those collected by Keil (1939), Kinnear-Brown and Stone (1958), and other authors, not quoted, cannot be accepted for discussion. For instance, the five like-sexed twin pairs described by Keil (1939) have not been investigated even as far as their physical appearance is concerned, and two among them have been classified as concordant because they were not affected.

The twin pair data collected in India (Mohamed-Ali 1965b, Mohamed-Ali and Ramanujam 1966) are also open to criticism either because of the sampling technique adopted, or due to the fact that the affected twins have been classified in two classes only: lepromatous and nonlepromatous cases. Another obscure point that should be commented on is that Mohamed-Ali (1965b) has mentioned first that 2 out of 5 DZ pairs were concordant for leprosy. In the second paper (Mohamed-Ali and Ramanujam 1966), that sample was enlarged to 12 DZ pairs, but all of them were referred to as discordant.

At any rate, the twin pairs studies on leprosy which have been performed up to the present, have not been in opposition to the hypothesis of the existence of an important genetic component of the host responsible for leprosy manifestation. Furthermore, they have pointed out that much is to be learned by applying correctly the twin pair methodology to leprosy studies.
RACIAL DIFFERENCES ON LEPROSY PREVALENCE AND LEPROMATOUS RATE

The racial differences on either leprosy prevalence or distribution of leprosy forms, pointed out since long by many authors, were used by Spickett (1962a and 1962b) as arguments to suggest genetic influence of the host on susceptibility to leprosy.

Nevertheless, those arguments can be put into doubt on basis of some epidemiological data. Thus, it is thought that the lepromatous rates observed in highly endemic areas never surpass figures of 5 to 10 per 1,000 even when the prevalence of leprosy is larger than 20 per 1,000 (Doull et al. 1942, Fonte 1967). This indicates that leprosy prevalence depends on both the existence of open cases and the opportunity of exposure to leprosy infection. As a consequence, genetic interpretation ascribed to variable leprosy prevalence among different populations is open to criticism. The same is true with regard to the analyses made on multiracial communities, because they only minimized the climate among the multiple nongenetic variables which may be acting in the different racial groups.

Concerning the comparisons of the distribution of different populations, the epidemiological data suggest that the proportion of lepromatous cases diminishes as the prevalence of leprosy increases, the lepromatous rate tending to apparently stable values in highly endemic areas. The surveys made by Kapoor (1963) in 25 Districts of Maharashtra (India) and by Bechelli et al. (1966) in 9 sample blocks of Thailand serve to illustrate this suggestion. Table II presents the average per-

<table>
<thead>
<tr>
<th>Leprosy prevalence per 1,000</th>
<th>Lepromatous cases (%)</th>
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<tbody>
<tr>
<td></td>
<td>India</td>
</tr>
<tr>
<td>&lt; 5</td>
<td>47.8</td>
</tr>
<tr>
<td>5-10</td>
<td>35.5</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>23.5</td>
</tr>
</tbody>
</table>

Note. The average percentage of lepromatous cases for different leprosy prevalence values has been calculated using the data of Kapoor (1963) and Bechelli et al. (1966).

It seems, therefore, that comparisons of the distribution of leprosy forms in different populations, though important for epidemiologic purposes, might have no meaning for drawing genetic conclusions. They might lead, in fact, to wrong state-
ments when it is not taken into account that small leprosy prevalence values seem
to be associated to higher proportion of lepromatous cases, the opposite situation
occurring for large leprosy-prevalence figures. For instance, the statement that Eu-
ropeans and Mongoloids are proner to manifest the lepromatous form of leprosy than
Indian and Africans (Cochrane 1947), may be a consequence of biased information.

Another point that deserves comments is the study of genetic isolates. A higher
frequency of a rare constitutional disease in isolates is interpreted as favouring the
hypothesis of recessive transmission, since isolates will exhibit higher consanguinity
rates than nonisolated populations. The same, however, cannot be applied to infec-
tious diseases if it is not demonstrated that isolated and nonisolated groups are under
the same environmental influence.

Otherwise, a genetic isolate exhibiting high leprosy prevalence, like that of Ger-
man origin living in Colonia Tovar, Venezuela (Convit et al. 1952a), cannot be
compared to the native populations, since the isolated and nonisolated populations
are racially and socially different.

Even if it is proven that leprosy prevalence is higher in the consanguineous than
in the nonconsanguineous fraction within the isolate, it may be argued that the con-
sanguinity may be an effect rather than the cause of the disease. In fact, the frequency
of consanguineous marriages among parents of leprosy patients observed in the State
of Sao Paulo, Brazil (Beiguelman 1962b) did not differ from that observed in the
general population by Saldanha (1960).

As it can be seen, most of the population studies are inconclusive for some ge-
etic purposes. Nevertheless, they have provided a valuable indication for genetic
work, suggesting that the lepromatous rate tends towards stable values as leprosy
prevalence increases. This may suggest that susceptibility to lepromatous leprosy
is genetically determined in a fraction of the population.

PEDIGREE STUDIES

The existence of families with high concentration of leprosy cases has stimulated
some authors to apply formal genetic methods to test the hypothesis of monogenic
inheritance for susceptibility to leprosy. For this purpose, Spickett (1962a) used
pedigrees published in the pertinent literature (Aycock and McKinley 1938, Steiniger
1941), while Prasad and Mohamed-Ali (1966b) analysed families collected in an

The use of pedigrees of leprosy cases for testing the possibility of attributing Men-
delian inheritance to the susceptibility to this disease have already been criticized,
although briefly (Beiguelman 1967). Therefore, it may be worthwhile to discuss only
some facts which show that pedigree analyses may lead to contestable results in
leprosy studies.

In this discussion the imprropriety of using pedigrees collected from the literature will
not be considered. The source of erroneous conclusions that those data provide, even
when one is dealing with rare constitutional diseases, have been thoroughly discussed
in textbooks of human genetics. Nevertheless, it will be shortly reminded that, besides other sources of distortions, such pedigrees might have been selected for the demonstration of some particular situation and that the information on the number of individuals, affected or not, the sex-ratio or the clinical status, at least in the elder generations, are usually poor. In the present appraisal, our considerations will concern the analysis of pedigrees which are assumed to be sampled with the specific purpose of verifying if leprosy distribution in families fits a genetic hypothesis of Mendelian inheritance.

First of all, it must be considered that, at the present state of knowledge, when different forms of leprosy are recognized, it seems unreasonable to pool together families in which all affected individuals are simply labelled as leprosy cases, as if they pertained to a single clinical entity. It seems obvious that a family with several lepromatous cases is strictly different from another in which all forms of leprosy are equally represented. The same is true for two families, each with a single case, if one of the affected individuals is a lepromatous patient and in the other the leprosy case is of the tuberculoid type. The possibility of future cases of leprosy in these families will not be \textit{a priori} comparable, since the risks of intrafamilial contagion offered by the affected cases are different.

A second point that must be considered is that pedigree studies are based on the minimization of the environmental influences. As a consequence, the reliability of the results offered by this method depends on the degree of minimization which can be attributed to the environmental influences on the trait under study. This is the reason why pedigree studies give good results for constitutional diseases, not so good results for degenerative diseases, and are not appropriate for infectious diseases.

In order to illustrate the latter statement, let us assume, for instance, that lepromatous leprosy would have proven to be completely associated to an enzyme deficiency of the host, observed even among healthy individuals either of endemic or nonendemic areas, with variable frequencies according to racial groups and whose expression would be independent of age and sex. Let us also suppose that segregation analysis performed in an appropriate sample of families had indicated that this trait should be included among the inborn errors of metabolism, of, say, simple autosomal recessive inheritance. In such a situation, a high confidence would be attributed to the conclusion that both the enzyme deficiency and the susceptibility to lepromatous leprosy are recessive autosomal traits because the former is considered as endogenously determined.

In the above-mentioned example, the environmental influences on the intrafamilial distribution of the trait, though not excluded, are minimized without introducing distortions in the data. Obviously, the same conclusion could not be extended to families investigated only on clinical grounds.

As a matter of fact, it cannot be overlooked that leprosy manifestation must be a function of both genetic and nongenetic factors, and that both of them need be investigated carefully. Leprosy cannot be handled as a constitutional disease in reference to which consanguinity plays a more important role than contiguity.
Otherwise stated, one cannot ignore \emph{a priori} the factors which promote differential exposure to leprosy infection, such as the influence exerted by: extrafamilial \emph{foci} living or not in the same household; frequency of exposure or period of cohabitation of the \emph{focus(i)} and the contacts; ratio between the number of \emph{foci} and the number of contacts; sex and age of both \emph{foci} and contacts; living conditions and size of the house; clinical condition, period and regularity of the treatment, rate of decrease of the bacteriological index, absence or presence of sporadic or recurrent reaction episodes of the \emph{focus}; nutritional status and social, cultural, professional, and religious habits of the individuals. By the way, it is interesting to note that objective methods for evaluating these influences begin now to be developed (Hausfeld 1970).

Taking into account the comments made either in this or in the previous sections of this paper, it may be easily concluded that the results of the analyses of pedigrees of leprosy cases found in the pertinent literature hardly call for any theoretical discussion, since correct statistical analyses have no meaning if applied to inappropriate data.

**GENETIC POLYMORPHISMS**

Several genetic systems have been analysed in samples of leprosy patients with the hope of detecting their possible pleiotropic effect on the susceptibility to this disease. Otherwise stated, an association between leprosy and some genetic polymorphisms was searched for.

The following genetic markers have been analysed: taste sensitivity to phenylthiourea (Beiguelman 1962a, 1964a and 1964b; Beiguelman and Marques 1964); ABO blood groups (Beiguelman 1963 and 1964a, Hsuen et al. 1963, Yankah 1965, Verma and Dongre 1965, Povey and Horton 1966, Sehgal et al. 1966, Prasad and Mohamed-Ali 1966a, Gupta and Gupta 1966, Vogel and Chakravarthi 1966, Salzano 1967, Salzano et al. 1967, Singh and Ohja 1967, Saengudom and Flatz 1967, Lechat et al. 1967 and 1968a, Vogel 1968, Vogel et al. 1969 and 1971); ABH substances in the saliva (Sehgal and Dube 1967); Rh blood groups (Beiguelman 1963, Yankah 1965, Salzano et al. 1967, Lechat et al. 1968a); MNSs system (Salzano et al. 1967, Lechat et al. 1968a); Kell, Kidd, Duffy, and P systems (Lechat et al. 1968a); group-specific component (Salzano and Hirschfeld 1965, Lechat et al. 1968b); haptoglobins (Povey and Horton 1966, Schwantes et al. 1967, Lechat et al. 1968b); transferrins (Povey and Horton 1966, Lechat et al. 1968b); beta-lipoprotein Ag\(^a\) (Lechat et al. 1968b); Inv groups (Vogel et al. 1971); glucose-6-phosphate-dehydrogenase deficiency (Pettit and Chin 1964, Beiguelman et al. 1966 and 1968b, Lechat et al. 1968b).

In discussing critically most of the papers written before 1966, the author has commented that the study of the above-mentioned polymorphisms has no importance for practical purposes, since association of genetic markers to leprosy is not useful in the diagnosis or prognosis of individual susceptibility or resistance to leprosy (Beiguelman 1967). Though this point of view has not been supported by other investigators (Lechat 1969), the articles which came onto light from 1966 onwards seem to reinforce the author's opinion.
Actually, the usefulness of some of the collected data on this matter may be put into doubt even for heuristic purposes, that is to say, to conclude whether leprosy is one of the several forces that maintain different polymorphic systems discovered by hazard. The controversial or negative results which existed up to 1966 have not only been maintained, but, in fact, have been increased by others.

Concerning the ABO system, it may be said, in brief, that the results have varied from absence of association between ABO groups and leprosy to associations of either O, A, or AB groups, leprosy forms being discriminated or not.

With respect to the Rh system, the only concordance concerns the conclusion that D negative group (dd) is not associated to leprosy. However, while Lechat et al. (1968a) have suggested deficiency of cc and EE groups among leprosy patients, Salzano et al. (1967) did not observe this difference. The latter have also observed absence of association between MNSs system and leprosy, while the former have pointed out that lepromatous patients exhibited an excess of MM group and a deficiency of Ss genotype.

Concerning the serumproteins, Lechat et al. (1968b) observed an excess of Hp1 gene among leprosy cases, while Schwantes et al. (1967) did not favour such difference.

Discordant results have been found for Inv groups by Vogel et al. (1971). Thus, in a series of patients from Thailand, Inv(1) was found to be associated to A and AB groups among lepromatous leprosy cases, but no associations could be assigned when Indian material was analysed.

For the other genetic markers, a significant excess of phenylthiourea tasters either among lepromatous or tuberculoid samples has been found, with no clinical significance. No differences between leprosy and control samples have been observed for group specific component, transferrins, beta-lipoproteins, G6PD deficiency, and secretors of ABH substances in the saliva.

AUSTRALIA ANTIGEN

The so-called Australia or Au(1) antigen has its name derived from the fact that it was first detected in the serum of an Australian aborigine by means of an antibody produced in the blood of a transfused hemophilia patient (Blumberg 1964, Blumberg and Alter 1965, Blumberg et al. 1965).

The Au(1) antigen was found to be more frequent among lepromatous patients than among tuberculoid leprosy cases or nonleprous individuals living in the Philippines (Blumberg and Melartin 1966). Taking into account the variable distribution of the Au(1) antigen in human populations, as well as the type of family aggregation, Blumberg and coworkers have suggested that this trait could be a genetic polymorphism associated to leprosy, Au(1) antigen being determined by an autosomal recessive gene (Blumberg and Melartin 1966, Blumberg et al. 1966).

This hypothesis has been considered as risky (Beiguelman 1967) and a series of
observations which appeared after 1966 have brought more obstacles to its acceptance. They may be summarized as follows:

a) The presence of Au(1) antigen has been demonstrated to be associated with acute viral hepatitis, chronic anicteric hepatitis and with hepatitis carriers (Blumberg et al. 1967, London et al. 1969a).

b) Acute viral hepatitis was manifested by individuals who were transfused with blood containing Au(1) antigen (Okochi and Murakami 1968).

c) Both hepatitis and Au(1) antigen were manifested by feeble-minded patients to whom Au(1) prepared from blood of hepatitis patients was administered either orally or parenterally (Giles et al. 1969).

d) Other antigens described as hepatitis antigens were demonstrated to be identical to Au(1) antigen (Prince 1968).

e) Electron-microscope studies have indicated that Au(1) antigen is a virus (Bayer et al. 1968, Hirschman et al. 1969).

f) Nuclei of liver cells of patients with acute and chronic hepatitis who have Au(1) antigen in their serum exhibit granules when submitted to fluorescein-labelled anti-Au(1) (Millman et al. 1969).

All these facts taken together lead to conclude that the high frequency of Au(1) antigen observed in lepromatous leprosy, Down syndrome, leukemia, Hodkin disease, as well as among patients and members of the staff of a chronic hemodialysis unit (Blumberg and Alter 1965, Blumberg 1966, Blumberg et al. 1967, Sutnick et al. 1968, London et al. 1969b) may be interpreted as a consequence of a higher risk of viral infection to which they are submitted due to institutionalization or transfusion, rather than to genetic causation. Here it should be remembered that in 218 leprosy and 50 leukemic Brazilian patients no cases of Au(1) antigen have been found (Salzano and Blumberg 1970).

Notwithstanding, Blumberg et al. (1970) have proposed a more risky hypothesis. In this new hypothesis they postulate the existence of a recessive autosomal gene Au¹ and that individuals with Au¹Au¹ genotype are more susceptible to chronic infections either by Au(1) virus or by other organisms including M. leprae. When infected by Au(1) virus they would not become obviously ill and would appear to be carriers of hepatitis. However, when infected by M. leprae they would manifest the lepromatous type of leprosy.

The above-mentioned hypothesis has been presented in order to conciliate the evidences that Au(1) antigen is a virus, with the results obtained by Blumberg et al. (1966, 1969) on segregation analyses performed on two sets of family data which have fitted the hypothesis of simple autosomal recessive inheritance. Nevertheless, the family data may have been distorted by the heterogeneity of the sampled families, the postulation of Au(o) phenotype for nonexamined individuals of the parental generation, the variable persistence of the antigen in the serum, the association of Au(1) antigen to age and to sex (male) and to incorrect classification of the reactions.
Riskier still, however, is to accept Blumberg et al.'s (1970) postulate that their hypothesis will be reinforced if lepromatous leprosy would follow a pattern of autosomal recessive inheritance in areas of high leprosy prevalence.

DERMATOGLYPHIC STUDIES

Interest on dermatoglyphic studies in different clinical disorders was renewed in the last decade because of the possibilities they were thought to have as a diagnostic tool, mostly for congenital abnormalities due to chromosomal aberrations. Nevertheless, since it was verified that the same pattern of dermatoglyphics could be found in different clinical disorders, as well as in normal individuals, the limitations of these studies for diagnostic purposes became obvious. For instance, an excess of whorls in the fingerprints may be found in Turner's syndrome, in cases with translocations involving D and E chromosomes associated or not to a G-chromosome trisomy, in patients with a deletion of an E or X chromosome, in Huntington's chorea, in Fallot's tetralogy, in patients with coarctation or stenosis of the aorta, in Wilson's disease, as well as in cases with skeletal abnormalities associated to anomalies of the face and genitalia (Hodges and Simon 1962, Holt and Lindsten 1964, Breibart et al. 1964, Lie et al. 1964, Sanchez-Cascos 1964, Engel and Forbes 1965, Townes and Ziegler 1965, De Grouchy 1965, Barbeau et al. 1965, Pinsky and George 1965).

Another example is provided by the excess of arches found in cases with E chromosome trisomy, Klinefelter's syndrome, different types of chromosomal translocations (B/B, B/C and E/G), enlarged chromosomal satellites, deletion of a D chromosome, pulmonary stenosis, pseudohypoparathyroidism and pseudopseudohypoparathyroidism (Gibson et al. 1963, Lele et al. 1963, Forbes 1964, Uchida et al. 1964a and 1964b, Farquhar and Walker 1964, Clarke et al. 1964, Lee et al. 1964, Jacobsen et al. 1964, Sanchez-Cascos 1964).

Therefore, at the present state of knowledge the idea of studying dermatoglyphics of leprosy patients in order to “determine if there might be significance in the analysis of dermal patterns of the hand as related to the hereditary susceptibility to the disease” (Enna et al. 1970) may be considered of doubtful validity.

Concerning the conclusions presented by Enna et al. (1970) it may be said that they can hardly call for any theoretical discussion. The alleged differences cannot be taken into account since both the leprosy and the control samples were small and heterogeneous for race, age, and sex, the former being also heterogeneous for leprosy forms. Moreover, with respect to the traits studied, it should be mentioned that the distance from distal flexion crease of the wrist either to the axial triradius or to the base of the middle finger depends on the size of the hand.

Here it seems relevant to point out that dermatoglyphic studies in leprosy are not new, the first paper on this subject having been published in Cuba by Castellanos in 1923 (cf. Ribeiro 1936). Otherwise, Ribeiro’s investigations (1934, 1935) have demonstrated clearly that leprosy is able either to change or to destroy completely the dermopapillary configurations of the fingers even of patients whose hands
were apparently normal. In one of his papers (Ribeiro 1935), published in the same issue as Enna et al.'s article (1970), the data are accompanied with photographic documents.

Taking into account those considerations, it seems more interesting, for practical purposes, to investigate the causes of the changes of dermopapillar patterns in different leprosy forms, rather than to search for questionable associations between leprosy and dermatoglyphic patterns.

**THE LATE LEPROMIN REACTION (MITSUDA REACTION)**

The lepromin reaction is a consequence of events that follow the phagocytosis of heat-killed leprosy bacilli by the histiocytes of the skin. Since *M. leprae* are intracellular parasites which are able to survive and to multiply within the macrophages of some individuals, the lepromin reaction has also been used as an approach to genetic studies on leprosy.

Before going on further in reviewing genetic studies on lepromin reaction it seems advisable, for the sake of clarity, to allot some space to the discussion of the lepromin reaction itself.

Lepromin is a sterile suspension of leprosy bacilli extracted mechanically from lepromata into an isotonic solution of sodium chloride. In the last few years great efforts have been directed towards its standardization. The interested reader will find valuable information on this matter in the papers of Hanks and coworkers (Hanks 1968a-c, Hanks et al. 1964 and 1970).

The intradermal injection of 0.1 ml of lepromin may provoke an early reaction, which is read in 48 hours and/or a late response, which is examined clinically at 4 weeks on both leprosy cases and contacts. Noncontacts of leprosy cases may exhibit greater delay in showing the maximal clinical late response to lepromin injection (Bechelli et al. 1963).

The early and the late reaction to lepromin are also known as Fernandez and Mitsuda reactions, but both of them were first described by Mitsuda among leprosy and healthy individuals (Mitsuda 1916, cf. Hayashi 1933 and Mitsuda 1924).

The Mitsuda reaction, as clinically read, is usually classified in five classes: 1) absence of an observable or palpable element (—); 2) presence of a perceptible element with a diameter smaller than 3 mm (±); 3) presence of a conspicuous infiltrated element with a diameter of 3 to 5 mm (+); 4) presence of a conspicuous infiltrated element with a diameter larger than 5 mm (++); 5) presence of an ulcerated nodule (+++).

A simplified classification of the Mitsuda reaction for practical purposes has been recently proposed (Beiguelman 1971), based on the variability of the above-mentioned classes throughout life. That classification considers as class 0 the negative and doubtful reactions, as class 1 the + reactions, and as class 2 the ++ and +++ responses.

Histological analyses of the Mitsuda reaction were made by a great number of
authors (Mitsuda 1919, cf. Hayashi 1933, Mariani 1924 and 1925, Schujman 1936, Nagai 1938, Alayon 1939, Alayon and Souza-Lima 1940, Nolasco 1940, Büngeler and Fernández 1940, Rodríguez 1950, Yokota 1953, de Faria 1953, Bechelli et al. 1959, Azulay et al. 1960, Andrade 1962, inter alia). From those studies it was concluded that a typical histologically positive Mitsuda reaction is represented by a granulomatosus infiltrate composed chiefly of epithelioid cells assuming a tuberculoid structure, where acid-fast bacilli are absent or scarcely found.

Some authors (Azulay et al. 1960, Andrade 1962) consider also as positive reactions those which demonstrate a tuberculoid-like structure, that is to say, those showing an incomplete granulomatous infiltrate in which the epithelioid cells are scattered and sparsely grouped, tending to form nodular structures, with few or no giant cells, and acid-fast bacilli absent or rarely found. Bechelli et al. (1959) have preferred to classify this type of reaction as favouring the hypothesis of a positive reaction.

A typical histologically negative Mitsuda reaction is represented by an infiltrate with histiocytes full of undigested phagocytized bacilli. Nevertheless, among the histologically negative Mitsuda reactions there are also included the simple inflammatory infiltrates in which acid-fast bacilli are absent or rarely found (Schujman 1936, Bechelli et al. 1959, Andrade 1962). The speculations on the origin of this type of reaction which cannot be included among the positive responses, as a consequence of the absence at least of a tuberculoid-like structure, have been discussed elsewhere (Beiguelman 1971).

The analyses of the biopsies of the different classes of clinically read Mitsuda reactions among leprosy patients have shown that both the positive and negative reactions usually have a histological correspondence, the doubtful reactions disclosing, as a general rule, a negative histological picture (Bechelli et al. 1959).

Concerning the reactions exhibited by contacts of leprosy cases it may be said that the positive reactions usually have a histological correspondence, as in leprosy cases. The same is not true for the negative and doubtful responses. Pooling together the data of Bechelli et al. (1959) and Andrade (1962), who extended the data of Azulay et al. (1960), it is found that 84.12% of tuberculoid or tuberculoid-like structures were disclosed by 63 clinically positive reactors. In opposition, 1 out of 4 clinically Mitsuda negative and 9 out of 12 doubtful Mitsuda reactors exhibited histological positivity. Unfortunately, similar analyses have not been performed for healthy samples of noncontacts of leprosy cases.

In spite of its little theoretical understanding, the Mitsuda reaction has gained an indisputable importance for leprosy studies because of the following facts:

a) The polar forms of leprosy exhibit an opposite behaviour with respect to the lepromin test: lepromatous cases are Mitsuda negative, while tuberculoid patients are usually Mitsuda positive. Concerning the unstable groups, border-line patients usually behave as lepromatous individuals or as weak reactors, while indeterminate cases include negative and positive reactors.

Negative or doubtful reactions among tuberculoid cases seem to be associated to those who have suffered reactional episodes. Thus, formerly Mitsuda positive tuber-
culoid cases may lose, at least temporarily, their capacity to respond positively to lepromin (Bechelli and Quagliato 1953, Basombrio 1956). However, negative reactions among tuberculoid cases seem not to occur if 15 or more months have elapsed since the last reactional episode (Bechelli and Quagliato 1953).

Weak or doubtful Mitsuda reactions among lepromatous cases, which are sometimes referred to in the pertinent literature, are not considered of histological significance. According to Bechelli et al.'s (1959) data those reactions do not reveal a tuberculoid granuloma among lepromatous cases. They seem to be a consequence of foreign body reactions provoked by tissular elements present in excess in some lepromins.

b) The Mitsuda reaction has a high prognostic value. A positive response exhibited by a healthy individual indicates that he has practically no probability of acquiring lepromatous leprosy. In opposition, the persistence of a negative or doubtful reaction after repeated lepromin tests in an individual exposed to infection indicates a high risk of manifestation of that polar form (Dharmendra and Chaterjee 1955, Quagliato 1962).

Otherwise, it is also known, since Hayashi's study (1933), that good prognosis may be ascribed to indeterminate Mitsuda positive cases. Of course, this does not mean that Mitsuda-negative indeterminate patients cannot have a good evolution when under sulphone therapy. As a matter of fact, in a 5-year follow-up of 34 indeterminate cases, Souza-Lima and Souza-Campos (1950) have verified that 11 became clinically cured, 2 continued as indeterminate, and 1 manifested the tuberculoid type of leprosy.

c) The reactions provoked by suspensions of other acid-fast bacilli or its extracts are not comparable to those elicited by lepromin (Hayashi 1933, Floch 1952, Yokota 1953, Yanagisawa et al. 1960, Guinto et al. 1962, Coudert et al. 1965, Bullock 1968). Moreover, in opposition to lepromin, injections of other suspensions of killed mycobacteria are able to provoke tuberculoid granulomata in lepromatous patients. Also not comparable to lepromin reaction are the organic compounds which have been tried. For instance, the alleged concordance of the reactions to intradermal injections of 2,4-dinitrochlorobenzene (DNCB) and lepromin described by Basombrio et al. (1943) and Mom and Basombrio (1944, 1945) has not been confirmed by Azulay (1948). More recently, Turk and Waters (1969) have shown that patients with lepromatous leprosy have a decreased ability to be sensitized to show contact sensitivity to DNCB. Nevertheless, the results were not comparable to those obtained with lepromin injections, since 12 out of 24 lepromatous cases exhibited positive reactions to DNCB.

FAMILY AND TWIN PAIR STUDIES ON MITSUDA REACTION

The Mitsuda reaction has proven to be a familial trait either in samples free of leprosy (Beiguelman 1962b and 1971, Beiguelman and Quagliato 1965) or in a sample including families with at least a parent affected with a polar form of leprosy (Bei-
Otherwise stated, family studies on the distribution of the late lepromin reaction have demonstrated that the late lepromin reactions in the offspring generation are associated to those presented in the parental generation.

A monogenic interpretation to explain the family distribution of the Mitsuda reaction (Beiguelman 1965) should, however, be considered with caution, because of the postulates and secondary hypothesis it has involved. The restrictions to accept that interpretation have already been discussed elsewhere (Beiguelman 1967).

A quantitative study of the Mitsuda reaction clinically evaluated in 127 healthy twin pairs, noncontacts of leprosy cases, has shown that MZ and DZ twin pairs presented similar intraclass correlation coefficients. This result allows two interpretations:

a) The Mitsuda reaction does not depend upon important genetic factors.

b) The Mitsuda reaction depends upon important genetic factors, but its clinical expression among genetically lepromin-positive individuals depends mostly on environmental agents.

At the present state of knowledge no elements exist to assure a definitive conclusion. As a matter of fact, the demonstration that the proportion of positive Mitsuda reactors increases with age (Del Favero 1948, Martinez-Dominguez 1953, Convit and Rassi 1954, Guinto et al. 1955, Beiguelman 1962a, Beiguelman and Quagliato 1965, Bechelli et al. 1970), BCG vaccination (Quagliato 1957b, Souza-Campos et al. 1962, Beiguelman et al. 1967, Bechelli et al. 1970), or repeated lepromin injections (Paula-Souza et al. 1953, Ignacio et al. 1955, Silva et al. 1955, Doull et al. 1957, Olmos-Castro and Arcuri 1957, Rosenberg et al. 1960, Beiguelman et al. 1965) does not rule out the second interpretation of the twin pair results because of the following facts.

a) The absence of negative reactors seems to be an exceptional phenomenon in any human population, even in the highest age-groups.

b) Some individuals are unable to disclose a positive Mitsuda reaction in spite of being submitted to BCG vaccination and to repeated lepromin injections. In opposition, individuals disclosing a positive Mitsuda reaction in early childhood have been described by Paula-Souza and Bechelli (1960) and Souza-Campos et al. (1962).

c) The attempts to induce positive late lepromin reactions in lepromatous patients vaccinated with BCG have failed. At most, doubtful and weak responses were induced in some cases (Convit et al. 1952b, Azulay et al. 1952, Quagliato and Bechelli 1953, Lowe and McNulty 1953, Jonquieres and Masanti 1954, Schujman 1956, Contreras et al. 1958) which are considered transient (Schujman 1956) and with no histological correspondence (Bechelli et al. 1959).

d) The proportion of strong positive Mitsuda reactors after BCG vaccination of children of lepromatous patients and of healthy parents was found to be significantly lower in the former (Beiguelman et al. 1967). If children of lepromatous parents would represent individuals with a genetic component favouring susceptibility to leprosy infection, those results may suggest that BCG, as a modifier agent of the
clinically late lepromin reaction, would be more effective among children with a genetic component favouring the manifestation of tissular resistance to *M. leprae*.

The studies on biopsies of Mitsuda reaction of leprosy cases and their contacts, reported in the foregoing section, together with the above-mentioned experiences, suggest that a clinically positive lepromin reaction depends both on the lysing ability of the macrophages for phagocytized bacilli and on the influence of sensitizing agents. The latter could be represented by specific (*M. leprae*), paraspecific (other mycobacteria) and broad-specific stimuli (nonmycobacterial intracellular parasites) which would enhance both the lysing ability and the clinical expression of the reaction.

Therefore, the crucial point hindering a definitive conclusion from the twin pair data on Mitsuda reaction is a consequence of the present day impossibility of distinguishing among negative, doubtful, or weak reactors of a healthy sample, those who will permanently react as such, from those who may exhibit a clinically strong positive reaction after being properly stimulated are in fact genetically endowed to produce this response.

If it could be admitted that a fraction of the healthy populations had macrophages genetically unable to destroy phagocytized *M. leprae*, it could also be supposed that those nonlysers would not be frequent in the population. The assumption of a low frequency of nonlysers of leprosy bacilli is based on the epidemiologic information that lepromatous leprosy never rises to frequencies higher than 1% even in endemic areas where leprosy is highly prevalent (Doull et al. 1942, Fonte 1967).

Thus, if the macrophages' permanent inability to digest phagocytized leprosy bacilli were a rare genetic trait, then, samples of healthy twin pairs collected at random would be chiefly composed by concordant cotwins for the lysing ability, irrespectively of their zygosity state. Moreover, a similarity of the intraclass correlation coefficient, or of the concordance rate of the Mitsuda reaction of MZ and DZ twin pairs, would in fact demonstrate that the clinical expression of the late lepromin reaction among genetically lepromin positive individuals does not depend on hereditary influence. It would not signify that Mitsuda reaction is not a genetic trait, but that environmental factors are able to influence macroscopical lepromin reactions only among individuals who are genetically endowed to exhibit them.

Light might be thrown on this subject by studies on the lepromin reaction clinically and histologically analysed in a sample of like-sexed twins (MZ and DZ male and female pairs) ascertained by starting from lepromatous probands. In such a sample the sources of errors are supposed to be strongly reduced because all the pairs will include at least a lepromatous cotwin who undoubtedly has a negative Mitsuda reaction. Otherwise, the cotwins who are not index cases are also expected to exhibit a Mitsuda reaction which should express the lysing ability of their macrophages to phagocytized *M. leprae*. As a matter of fact, they will be either affected by leprosy or be represented by healthy individuals supposed to be intensively submitted to specific sensitizing stimuli.
IN VITRO BEHAVIOUR OF BLOOD MACROPHAGES AGAINST KILLED *M. LEPRAE*

The first report on phagocytosis in vitro of leprosy bacilli by blood macrophages seems to be that of Benewolenskaja (1932). Fifteen years later, Hanks (1947) described the fate of leprosy bacilli in cultures of lepromatous and tuberculoid lesions.

In recent years, the studies on the in vitro lysogenic ability of blood macrophages for phagocytized leprosy bacilli have been retaken with the hope of obtaining a better clue to investigate both the mechanism of the late lepromin reaction and the possible participation of hereditary factors on the determination of susceptibility and resistance to leprosy. For this purpose Treo and Silva (1963) used total blood samples, as Benewolenskaja (1932), while other authors have extracted leukocytes from blood samples (Beiguelman and Barbieri 1965, Barbieri and Correa 1967, Beiguelman 1968, Godal and Rees 1970).

The attempts to standardize an in vitro test have lead the author to adopt the technique described below.

The in vitro reactions are performed in Leighton tubes provided with coverslips on which leprosy bacilli are adhered in aseptic conditions. Leprosy bacilli are extracted with chloroform from integral lepromin diluted 20 times in 0.9% NaCl solution using a 1:1 mixture, which is vigorously shaken in a test tube for 5 minutes. The chloroform is aspirated with a Pasteur pipette and transferred to a test tube maintained in an ice-bath. The final suspension is used within 20 minutes to avoid possible damage of the bacilli, due to chloroform. A volume of this suspension, sufficient to fill the capillary end of a Pasteur pipette is placed on each coverslip. Each volume was estimated to contain an average number of $7.5 \times 10^4$ bacilli.

A sample of 20 ml heparinized venous blood is distributed into 4 sterile tubes which are left for 30 to 40 minutes at 37°C and at 45° angle. In cases of slow hemosedimentation rate the tubes are centrifuged at a low speed (300 rpm).

The plasma of the 4 tubes containing a large number of leukocytes is aspirated by using a Pasteur pipette and transferred to a conical graduated tube. Hanks BSS supplemented with 0.5% lactalbumin hydrolysate and antibiotics (100 I.U. of penicillin and 100 µg of streptomycin per ml) is added to the plasma in the proportion of 6v/4v.

The amount of leukocyte suspension in the final medium is sufficient to be distributed at least in 5 Leighton tubes which are incubated at 37°C. The tissue culture medium is renewed twice a week with a solution of Hanks BSS containing lactalbumin hydrolysate and antibiotics (60%) and foetal bovine serum (40%).

The phagocytic and lysing ability of the macrophages which develops from monocytes and perhaps of some lymphocytes as well, are followed during 25 days by removing a coverslip from each set of Leighton tubes.

The coverslips are fixed for 5 minutes in absolute methanol and dried at room temperature. Bacilli are stained with carbol fuchsine at room temperature for 20 minutes. After rinsing with 1% hydrochloric alcohol, the coverslips are washed in 3 baths of tap water, one minute each. The cellular elements are stained for a few seconds in a 0.5% methylene blue aqueous solution and washed in running tap water. The coverslips are dried at room temperature, cleared in xylol, and mounted in balsam.
Three bacterioscopic scores are used to quantify the in vitro behaviour of the macrophages against killed *M. leprae*. The criteria used for attributing scores are synthesized in Table III. Concerning this table, one explanation with regard to the meaning of the name *epithelioid cells* seems to be necessary. This term has been adopted to distinguish the cells which differ from the macrophages because of their sharp outline and their homogeneous or thinny granulated cytoplasm. As a matter of fact, these cells are comparable to the epithelioid cells of the tuberculoid granulomata because they are after-lysing activity macrophages.

A simple glance at Table III makes it clear that the three scores are mainly based on the decrease of the bacilli concentration in the inoculum, as an indirect evaluation of the rate of phagocytic and lysing activity of the macrophages.

**Table III. In Vitro Reactions of Blood Macrophages Against Heat Killed M. leprae**

<table>
<thead>
<tr>
<th>Score</th>
<th>Characteristics</th>
</tr>
</thead>
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| **2** | **Bacilli concentration in the inoculum practically unchanged.**  
Macrophages usually with low tactism for the leprosy bacilli are represented commonly by histiocyte-like cells, giant-cells being absent or scarcely found. Epithelioid cells are absent or scarcely found. Macrophages surrounding the bacillary inoculum are either full or free of undigested bacilli, while signs of lysis are usually absent or insignificant. Necrotic masses containing undigested bacilli are usually found after 15 days incubation. |
| **1** | **Bacilli concentration in the inoculum reduced in variable degree.**  
Macrophages are represented by histiocyte-like cells as well as by giant-cells, but the frequency of the latter may decrease considerably after a long incubation interval. Epithelioid cells are frequently found. Macrophages with undigested bacilli may be frequent or rare, but signs of lysis are ever clearly observed within the macrophages. Necrotic masses containing undigested bacilli may be present or not. |
| **0** | **Free acid-fast bacilli absent or very scarcely found.**  
Macrophages are represented by histiocyte-like cells as well as by giant-cells, but the frequency of the latter may decrease considerably after a long incubation interval. Epithelioid cells are frequently found. Macrophages with undigested bacilli may be absent, rare or frequent, while signs of lysis may be present or have disappeared. Necrotic masses containing undigested bacilli are usually absent. |

In an unpublished paper Pisani et al., by analysing the distribution of the average bacterioscopic scores presented by leprosy cases, have shown that both the 20th and 25th incubation day scores are the most critical for classifying the in vitro reactions among them. Moreover, by combining those two scores (combined bacterioscopic score or CBS) it was possible to consider three classes of in vitro responses: a *lytic reaction* indicated by a 0-0 CBS, a *weakly lytic reaction* expressed by a 1-0 CBS, and a *nonlytic reaction* represented by either 2-2, 2-1 or 1-1 CBS. The last two CBS have been con-
sidered as expressing a nonlytic reaction because they indicate a slow rate of phagocytosis and lysis.

By applying this technique to a sample of leprosy cases (10 lepromatous, 10 tuberculoïd, 10 borderline and 17 indeterminate) it was observed that all lepromatous cases were classified as nonlytic reactors (2-2 CBS), while all tuberculoïd patients behaved as lytic reactors since all of them exhibited a o-0 CBS. Nine out of ten borderline cases were weakly positive reactors (1-0 CBS), the remainder being classified as a lytic reactor (o-0). However, with respect to this case it may be argued whether the lytic response could have been influenced by the corticoid therapy to which he was submitted and/or the reactional state he manifested. The 17 indeterminate patients presented the following distribution: 8 lytic reactors (o-0 CBS), 5 weakly lytic reactors (1-0 CBS), and 4 nonlytic reactors, 3 of them with a 2-2 CBS and one with a 1-1 CBS.

Good agreement was also found when the in vitro results were compared to the Mitsuda reaction either clinically or histopathologically analysed among both the lepromatous and the tuberculoïd patients.

Exception made for Godal and Rees (1970), all authors have obtained comparable results concerning the in vitro reactions of the polar forms of leprosy (Treo and Silva 1963, Beiguelman and Barbieri 1965, Barbieri and Correa 1967, Beiguelman 1968b). With regard to indeterminate cases, Treo and Silva (1963) have observed only two types of reactions among 7 individuals showing indeterminate leprosy (lepromatous-like and tuberculoïd-like), as well as a lepromatous-like reaction in 2 borderline cases.

The in vitro test applied to 57 healthy individuals (40 noncontacts and 17 contacts of leprosy cases) has shown that the reactions disclosed by their blood derived macrophages are not comparable to those seen among leprosy patients, since most of the reactions should be classified as nonlytic if the same criteria would be adopted. As a matter of fact, no o-0 CBS was observed among the 57 healthy individuals, while only 13 (22.8%) among them were able to present a 1-0 CBS.

The distribution of the healthy individuals according to both the Mitsuda reaction and the CBS has shown that among 10 negative or doubtful Mitsuda reactors, 6 presented a 1-1 CBS and 4 a 2-2 CBS; among 12 weakly Mitsuda positive reactors, 1 showed a 1-0 CBS, 6 a 1-1 CBS, and 5 either a 2-1 or a 2-2 CBS; among 35 strong Mitsuda positive reactors, 12 disclosed a 1-0 CBS, 14 a 1-1 CBS, and 9 either a 2-1 or 2-2 CBS.

As it can be seen, the results mentioned above are in disagreement with those published by Barbieri and Correa (1967) who claimed to have found two alternative types of reactions among healthy individuals, i.e. those of the tuberculoïd and those of the lepromatous type, as well as complete association between the Mitsuda and the in vitro reactions.

No obvious explanation can be found for the slow rate of phagocytosis and lysis of killed M. leprae by the macrophages of the healthy individuals. However, the data of Bechelli et al. (1959) favour the hypothesis that the macrophages of healthy
individuals exhibit a lower degree of in vitro lysing activity against killed *M. leprae* than macrophages of tuberculoid and Mitsuda positive indeterminate cases. Thus, pooling together Bechelli et al.’s (1959) data on tuberculoid and indeterminate cases with histological positive late lepromin reaction it is seen that 48 (36.1%) out of 133 patients have exhibited a tuberculoid-like response and not a tuberculoid granuloma. In opposition, among 48 healthy contacts exhibiting a histologically positive reaction the proportion of tuberculoid-like responses (30 or 62.5%) was significantly higher ($\chi^2 = 10.031; 1 \text{ DF}; P < 0.001$).

Otherwise, the observation that the peak of the Mitsuda reaction may be delayed among healthy noncontacts of leprosy cases (Bechelli et al. 1963) seems also to favour the assumption that the lysing ability of the macrophages of healthy individuals is expressed in a lesser degree than among leprosy patients whose macrophages are able to destroy *M. leprae*. Histological analyses of biopsies of different classes of Mitsuda reaction expressed by healthy noncontacts may throw light on this problem.

**CONCLUSIONS**

From the foregoing sections it may be concluded that the efforts applied to evaluate the importance of genetic factors of the host in leprosy manifestation have shed less light on this problem than it was desired. At any rate, those studies have made clear that geneticists are dropping simple speculations on hypothetical hereditary factors which would confer resistance or susceptibility to leprosy and beginning more objective work. As a matter of fact, the creation of symbols like *N factor* (Rotberg 1937), *P factor* (Souza-Campos 1953, Saúl and Díaz 1965), or *S factor* (Blumberg and Melartin 1966), which do not designate observed entities but merely stand for *natural resistance*, *predisposition*, and *susceptibility*, while satisfying the human psychological need of representing abstract notions, adds nothing to the better understanding of the genetic involvement of the host in leprosy manifestation.

The literature on human genetics is shy of a methodology for evaluating the importance of the genetic component of the host in the manifestation of infectious diseases. Nevertheless, the experience which has been accumulated by genetical workers in leprosy during the last decade allows the outlining of some areas in which further research may be considered as prioritary.

Thus, twin pair studies to investigate the heritability of either leprosy or Mitsuda reaction should be made in samples of MZ and like-sexed DZ pairs, ascertained by starting from lepromatous index cases. It seems superfluous to stress that these studies will require careful zygosity investigation, as well as detailed clinical and histopathological documentation of all affected cases. The healthy cotwins cohabiting for less than five years after the presumed onset of the disease on the index twin, as well as those disclosing unstable forms, should be excluded only from the sample destined to investigation of the heritability of leprosy. In this study, concordant pairs will consist of lepromatous twins, while discordant pairs will include a lepromatous and a tuberculoid or a healthy cotwin.
With respect to the study of the heritability of the Mitsuda reaction, it seems obvious that all collected sets should be submitted to tests performed with a standard lepromin. It is also advisable to record the results at four weeks. The average diameter of firm enduration should be expressed in millimeters, letter \( u \) being added as an exponent if ulceration is observed. Biopsies of all reactions, even of the negative responses, should be histologically examined.

Some techniques for analysing the in vitro reaction of blood macrophages against killed leprosy bacilli gave hopeful results concerning the study of macrophage function of leprosy patients. Nevertheless, the alleged correspondence between the Mitsuda and the in vitro tests among healthy individuals (Barbieri and Correa 1967) could not be confirmed (Pisani et al., to be published). Therefore, it may be concluded that with present-day techniques the above-mentioned in vitro test cannot substitute the Mitsuda reaction. While new techniques might be tried on this field, it seems that efforts should be directed to verify the histological pattern of the biopsies of different classes of Mitsuda reaction clinically read in samples of healthy noncontacts of leprosy cases. Up to the present such investigations have been restricted to leprosy cases and healthy contacts (Bechelli et al. 1959, Azulay et al. 1960, Andrade 1962) and no proofs exist that the high correspondence found between the clinical and histological late lepromin reactions among them may be extended to healthy noncontacts.

Concerning genetic polymorphic systems it seems advisable to restrict research to those which may offer promising results of practical importance. At present, investigations on the patterns of histocompatibility antigens in leprosy and healthy control samples by typing leukocytes for HL-A antigens seem to be stimulating. Otherwise, since leprosy and sickle-cell anemia are highly prevalent in some areas of the world, as in Africa, it seems also of great interest to verify to what extent may the clinical evolution of leprosy cases be influenced by the abnormal hemoglobin.

Complete leprologic surveys in several endemic areas with leprosy prevalence ranging from low to very high figures may provide valuable epidemiologic and genetic information. These studies may allow data on the distribution of lepromatous rate according to different values of leprosy prevalence, as well as to sex and age groups; the concentration of different leprosy forms in sibships belonging to populations with different figures of leprosy prevalence; the family association of leprosy forms; the risk of leprosy contagion among relatives with different coefficients of relationship to the focus; the influence on leprosy contagion exerted by factors like the family-size, ratio between the number of \( foci \) and contacts, period of co-habitation or frequency of exposure among them, sex, age, and birth rank of both index cases and contacts. These surveys may also allow to relate leprosy incidence to living conditions, nutritional status, social, cultural, alimentary, professional, and religious habits of the individuals, as well as to the clinical conditions, period and regularity of treatment, rate of decrease of the bacteriological index, and absence or presence of reaction episodes of the index cases.
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RIASSUNTO
Questo lavoro passa in rassegna le linee di ricerca finora esplorate nel valutare l'entità dell'intervento dei fattori genetici nei meccanismi di resistenza e suscettibilità alla lebbra.
Vengono discusse criticamente gli studi condotti sull'associazione familiare della lebbra e dei tipi di lebbra, sul contagio intrafamiliare, concordanza in gemelli, differenze di incidenza nelle diverse popolazioni, genealogie, e l'associazione a marcatori genetici, l'antigene Australia e i dermatoglifi. Sono anche stati esaminati studi familiari e gemellari sulla reazione di Mitsuda nonché studi sul comportamento in vitro dei macrofagi nei riguardi del \textit{M. leprae}.
Vengono infine delineati quei settori in cui le ricerche in proposito dovrebbero essere ampliate.

Résumé
Cet article passe en revue les lignes de recherche suivies jusqu'à présent dans l'évaluation du rôle des facteurs génétiques dans les mécanismes de résistance ou prédisposition à la lepra.
Une analyse critique est conduite sur l'association familiale de la lepr e et des types de lepra, sur la contagion intrafamille, la concordance en gémellés, les différences de fréquence d'après la population, les généalogies, et l'association à des marqueurs génétiques, l'antigène Australia et les dermatoglyphes. Les études familiales et gémellaires sur la réaction de Mitsuda ont aussi été examinées, ainsi que les études sur le comportement en vitro des macrophages vis-à-vis du \textit{M. Leprae}.
Les secteurs où les recherches sur ces sujets devraient être développées sont enfin délimités.

Zusammenfassung
Die Arbeit zeigt die verschiedenen Forschungen auf, die das Problem untersuchten, wie weit die Resistenz oder Anfälligkeit für Lepra erbbedingt ist.
Es folgt eine kritische Diskussion der Untersuchungen über familiäres Auftreten der Lepra u. Lepraformen über die Ansteckung innerhalb der Familie, die Konkordanz bei Zwillingen, die Frequenzunterschiede bei den verschiedenen Populationen und Stämmen sowie über die Assozierung mit Genanzeigern, dem Australia-Antigen und den Hautleisten. Auch die Familien- und Zwillingsuntersuchungen über die Mitsuda-Reaktion wurden betrachtet sowie die Untersuchungen über das Verhalten der Makrophagen gegenüber dem \textit{M. Leprae} in vitro.
Zum Schluss werden die Gebiete angegeben, auf denen noch weitere Untersuchungen anzustellen wären.

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