"N-factor/Anergic Margin" or resistance/susceptibility to hanseniasis I. The foundations of the theory

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ABSTRACT — The foundations of the pathogenetical theory "N-factor/Anergic Margin" postulated in 1937 and completed in 1957 are given. Based on the observation of 2.160 persons who had been tested by the author with Mitsuda's and frequently with other allergens, it was hypothesized that all individuals are born Mitsuda-negative but gradually become Mitsuda-positive with age, after stimulation by Hansen's mycobacterium, Myco tuberculosis BCG or other unknown agents, if they are capable of reacting, depending on the presence of a constitutional "natural" factor of resistance ("N-factor"). If infection occurs, the "N-factor" bearers resist, but tuberculoid lesions may appear if "accessory factors" coadjuvate. The minority, lacking the "N-factor", remains Mitsuda-negative throughout life ("Anergic Margin") and, if infected, may progress to the Virchowian aspects after the same coadjuvation. An extensive range of intermediate reactivities should be placed between both extremes of maximal hyperergy and maximal energy. The erythematous and/or hypochromic macule would be the theoretical initial stage of the tuberculoid, Virchowian and intermediate lesions.

The theory contradicted Mitsuda's hypothesis of "exhaustion" of the Mitsuda-positivity to explain the energy of "nodular" (Virchowian) patients. It gave support to a genetical basis for predisposition to hanseniasis and was pessimistic about the preventive possibilities of BCG. The general acceptance of the theory — under its original or modified terminology (v.g. "**Potential immunity', "Defect of cell-mediated immunity**", etc.), the pending questions, and the field it represents for future research will be the subjects of following articles of this series.

Key words: Hanseniasis. Immunity. Resistance. Predisposition. Heredity. Genetics. N-Factor. Anergic Margin.

In 1923, Mitsuda reported to the 3rd International L e p r o s y Conference (Strasbourg) a fact that was to become historical : "neuro-macular" hanseniasis patients reacted strongly to an intradermal injection of an "emulsion of leprous nodules", whereas "nodular" patients did not react at all (11) .

There was another statement in Mitsuda's report — that the "nodular" (i.e., Virchowian or "lepromatous") patients did not react to that "emulsion" because "they had lost their immunity in the long fight against the bacilli". In other words, according to Mitsuda, all patients would be Mitsuda-positive,

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but those overpowered by the bacilli would become negative reactors.

In a series of investigations made in S. Paulo, Brazil (13, 14, 15) , this last statement was not confirmed. It was reasoned that if the nonreactivity to the antigen was a "consequence" of the aggravation of the disease, there would be a gradual decline in reactivity, from a maximum in early cases to the zero observed in "nodular" patients.

To check this point, patients were divided according to degree of bacillation : negative, slightly bacillary and heavily bacillary, as measured by examinations of the skin lesions and the nasal mucosa. The Mitsuda reaction, read according to Hayashi's system (10) was positive (++ and +++) in 71,3% of 491 bacterioscopically negative patients, a percentage which *sharply* fell to 2,6% and 3,1% of 151 *slightly* and 315 *heavily* bacillary pa- tients. The gradual decline which would confirm Mitsuda's explanation was not observed (Fig. 1).

A second fact disagreed with Mitsuda's interpretation. Patients included in the Brazilian research were no longer divided according to the usual topographical classification of the time (neural, etc.), but according to arbitrarily chosen "elementary types of lesion" : 1 — "leproma", 2 — "macular leproma", 3 — "diffuse leprosy", 4 — "bacillary erythematous macule", 5 — "bacillary hypochromic macule", 6 — "bacillary edematous macule", 7 — "non-bacillary edematous macule", 8 — "non-bacillary erythematous macule", 9 — "non-bacillary, hypochromic macule", 10 — "involuted macule", 11 — "clinically tuberculoid macule", 12 — "tuberculoid lesion, pathologically tuberculoid", 13 — "tuberculoid lesion, pathologically sarcoid", 14 — "atrophic macule or spontaneous scar", 15 — "lesions or nerve trunks, no clinical lesions or bacilli on the skin" (Fig. 2).

Using only grades ++ and +++ of Hayashi's reading method it was observ- ed: a) a "minimum of reactivity" in classes 1 to 7 (only 11 -1-± reactions in 485 patients, i.e., 2,3%, with *no appreciable differences between those groups;* b) a maximum of reactivity at the other extreme, from groups 11 to 15 (233 ++ and +++ in 243 patients, i.e., 95,9%) and *c)* an "inter- mediate" degree of reactivity, from groups 8 to 10 (129 ++ and +++ reactions in 245 patients, i.e., 52,6%).

The fact that the "energy" was of the same grade in the groups 4 to 7 ("bacillary macular" cases and "non--bacillary edematous macules") and in the groups 1 to 3 (frankly Virchowian, then called "lepromatous" cases) . was another proof against the gradual "loss of reactivity" advocated by Mitsuda.

The only possible explanation was that the "energy" to "lepromin" of *all* groups from 1 to 7, *preceded* the development of bacillary and "lepromatous" lesions, the difference of clinical and bacterioscopical aspects being attribut- ed to time of evolution or "accessory factors".

On the other extreme, the "immuno--allergic" condition revealed by the positivity of Mitsuda's test would protect the host against the development of bacillary lesions of any clinical type. However, non-bacillary "tuberculoid" lesions of the skin or nerves might appear.

The "non-bacillary erythematous, hy- pochromic or edematous macules" (groups 8 to 10) whose bearers were found to be either positive or negative to the test, would possibly represent the initial mass from which both extremes, the "anergic" and the "immuno-aller- gic" (or "tuberculoid") would derive.

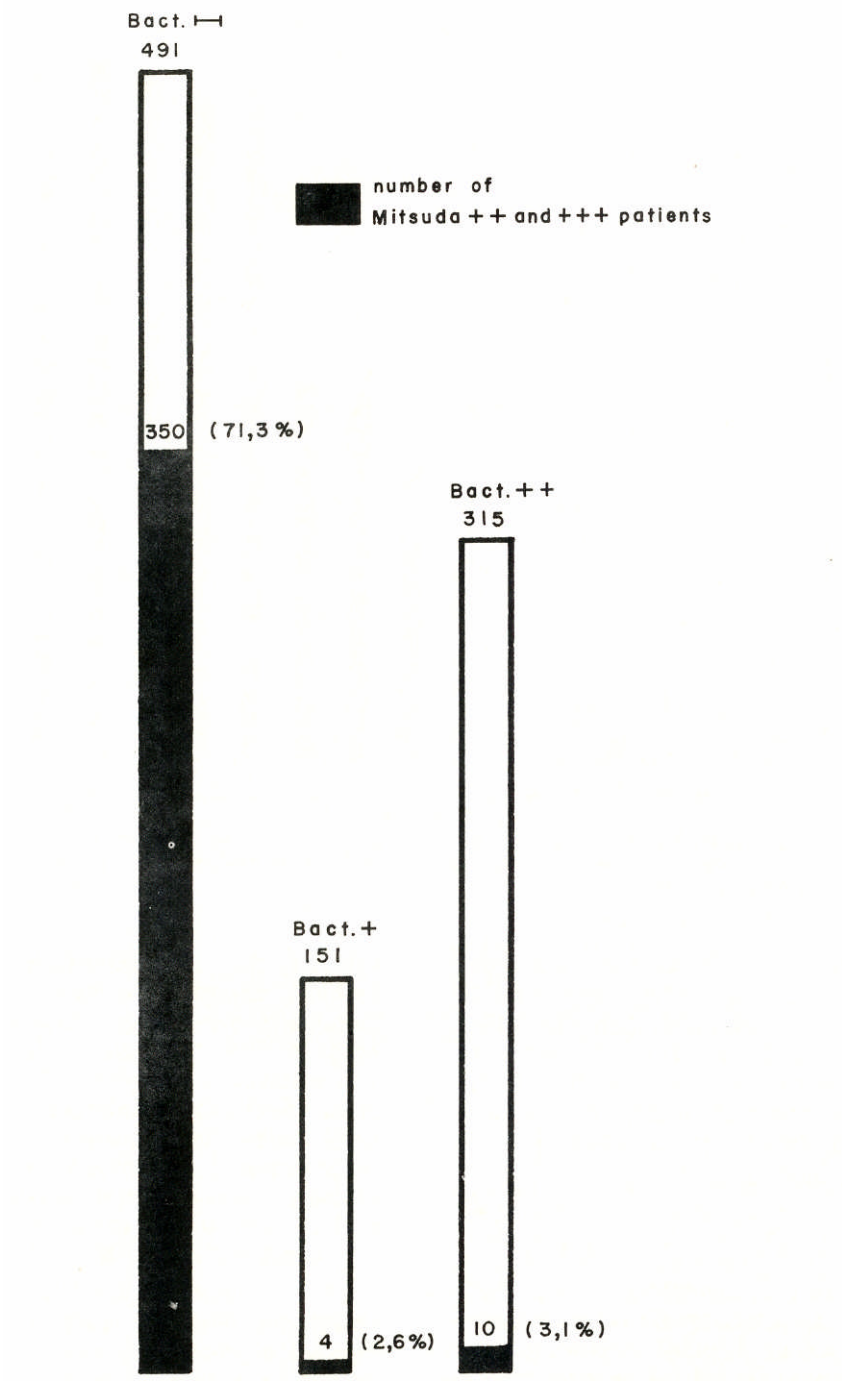

Fig. 1 — Mitsuda-positivity(++ and +++) in bacteriologically negative, lightly and heavily bacillary patients. No appreciable difference between the latter.

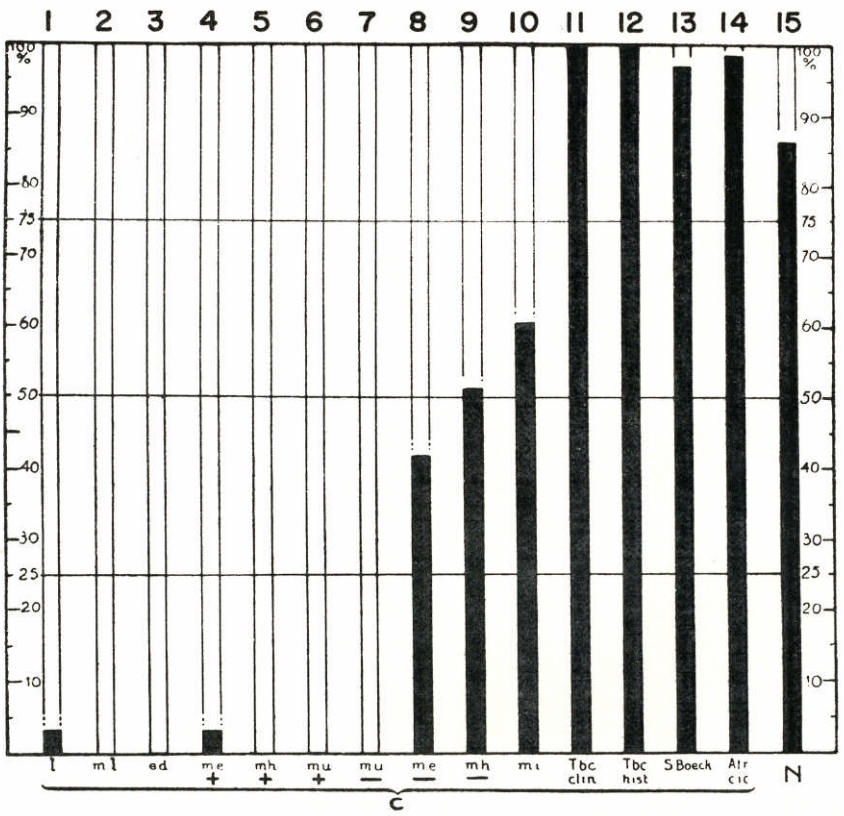

Fig. 2 — Mitsuda-positivity (+ + and +++) in patients showing different "elementary aspects'' of "cutaneous" hanseniasis (C) and neural involvement (N). Numbers of columns according to text.

Group 10 of "involuted macules" (checked both by *history* and *clinical records,* as no atrophy, scar or other signals could be seen at the time of testing) would be the result of in-volution of macules of groups 7 and 9, conserving their initial reactivity.

Out of 323 contacts or all age groups (284 under 15), 152 were Mitsuda - positive 47.1%) , 171 negative (52.9%).

A general pathogenetical and evoluti-ve hypothesis of hanseniasis was there-fore proposed, briefly : non-bacillary macules would be initial lesions, which, appearing in "lepromin-positive" contacts, would eventually progress towards the "immuno-allergic", "tuber-culoid" aspect. In the presence of "lepromin-negativity" they would prog-ress to the "anergic", "lepromatous" (Virchowian) aspects. Stability and even involution might be observed on both sides ; progress to the diametri-cally opposed "anergic" and "immuno--alergic" areas would depend on time and on the action of unknown "acces-sory factors". Fig. 3).

It is presumed that this N-factor of resistance does not obey to the law of "all or nothing". On the contrary, an extensive range of intermediate reac-tivities should be admitted between both extremes of maximal hyperergy

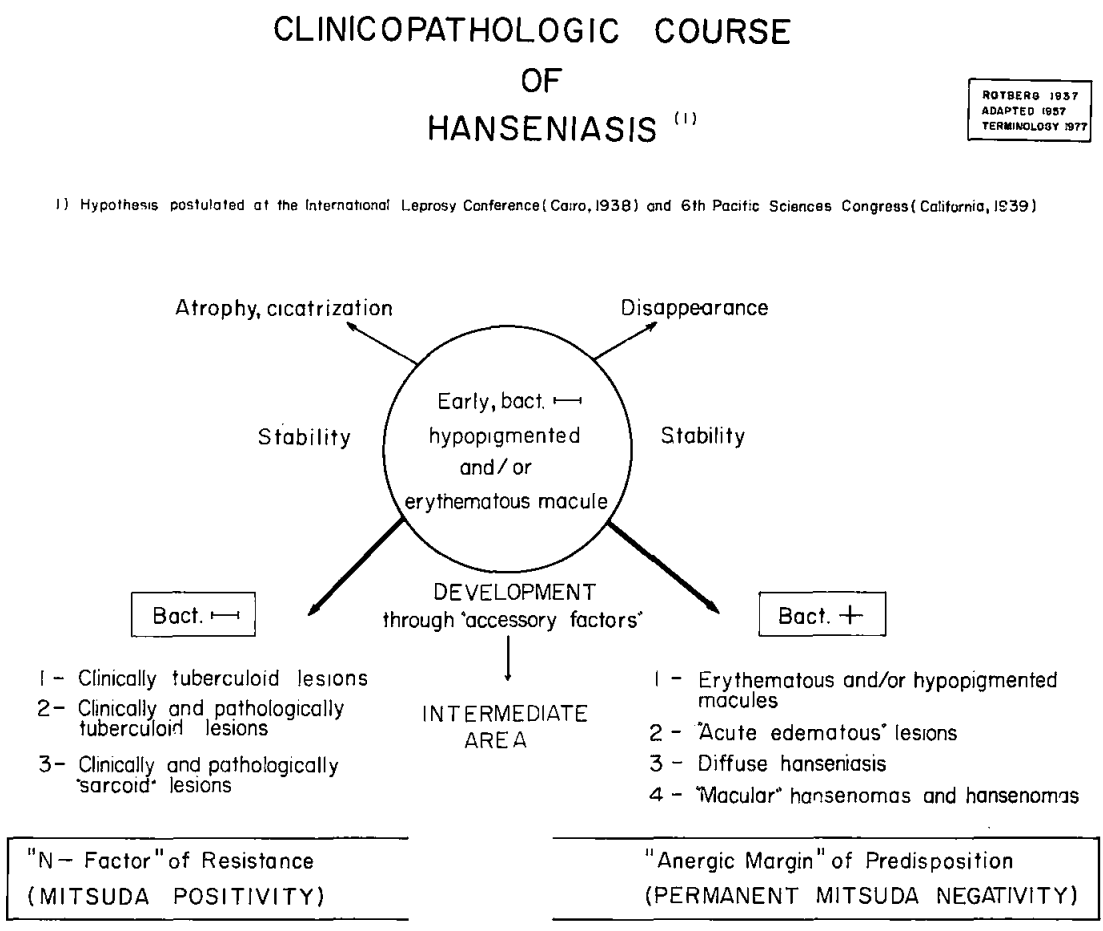

Fig. 3 — Pathogenesis of hanseniasis, as postulated at the Cairo Conference (1938) and 6th Pacific Science Congress (California, U.S., 1939).

and maximal anergy. This would *account* for the ample variety of clinico--pathological aspects and evolutive possibilities. (17).

THE NATURE OF THE MITSUDA REACTION

Accepting the viewpoints of Bargehr (3), De Langen (7) and Mitsuda (11) , the Mitsuda-positivity was attributed to sensitization to Hansen's mycobacterium. *The problem was the Mitsuda-negativity of patients and contacts,* which evidently could not be attributed to a lack of bacterial stimulation.

As stated, negativity could not be attributed to an "exhaustion" of previous positivity due to hanseniasis itself, and the negativity of contacts further contradicted Mitsuda's explanation. At the time the above pathogenetical theory was proposed, the current "pre-

disposition" to hanseniasis was debilitation to conditions like malnutrition, alcoholism, malaria, tuberculosis, verminosis, venereal and other diseases. However, in none of the Mitsuda-negative contacts, non-contacts and early cases were such conditions present or different from the Mitsuda-reactors. On the other hand, Mitsuda tests performed to check the point, in debilitated adult persons were more often positive: 60 out of 70 cases of open pulmonary tuberculosis (85.7%), 6 out of 7 cases of grave South American blastomycosis (85.7%), 7 out of 8 cases of American leishmaniasis (87.5%), all 3 cases of malaria. Of the 6 blastomycosis Mitsuda-positive cases, 3 were 3-plus and the sedimentation indexes of their red blood cells were 87,96 and 107, in one hour, by Westergreen's technique.

Other parameters were researched. In Brazil, the general impression at the time that European immigrants were more susceptible to hanseniasis than natives, led to investigations about their possibly lower degree of Mitsuda--reactivity. However, 48.2% adult immigrant patients (81 out of 168) reacted positively, against 33.3% among natives, who were children of immigrants (132 out of 396) and 33.9% among natives who were children of natives (75 out of 221).

Among their Brazilian-born contacts, 40.1% were Mitsuda-positive if children of immigrants (62 out of 154), against 46.9% if children of natives (61 out of 130).

The sex factor was not an explanation either. Among patients of all ages, 33.1% of the males (194 out of 585) and 42.5% of the females (203 out of 477) reacted positively to Mitsuda's antigen. Of their healthy children, 50.2% of the males and 42.6% of the females were Mitsuda-positive (respectively 90 out of 179 and 58 out of 136). Out of 39 male pulmonary tuberculosis patients 35 (89.7%) were Mitsuda-positive, against 25 out of 31 females (80.6%).

Age, however, seemed to be an important factor, as only 1 out of 19 contacts (5.7%) of the 0-3 age group reacted, whereas 33 out of 79 (41.7%) and 29 out of 39 (74.3%) reacted in the 7-9 and "16 or more" age groups, respectively, in a Curve similar to that of tuberculin-positivity, supporting the hypothesis of a bacterial stimulant.

That curve would only reflect the higher frequency of exposure and sensitization to Hansen's mycobacterium with age, not a special immunitary condition inherent to age itself. In other words, the age-group "16 or more" reacted more often than the age-groups 0-3 or 7-9 presumably because a longer exposure to the bacterial agent had taken place, not because persons over 16 are necessarily more resistant to hanseniasis than younger children. If this were the case, patients whose disease had appeared in early childhood would more often present Virchowian aspects, whereas tuberculoid lesions would have been the general characteristic of the disease appearing in older children and specially in adults.

This was not confirmed by the clinical study. Out of 66 children whose disease appeared under 10, only 37 (56.1%) showed bacillary lesions against 29 (43.9%) who exhibited tuberculoid and "sarcoid-like" bacteriologically negative lesions. On the other hand, out of 446 patients whose disease became apparent over 16,307 (68.8%) showed the bacillary "elementary" aspects, against 139 (31.2%) who manifested tuberculoid and "sarcoid-like" non-bacillary lesions.

A "generalized hyperergy" or a "generalized anergy" of the skin were

other explanations for the reactivity or non-reactivity to antigens derived from hansenic nodules. Ambrogio (1) , for instance, found that reactions to a "hidro-alcoholic bacillary protein extract" were positive in "neural" cases who also strongly reacted to tuberculin and a gonococcus vaccine. Tests were usually negative in "cutaneous" or "nodular" patients in whom the inespecific tests were also negative or much weaker. Bernucci (4) tested extracts of hansenic nodules and normal skin on "nodular" and "mixed" patients. All tests were negative. His explanation was a "general anergizing activity of the leprous virus" aggravated by skin anesthetic conditions.

These views had been contested in an earlier work (12). Eleven Mitsuda-negative advanced nodular patients reacted as strongly to suspensions of Deycke's *Streptothrix leproides* and of the acid-fast bacilli cultivated by Bayon as the less advanced and the "pure neural" and "maculo-anesthetic" cases. 75 . b% of Mitsuda-positive healthy children were negative to Dorset's synthetic tuberculin (16). Out of 122 bacteriologically positive and Mitsuda negative hanseniasis patients, 65 (53,3 %) reacted to the same tuberculin. These studies, as well as one conducted among patients of tuberculosis (20), also showed that tuberculin and Mitsu-da reactivities were not necessarily correlated.

THE INHERITED "NATURAL" FACTOR ("N-FACTOR") OF MITSUDA-REACTIVITY

From the above facts and considerations, it was concluded that the Mitsuda-negativity of contacts and hanseniasis patients neither depends on any of the investigated parameters — age, sex, national background, general skin- -reactivity, malnutrition and other debilitating conditions, nor could it be attributed to an "energizing" activity of Hansen's mycobacterium itself.

For lack of a better explanation and with the observation that extreme disparities of reactivity often occur among the youngest contacts of the same focus, with the same habits and under the same environmental conditions, the theory was advanced that the incapacity to react *"is already present at birth and seems to depend on exclusively hereditary factors".*

The citation continues (13) : "The controverted inheritance of predisposition takes on thus a new objective aspect, which was already suspected by Jadassohn when he stated that there is undoubtedly an individual difference in the capacity of allergization in contact with the bacillus of Hansen". "To avoid repetitions I shall *give* to this factor, or the conjunction of factors which give capacity of allergization the name of natural factor, abridged to Factor N." "Therefore, the individual not inheriting the Factor N will not develop allergy in contact with the bacillus, and will remain always anergic. Among these anergic cases are the candidates to the bacillary forms of leprosy, once there are accessory factors, as superinfections, organic debilitation, etc."

TUBERCULIN-REACTIVITY AND BCG RESHAPE AND CONFIRM THE THEORY

As stated, Mitsuda-positivity was first attributed to "infection by Hansen's mycobacterium *plus* "N-Factor". Consequently, the Mitsuda-positivity very often observed in non-contacts would imply a more widespread dissemination of the infection than was admitted. On the other hand, no Mitsuda-positivity should be observed in non-endemic areas. The positive

reactions observed in England (6), Belgium (8) and non-endemic areas of Italy (5) were supposed to be doubtful, considering their small size and/or their different aspects.

Further studies (2, 18, 19), however, showed that positive Mitsuda-tests, similar to those seen in endemic areas, could be observed on persons who had never left their non-endemic countries. Most of those persons were tuberculin -positive (18, 19) and a crossreaction to *Myco. tuberculosis* was admitted, having in view the conversion of Mitsuda-negativity to positivity by BCG, first observed by Fernandez (9). As some of the Mitsuda-positive reactions of non-endemic countries were not accompanied by a tuberculin-positivity, other stimulants, probably dif- ferent mycobacteria, might be involved in the process. The original theory had to be adapted to conform to the newly observed facts.

On the other hand, both *BCG and tuberculin studies have brought a confirmation of a constitutional "N-Factor" of immuno-allergization against Hansen's mycobacterium.*

There is no longer any doubt that BCG has the capacity to convert Mitsuda-negative individuals to positivity. However, *this capacity is not universal.* In a review of the literature up to 1957 (17) a "margin of exceptions" to the conversion could be observed in 17 articles, ranging from 1 to 69,6% mostly within the 12.5-25% range. Those "exceptions" continued to appear in the vast majority of later articles about Mitsuda conversion by BCG and other mycobacteria.

Natural infection by *Myco. tuberculosis* also leaves a "margin of non--reactors" to Mitsuda's antigen.. In that same article (17) 29 papers were reviewed and showed that persons with clinical tuberculosis or only tuberculin-positive were Mitsuda-negative, in percentages ranging from 1.6 to 55.4%, mostly within the 1070-25% range.

In some articles referring to tuberculosis, tuberculin positivity or BCG, the permanent Mitsuda negative individuals were also contacts of hanseniasis patients, which shows that a double stimulation by both mycobacteria — Koch's and Hansen's — did not result in a conversion of the "anergic margin".

Figure 4 shows the definite state of the "N-Factor/Anergic Margin" hypothesis : the percentage of Mitsuda--reactors augments from zero at birth to a maximum of schematically 80% in adults, due to stimulation of the constitutional "N-Factor" bearers by Hansen's and Koch's and possibly other mycobacteria. The curve does not reach the 100% due to the fraction of population who do not inherit the "N-Factor" and constitute the "Anergic Margin".

The percentages of 80% and 20% for the "N-Factor" bearers, and the "anergic margin", respectively, were only schematic and roughly reflected the averages of Mitsuda-positivity and negativity observed by various authors on adults of endemic and non-endemic countries, or in children given BCG.

The preventive possibilities of BCG were thus put in doubt. If, according to the theory, the infectious Virchowian patients are recruited among the "constitutional anergic margin" and if BCG only accelerates the Mitsuda--positivity of the bearers of "N-Factor", not much hope could be laid on its prophylactic value. The inconclusive results about BCG as a preventive measure in the field might be taken as an undesired confirmation of the pessimistic forecast intrinsic to a theory of a gene-conditioned susceptibility.

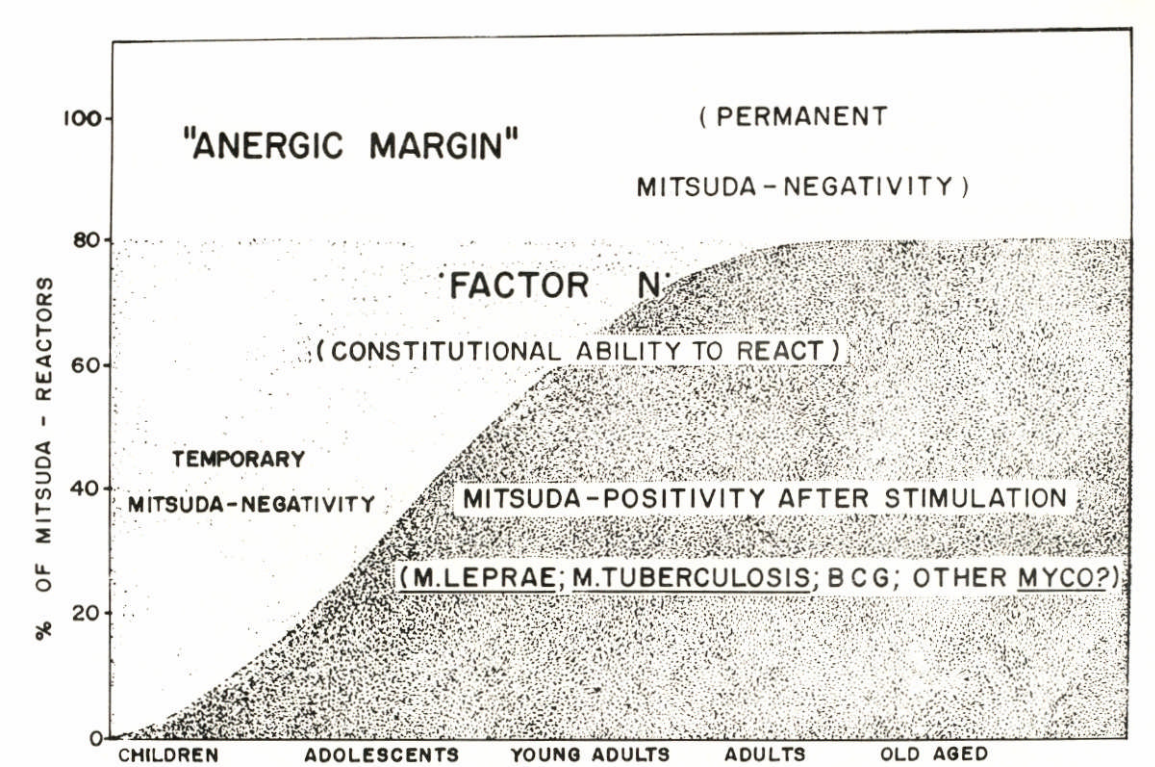

Fig. 4 — The "Anergic Margin" of the population, after adequate stimulation, remains Mitsuda-negative.

RESUMO

São apresentados os fundamentos da teoria patogenética "Fator-N/Margem Anérgica", apresentada em 1937 e completada em 1957. Com base na observação de 2.160 pessoas que tinham sido testadas pelo autor com antígeno de Mitsuda e, freqüentemente, com outros antígenos, foi proposta a hipótese de que todos os indivíduos nascem Mitsuda-negativos mas gradativamente se tornam Mitsuda-positivos com a idade, após estímulos pela micobactéria de Hansen, **Myco. tuberculosis**, BCG e outros agentes desconhecidos, se forem capazes de reagir, dependendo da presença de um fator constitucional "natural" ("Fator-N") de resistência. Se ocorrer a infecção, os portadores do "Fator-N" resistem, mas podem surgir lesões tuberculóides, pela ação de "fatores acessórios". A minoria, sem "Fator-N", permanece Mitsuda-negativa pelo resto da vida ("Margem Anérgica") e, se infectada, pode evoluir para os aspectos virchovianos, na presença dos mesmos "fatores acessórios". Extensa gama de reatividades intermediárias deve ser colocada entre ambos os extremos de hiperergia máxima e anergia total. A mácula eritematosa e/ou hipocrômica seria o estágio inicial teórico das lesões tuberculóides, virchovianas e intermediárias.

A teoria contrariava as idéias então dominantes sobre a predisposição à hanseníase. atribuída à debilitação geral por diversas doenças, alcoolismo, etc. Também contradizia a hipótese do próprio Mitsuda de que a Mitsuda-negatividade dos doentes "nodulares" (virchovianos) era o **resultado** da "exaustão" da reatividade depois de "longa luta contra o bacilo de Hansen".

Termos índice: Hanseníase. Imunidade. Resistência. Predisposição. Hereditariedade. Genética. Fator-N. Margem Anérgica.

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