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THE MITSUDA REACTION IN A NONLEPROUS AREA ¹

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The initial observations of Mitsuda (1) on the use of his "lepromatous tubercle emulsion" were of purely immunological character, and they revealed the difference in behavior between cases of the lepromatous ("tuberculous") and the benign "maculoneural" types. The evolution of such cases observed by Hayashi (2) after testing demonstrated the prognostic value of the reaction, since only those which were Mitsuda negative progressed and became worse. Neither Mitsuda nor Hayashi claimed this reaction to be anything more than an index of resistance to infection, such as is seen in the benign cases and in healthy persons who do not become sick after years of contact with the disease.

Bargehr (3), using a paste of triturated lepromas and applying it to skin scarifications, observed the same positivity in "extinct" and bacillus-free cases, but he laid stress especially on the positive results obtained in persons residing in endemic areas and with more or less prolonged contact with leprosy patients. In contrast with those findings were the negative reactions obtained later by de Langen (4) in individuals newly arrived in the Netherlands Indies from Holland, which is free from leprosy. These investigations of Bargehr and de Langen gave rise to the concept of the "positive reaction due to minimal and repeated previous infections producing immunizing antibodies," a concept extended by later workers to the results of the Mitsuda reaction.

The question thus arose of whether positivity to lepromin,

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applied by either the Mitsuda or the Bargehr technique, besides indicating resistance as is generally accepted, also signifies a previous immunizing infection with leprosy. That opinion of Bargehr and de Langen was later adopted by other authors, among them Stein and Steperin (5), Cerqueira Pereira (6) and Rotberg (7).

The necessity became evident of applying the test to persons certainly free of previous contact with leprosy, as for example in the natives and residents of European countries or the United States, individuals who had never been away either to reside or to travel in areas where leprosy is endemic. To confirm the hypothesis that infection by the leprosy bacillus determines positive reactivity, such individuals should always give negative results.

Such a study was made for the first time by Cummins and Williams (8) in psychopathic patients in England, with an antigen sent them by Muir for the purpose. The results were considered positive, and were therefore contrary to the hypothesis in question. Also considered positive were results obtained by Dubois in Belgium (9) and Boncinelli in Italy (10), which again lent support to the view that positivity to lepromin is not necessarily indicative of previous infection.

One of us (A. R.) still believed, however, that the hypothesis of previous infection was admissible for the following reasons: (1) Examination of the reports of Cummins and Williams, Dubois, and Boncinelli gave the impression that the reactions obtained were quite minor ones, and were less frequent than those obtained in endemic areas. For example Dubois, who tested 29 psychopathic patients with an antigen supplied by van Breuseghem from the Belgian Congo, obtained 14 negative reactions (0-2 mm. on the 15th-20th days) and 10 with 3-5 mm. reactions (1+ according to Hayashi's classification); only 5 reactions were stronger, although "some of these were very strong, going beyond 10 mm. with slight ulceration." These results were very different from those which are obtained in healthy adults of endemic areas, where reactions of 2+ or 3+ are very numerous, many of them with frank necrosis.

(2) In endemic areas the studies of Chiyuto (11), Muir (12), Bhattacharji (13), Burnet (14), Souza Campos (15), Cerqueira Pereira (6), Rotberg (7), and others have demonstrated the frequent negativity of the lepromin reaction in childhood. Rotberg, however, demonstrated that the frequency of positivity gradually increases in the older age groups up to 70-80 per cent in adults, which brings to mind the curve observed for the tuberculin reaction.

Further observations to clarify the matter became necessary. Wade (16), however, believes that there is no reason to deny the positivity seen in nonendemic countries. He thinks that it can be explained as an allergic phenomenon, even without previous infection, through sensitization by the injected lepromin itself. Therefore, the positive reaction in a healthy individual residing in a nonendemic area and without any previous contact with leprosy, may be due to a slow allergization to the substance with which he comes in contact for the first time on the occasion of the test. The positive results obtained by Rodriguez (17) and by Wade himself in healthy animals are arguments in favor of this hypothesis.

In the meantime, a new factor entered to complicate the situation, namely, the early or first-phase reaction studied by Fernandez (18). He observed that lepromin produced an erythematous-edematous reaction in 48 hours in the majority of the cases which showed the classical late (second-phase) reaction of Mitsuda-Hayashi in the third or fourth week. If this second-phase reaction can be attributed to sensitization by the injected material itself, the first phase, which is rapid and frequently evident within 6 to 8 hours after the injection and behaves like the tuberculin reaction, can only be attributed to a preexisting sensitivity. Fernandez and Olmos Castro (19) have observed a high frequency of the "early" positivity in contacts but rarely in noncontacts, and they believe that this reaction may actually indicate previous infection, in contrast with the classical late reaction.

Another factor worthy of study is with reference to tuberculin sensitivity. Lepromin reactions have very often been compared with those to tuberculin in man and in experimental animals, on the ground that an infection by the Koch bacillus may determine cross sensitization to lepromin, and vice versa. If comparative studies of the two reactions in a leprosy endemic area contribute to the clarification of this question, more decisive should be such a study in a nonleprous area. In such areas one of the possible factors of sensitization is absent, there being only the one pertaining to the Koch bacillus, and that should constitute a favorable situation for the study of this phase of the problem.

PERSONAL OBSERVATIONS

To determine personally the results of the reactions of Fernandez and Mitsuda in individuals free of contact with leprosy, we performed in New York intradermal lepromin reactions in healthy and tuberculous persons born in the United States who had never been abroad, or in individuals born in European

countries where leprosy is nonexistent or extremely rare who had emigrated to the United States. Also tested were several cases from regions where leprosy is encountered sporadically, in small endemic foci, from which regions they had emigrated to the United States. Finally, a group of patients with pulmonary tuberculosis was subjected to this test. The antigen employed was of the same lot, prepared by one of us, with which numerous frankly positive reactions had been obtained in cases of tuberculoïd leprosy and in normal individuals residing in the endemic area of São Paulo. The results of the Fernandez reaction were classified as negative, 1+ or 2+, according to the criteria of that worker, and those of Mitsuda as from negative to 3+ according to Hayashi's criteria.

A part of this group was also tested for reactivity to tuberculin, using the "PPD" (purified protein derivative) tuberculin and the international classification (results graded from negative to 4+), and the tuberculous group was also given this test. This phase of the work was done to ascertain the influence of sensitization by the Koch bacillus on the responses to lepromin, since it is generally thought that many positive lepromin reactions are due to the phenomenon of cross sensitization.

As a basis of comparison, the results obtained by Souza Campos and Rotberg (20) with the same lot of lepromin in a group of healthy individuals residing in the endemic zone of São Paulo are used here. In these cases the results of the tuberculin refer to reactions to the crude tuberculin of Koch diluted 1:10,000.

Our first results were presented in a preliminary note to the Sociedade Paulista de Leprologia (21). At that time we noted that, of 36 individuals who had never resided in an endemic leprosy area, 2 had given strongly positive lepromin reactions (3+) and 8 moderately strong reactions (2+), representing 27.8 per cent of definite late reactions. Also, 6 early (Fernandez) reactions of moderate or strong degree were observed in this group. We therefore concluded that neither the early reaction of Fernandez nor the late one of Mitsuda could necessarily be considered an indication of previous leprosy infection; that the late reaction could be attributed to a progressive allergization to the injected lepromin itself, located at the site of injection, as suggested by Wade; but that this hypothesis was not applicable to the case of the early reaction, which could be better explained as a cross reaction due to sensitization to the Koch bacillus. It

was a fact that, with a single exception, all Fernandez positive patients were also positive to tuberculin.

A final report of the observations referred to, and of more recent results, is now presented.

THE MITSUDA REACTION

Considering first the incidence of late lepromin positivity, in the third and fourth weeks, the data are shown in Table 1.

TABLE 1.—*The Mitsuda (late) reaction to lepromin.*

Group	Number of cases	Results			
		Neg.	1+	2+	3+
A. United States, healthy	64	9 (14.1%)	15 (23.4%)	38 (59.4%)	2 (3.1%)
				62.4%	
B. United States, tuberculous	124	29 (16.1%)	30 (24.2%)	57 (46.0%)	17 (13.7%)
				59.7%	
C. São Paulo, healthy	161	3 (1.8%)	42 (26.4%)	83 (51.5%)	33 (20.2%)
				71.7%	

That the Mitsuda reaction is frequently positive in individuals without contact with leprosy is evident. If we consider as positive only the stronger ones (2+ and 3+), we see that the groups A and B, normal and tuberculous individuals in the United States, gave 62.4 and 59.6 per cent of such reactions respectively—an insignificant difference—whereas the figure for the São Paulo control group C, 71.7 per cent, is a little higher. Examples of these reactions are shown in Figs. 1 and 2.

Several of the reaction lesions of groups A and B were studied histologically. They showed the same tuberculoid picture as is seen in the reactions in tuberculoid leprosy patients (Fig. 3).

The frequencies of weak (1+) reactions are essentially the same in the three groups. As for the negative results, groups A and B (New York) gave 14.1 and 16.1 per cent, respectively—again an insignificant difference—whereas in group C (São Paulo) the negatives were only 1.8 per cent. On the other hand, considering the strongest (3+) reactions, group A gave only 3.1 per cent, group B gave 13.7 per cent, and group C gave 20.2 per cent. This last figure is materially higher than that of the

tuberculous group in New York, and in sharp contrast with that of the comparable New York group (i.e., the one without clinical tuberculosis).

It would thus appear that, although typical positive lepromin reactions were observed with a high degree of frequency in the individuals without any possibility of contact with leprosy (see Plate 10), yet strong (3+) reactions were somewhat more frequent and the negative reactions more rare in the healthy individuals living in an endemic area. The fact that, in the United States, strong reactions were observed more often in group B than in group A suggests a probable influence of the tuberculosis factor upon the intensity rather than the frequency of the Mitsuda reaction; the difference is due solely to a lesser frequency of 2+ reactions, since the incidence of weak reactions was the same in the two groups. The possibility of a tuberculosis factor in group C is remote, since all of the individuals involved were healthy and robust.

THE FERNANDEZ REACTION

The results of Fernandez' early reaction are shown in Table 2. These data are highly significant of a difference in this matter in people from nonendemic and endemic regions.

TABLE 2.—*The Fernandez (early) reaction to lepromin.*

Group	Number of cases	Results		
		Neg.	1+	2+
A. United States, healthy	64	52 (81.2%)	4 (6.5%)	8 (12.5%)
		18.7%		
B. United States, tuberculous	126	104 (82.5%)	11 (8.7%)	11 (8.7%)
		17.4%		
C. São Paulo, healthy	161	72 (44.7%)	39 (24.2%)	50 (31.0%)
		54.2%		

In groups A and B this reaction was found negative in 81.2 and 82.5 per cent respectively, whereas in the São Paulo cases only 44.7 per cent were negative. Taking all positive reactions together, we have only 18.7 and 17.4 per cent in groups A and B, respectively, against 55.2 per cent in group C.

These results suggest the presence, in a leprosy endemic

environment, of a factor which conditions a fairly high frequency of positive Fernandez reactions. This factor is evidently not related to *active* tuberculosis, for group C was free from it and the tuberculous group B gave results identical with those in the nontuberculous group A. However, it may be that the condition is due to cross sensitization with relation to the Koch bacillus. If so, it should be demonstrated by comparison of the lepromin and tuberculin reactions, in all groups.

THE TUBERCULIN REACTION

A preliminary analysis of the results of the tuberculin reactions (Table 3), shows the very low frequency of 19.5 per cent of tuberculin-positive individuals among the healthy persons tested in the United States, as compared with the very high one of 84.9 per cent in São Paulo. This leads us to suspect that the greater frequency of tuberculin reactivity in São Paulo was, by the mechanism of cross sensitization, the factor responsible for the equally greater frequency of positive Fernandez reactions in the same endemic environment.

TABLE 3.—*The tuberculin (Mantoux) reaction.*

Group	Number of cases	Results		
		Neg.	1+	2+ or 3+
A. United States, healthy	21	15 (71.4%)	2 (9.5%)	4 (19.0%)
			19.5%	
B. United States, tuberculous	127	48 (37.7%)	40 (31.4%)	39 (30.7%)
			62.1%	
C. São Paulo, healthy	106	16 (15.1%)	13 (12.3%)	77 (72.6%)
			84.9%	

To clarify this matter it is necessary to ascertain the role of that possible factor by analyzing the Fernandez and Mitsuda reactions, on the one hand in the tuberculin-negative cases in the United States and São Paulo, and on the other hand in the tuberculin-positive cases in those regions. This analysis is made in Table 4. Although difficulties are met because of the necessary multiplicity of groups, and sometimes the insufficient numbers of cases in individual groups, nevertheless the percentages arrived at permit us to draw several conclusions.

TABLE 4.—Relation of the Fernandez and Mitsuda reactions to tuberculin reactivity.

Grouping	Fernandez					Mitsuda				
	Cases		Neg.	1+	2+	Cases	Neg.	1+	2+	3+
1. Cases negative to tuberculin										
A. United States, healthy	15	11 (73.3%)	2 (13.3%)	2 (13.3%)	0	15	0	3 (20.0%)	12 (80.0%)	0
B. United States, tuberculous	48	43 (89.6%)	3 (6.2%)	1 (2.1%)	11 (23.9%)	46	11 (23.9%)	14 (30.4%)	15 (32.6%)	6 (13.0%)
C. São Paulo healthy	16	11 (68.7%)	3 (18.7%)	2 (12.5%)	2 (12.5%)	16	2 (12.5%)	5 (31.2%)	6 (37.5%)	3 (18.7%)
2. Cases weakly positive to tuberculin (1+)										
A. United States, healthy	2	1	0	1	0	2	0	0	1	1
B. United States, tuberculous	40	35 (87.5%)	2 (5.0%)	3 (7.5%)	3 (7.5%)	40	3 (7.5%)	10 (25.0%)	25 (62.5%)	2 (5.0%)
C. São Paulo healthy	13	9 (69.2%)	4 (30.7%)	0	0	13	0	5 (38.4%)	7 (53.8%)	1 (7.7%)
3. Cases strongly positive to tuberculin (2+ and 3+)										
A. United States, healthy	4	3	0	1	0	4	0	0	4	0
B. United States, tuberculous	39	26 (66.6%)	6 (15.4%)	7 (17.9%)	6 (15.8%)	38	6 (15.8%)	6 (15.8%)	17 (44.7%)	9 (23.7%)
C. São Paulo healthy	77	28 (36.4%)	15 (19.5%)	34 (44.1%)	0	77	0	22 (28.6%)	43 (55.8%)	12 (15.6%)

We see that in the tuberculin-negative cases the difference between those in the United States and Brazil with respect to the early or Fernandez-type reactivity disappears. In the absence of tuberculin positivity the Fernandez reaction behaves approximately alike in all three groups. Turning to the tuberculin positive cases, an increase of frequency of the positive Fernandez reaction is observed in the São Paulo group. Other data of this table cannot easily be analyzed.

The results, as well as the comparison of those of the Fernandez and Mantoux reactions in Tables 2 and 3, reveal that an increase in frequency of Fernandez positivity tends to accompany increase in frequency of positivity to tuberculin. It is possible that this is due to mere coincidence, and that the individuals who were sensitized to tuberculin had also had opportunity to become sensitized, in parallel, to an unknown factor which may condition the Fernandez reactivity. If, in an endemic environment, this factor may be assumed to be leprosy itself, this explanation does not apply to the Fernandez-positive cases in the United States.

Another hypothesis is that there was a direct influence of the tuberculin sensitization, because of which the Mitsuda antigen produced cross reactions of the Fernandez type. This hypothesis is plausible in view of the works of Bieling, Oelrich and Schwartz (22), Melsom (23), Fernandez (24) and others, who have demonstrated in human beings and in animals the possibility of cross sensitization to tuberculous and lepromatous antigens. Admitting this hypothesis there remain, however, two facts to be explained:

(1) For what reason, if sensitization to the tubercle bacillus gives rise to positive Fernandez reactions, do some of the tuberculin-positive cases—66.6 per cent of the United States cases and 36.4 per cent of those in São Paulo—fail to show that reaction?

This fact could be explained simply on the ground that cross sensitization is not obligatory.

(2) For what reason are positive Fernandez reactions observed in tuberculin-negative cases—26.6 per cent in healthy persons and 8.1 per cent in the tuberculous in the United States, and 31.2 per cent in healthy persons of São Paulo?

It seems to us that this discordance can be explained on the basis of the different concentrations of the antigens. The Mitsuda antigen with its soluble principle responsible for the

early reaction is employed without dilution,² whereas tuberculin is used in high dilutions. It is theoretically possible that, in given cases previously infected by the Koch bacillus, tuberculin diluted 1:5,000 or 1:10,000 may be incapable of producing reactions, whereas reactions due to cross sensitization may be induced by the concentrated Mitsuda antigen. To ascertain the validity of this explanation it would be necessary that, in the same cases, stronger concentrations of tuberculin (1:1,000 or 1:100) should reveal a reactivity not induced by the 1:10,000 dilution. This work could not be performed. In case this assumption should not be confirmed, the hypothesis of cross sensitization would be greatly prejudiced. It would be necessary then to ascertain some other factor to explain the Fernandez reaction in endemic and nonendemic environments.

SUMMARY AND CONCLUSIONS

This report deals with the results of the intradermal injection of 0.1 cc. of the Mitsuda antigen (lepromin) and of the Mantoux reaction, performed on 190 adult persons, healthy or tuberculous, who were born in the United States and had never lived in any leprosy endemic area. The results in these two groups are compared with each other and also with those similarly obtained with lepromin and tuberculin by Souza Campos and Rotberg in healthy adults living in the leprosy endemic area of São Paulo. The following conclusions are drawn:

1. The late positive result of the lepromin test (the Mitsuda reaction) was observed in healthy and tuberculous individuals in the United States in 62.4 and 59.7 per cent of cases, respectively. These percentages, which refer only to the frankly positive reactions (2+ and 3+), approximated the 71.7 per cent positive results observed in the endemic area, São Paulo. There was, however, an excess of strong (3+) reactions in the group in the latter area (20.2 per cent) in comparison with the American healthy group (3.1 per cent).

2. No correlation was found between the results of the Mitsuda and the Mantoux reactions.

3. The early, 48-hour reaction (Fernandez phenomenon) was seen in the United States in 18.7 per cent of the healthy group and 17.4 per cent of the tuberculous group, against 55.2 per cent of the São Paulo group. In the absence of any other known factor which would explain the Fernandez phenomenon in a nonleprosy

² It will be recalled that this antigen is a 5 per cent suspension of the leproma, less the coarser tissue elements.

environment, the hypothesis of cross sensitization with the Koch bacillus is accepted provisionally. It is suspected that the relative high frequency of positive Fernandez reactions in the persons of the endemic area probably results from the high frequency of positive tuberculin reactions in that group—84.9 per cent in São Paulo against 19.5 per cent in the healthy group and 62.1 per cent in the tuberculous group in the United States.

4. If the cross-sensitization hypothesis is admitted, it remains necessary to explain two types of disparities, (a) the Mantoux positive-Fernandez negative condition, and (b) the Mantoux negative-Fernandez positive condition. The former is probably explainable by the nonobligatory character of cross sensitization. The latter is explained, hypothetically, on the ground that tuberculin was used in high dilutions whereas the Mitsuda antigens was employed without dilution.

5. If these explanations are not satisfactory, or if cross sensitization cannot be proved in any other way, it will be necessary to search for another factor to explain the Fernandez reaction, in both endemic and nonendemic areas.

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DESCRIPTION OF PLATE

PLATE 10.

FIG. 1. Moderately strong positive lepromin (Mitsuda) reaction in a New York patient (L. H., aged 15 years, December 16, 1944).

FIG. 2. Strongly positive lepromin (Mitsuda) reaction, with desquamation and slight superficial ulceration, in a New York patient (T. H., aged 50 years, January 6, 1945).

FIG. 3. Showing the tuberculoid structure of a lepromin reaction lesion, consisting of epithelioid and lymphoid cells, in a New York case.

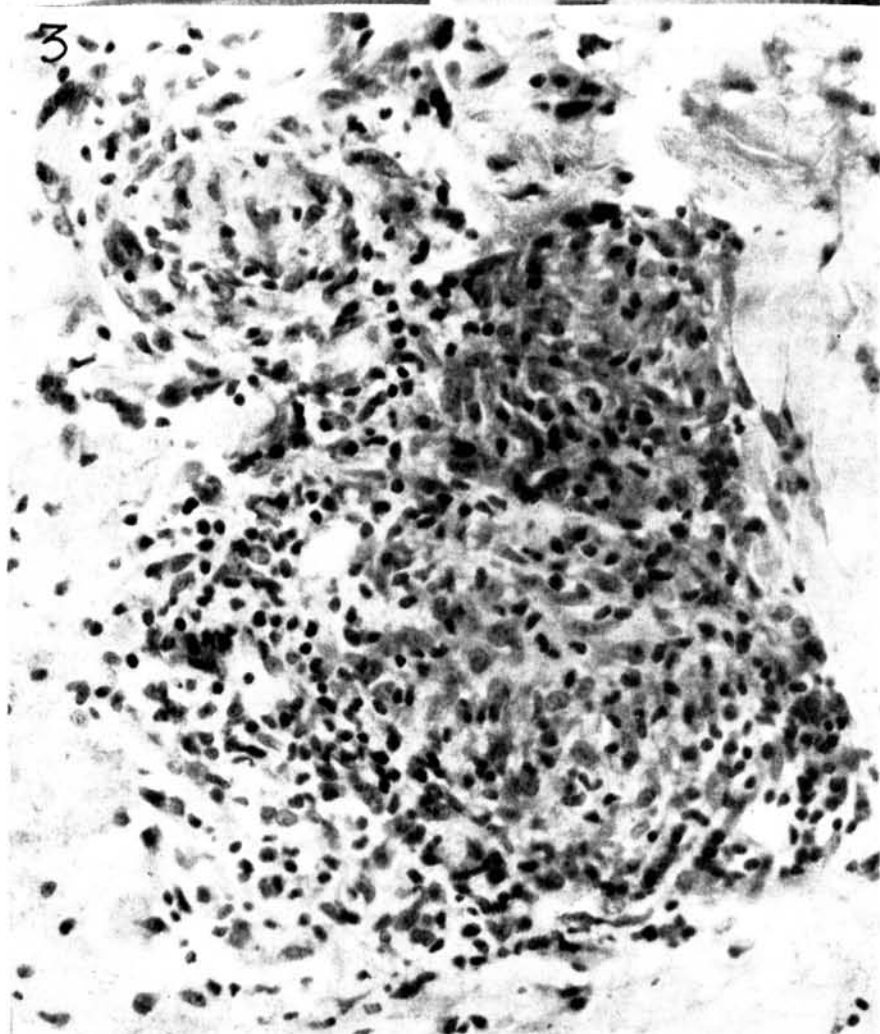
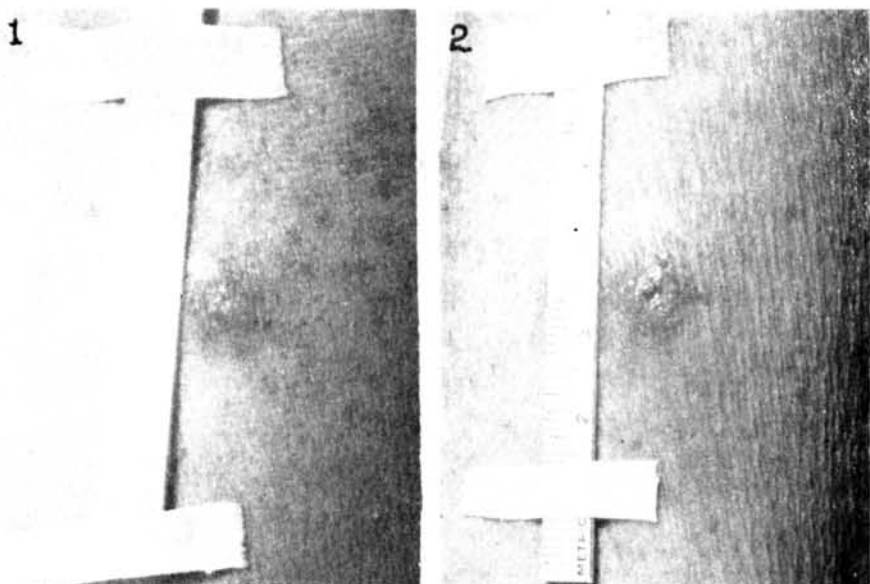


PLATE 10.