THE MITSUDA REACTION BY VACCINES TREATED WITH THE ULTRA-SUPERSONIC WAVE

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After relatively long experimentation and discussion, it has been recognized that the element of Mitsuda's leproma vaccine which causes the reaction known by his name is the bacillus itself, and not the tissue or the fluid in the preparation.

The first investigations of this matter were made by Fumio Hayashi (1). He prepared vaccines from lepromatous lymph nodes which contained varying numbers of bacilli, depending upon the stage of the disease. As the bacilli are destroyed more easily by this organ than by any other, there are found in it various concentrations of bacilli and of the lipoid which is characteristic of lepromatous lesions. It is, therefore, the most suitable organ to use in investigating the question whether the Mitsuda reaction is caused by the bacilli or by their dissolved elements. This experiment revealed that intensity of the reaction depended upon the number of bacilli present, and not upon the amount of the lepromatous lipoid.

There remains another problem in connection with this lipoid. It is not certain whether it represents the substance of degenerated bacilli, or is a product of the tissue itself. Mitsuda asserts that it is chiefly produced by the tissue, pointing to the fact that, in any stage of the disease, lepra cells found in the heart contain at most only two or three bacilli per cell, yet in time these cells become transformed into the usual large foamy cell with much lipoid. Assuming, however, that this lipoid does contain some proportion, whether large or small, of the dissolved bacillary substances, there is the question of whether all of these substances are present or whether some part of them have been transported outside the lymph nodes, and also that of whether or not the dissolved products that remain are changed in quality.

Having these questions in mind, the late Dr. Nagai attempted to dissolve the bacilli directly by chemicals in the test tube.

Lecithin and kephalin were found to be the most suitable ones for this purpose, but it was impossible to separate the dissolved bacillary substance from those chemicals, and the skin reaction was much disturbed by them. Consequently, this experiment failed.

There then appeared an apparatus that is ideal for dissolving bacilli, that of the "ultra-supersonic wave." A machine of this kind, of very high efficiency, was installed in Keiaien by Inoué in June, 1939, and a vaccine with completely dissolved bacilli was prepared for skin reactions and other purposes. A high-efficiency ultra-short wave apparatus was also installed.

METHOD

A Mitsuda emulsion is prepared from a fresh nodule in the usual way, divided into lots of 0.5 or 1.0 cc. in ampules, and treated by the ultra-supersonic wave at 600 or 1,500 thousands cycles until the bacilli are dissolved. The ultra-short wave apparatus, Inoué found, will also destroy and dissolve leprosy bacilli, but by this wave the tissue particles form clots within which undestroyed bacilli persist for a long time. On the other hand, with the ultra-supersonic wave the tissue particles and bacilli make perfect emulsions, resulting in the complete dissolution of the bacilli, and in consequence this modus has been employed.

In making tests with the vaccines so prepared, Mitsuda's original skin reaction has always been made with untreated vaccine, for comparison. The dosage employed and the grading of the reactions has been according to Mitsuda's original method.

EXPERIMENTS AND RESULTS

REACTIONS WITH INCOMPLETELY DISSOLVED VACCINE

It was found to be not easy to prepare completely dissolved bacillary emulsions by the ultra-supersonic wave treatment, and it was several months before Inoué succeeded in doing so. Before that was accomplished skin reactions were made with a vaccine containing four or five bacilli per field of the oil-immersion objective. It was thought that such small numbers of them might be negligible, in view of the fact that the usual vaccine contains several thousands of bacilli per field. These tests were made on two healthy men. The results are given in Table 1. There, and in succeeding tables, the "M-vaccine" is the standard one of Mitsuda, the "U-vaccine" the one produced by treatment with the ultra-supersonic wave.

Though in individual H.K. the U-vaccine gave the stronger reaction on the 4th and 6th days, after the 8th day the Mitsuda reaction became the stronger, until its infiltration was twice the size of that of the other. In F.H. both reactions were markedly stronger than those of H.K. in the first week, during which period the U-vaccine reaction was very strong. After the 8th day, however, the Mitsuda reaction grew stronger, and the two individuals came to give quite similar readings.

TABLE 1.—*Reactions produced in two healthy persons by the Mitsuda vaccine and by one treated by ultra-supersonic waves but still containing acid-fast bacilli.*

Person	Antigen [a]	Readings (in mm.) on days indicated [b]						
		2	4	6	8	15	20	31
F.H.....	M-V	3	4	5	5	8	7	7
		(25)	(16)	(15)	(15)	Pus	—	—
	U-V	5	5	3	3	4	4	4
		(40)	(25)	(6)	(4)	—	—	—
H.K.....	M-V	4	1	1	3	8	8	7
		—	(3)	(3)	(4)	Pus	—	—
	U-V	3	3	3	2	4	3	4
		—	—	—	(3)	—	—	—

[a] M-V = Mitsuda vaccine; U-V = vaccine treated with ultra-supersonic waves.
[b] First figures refer to infiltration, those in parentheses to erythema.

Even though the U-vaccine contained all of the bacillary elements, the primary skin reaction produced by it became weaker in the later days. This is in clear contrast with the true Mitsuda reaction, which does not appear until after eight days. The reactions in the first five days are allergic in nature, probably with a traumatic element. In work with filtrates of the Mitsuda vaccine, F. Hayashi found that reactions caused by it are strong only in cases in which the Mitsuda reaction itself is strong. The fact that at the beginning the U-vaccine reaction in F.H. was much stronger than the Mitsuda reaction indicates that he is allergic. He is much more so than H.K.

The early reaction with the U-vaccine evidently depends upon the dissolved bacillary substances. At first we were inclined to attribute this effect to an irritating factor, but after succeeding in making a vaccine that did not cause pain we found it still to give the stronger primary reactions.

A question with respect to this experiment is why there was not more difference between the two reactions in F.H., considering the fact that the Mitsuda vaccine contains innumerable bacilli and U-vaccine only three to five in an oil-immersion field. Two rea-

sons are to be considered: (*a*) As is known, the Mitsuda reaction is, so to speak, an "all or nothing" reaction, not greatly influenced by variations in the numbers of bacilli in the antigen. In the reactive condition of tuberculoid leprosy known as "akuter Schub," almost equally strong reactions are caused by the intracutaneous injection of 0.1 cc. of the Mitsuda vaccine and of only a single drop. In F.H. the Mitsuda reaction has been very strong for a long time, and this is the reason why he showed almost equal reactions to the two antigens, while in H.K. the reaction of the U-vaccine was much weaker than the Mitsuda reaction. (*b*) It is also necessary to consider whether or not the elements of the destroyed bacilli which cannot pass through a filter can cause a positive reaction, even though their acid-fastness is lost. This question will be dealt with shortly.

REACTIONS WITH COMPLETELY DESTROYED BACILLI

As has been said, it is very difficult to prepare a U-vaccine without acid-fast bacillary particles, and Inoué succeeded in doing so only after four months of work. With this preparation four healthy men and eleven patients were tested.

Results in healthy persons.—The reactions in the four healthy persons tested, shown in Table 2, were essentially similar. In general, the reaction to the U-vaccine was at first stronger than that to the Mitsuda reaction, a response ascribed to the soluble bacillary elements. With the passage of days, however, the infiltration of the Mitsuda reaction became the stronger, while that of the U-vaccine became weaker. In two instances the U-vaccine reactions increased again to reach a maximum in the third week, as did the Mitsuda reactions; this is absolutely different from what happens with a filtrate.

From these results it is evident that the U-vaccine without acid-fast bacillary elements can cause a positive Mitsuda reaction, though not as strong as does the original vaccine. This vaccine is turbid and contains bacillary particles which are neither acid-fast nor stainable by methylene blue. Notwithstanding this fact, these particles do not lose the property of causing the Mitsuda reaction. A similar observation was made by F. Hayashi, who experimented with a vaccine composed of leprosy bacilli that had been rendered nonacid-fast by hydrochloric acid. It was found to be capable of causing positive reactions, though not as strong as those due to the original antigen. On that occasion he suspected that the numbers of bacilli had decreased

during the washing after treatment with the acid, which might explain the weaker reactions. In our experiment it was certain that there had been no actual loss of bacilli, yet the reaction was weakened through their destruction and loss of acid-fastness.

TABLE 2.—*Reactions produced in four healthy persons by the Mitsuda vaccine and by one treated by ultra-supersonic waves, without acid-fast forms.*

Person	Antigen [a]	Readings (in mm.) on days indicated [b]							
		1	2	3	5	8	13	17	27
T.M....	M-V	5	5	6	10	8	7	15	17
		(15)	(23)	(20)	(17)	(13)	Pus	Ulc.	Ulc.
	U-V	7	7	12	5	4	4	5	7
		(25)	(33)	(30)	(30)	(25)	—	—	—
T.I....	M-V	4	7	6	7	8	7	9	12
		(10)	(14)	(15)	—	—	—	—	—
	U-V	5	3	3	4	3	3	4	8
		(30)	(30)	(30)	—	—	—	—	—
M.M....	M-V	3	7	8	10	8	7	10	15
		—	(20)	(15)	(4)	—	—	—	—
	U-V	3	5	12	4	4	3	3	0
		—	(35)	(25)	—	—	—	—	—
T.F....	M-V	4	7	3	4	..	7	6	7
		—	(7)	(?)	(7)		—	—	—
	U-V	7	7	6	3	..	3	2	3
		—	—	(8)	—		—	—	—

[a] M-V = Mitsuda vaccine; U-V = vaccine treated with ultra-supersonic waves.
[b] First figures refer to infiltration, those in parentheses to erythema.

Because the erythema produced by both vaccines in the fourth individual (T.F.) was so much less than in the others, we wondered if there might be some relation with the tuberculin reaction, and the Mantoux test was therefore applied to these individuals. No correlation with the initial vaccine reaction was found.

Results in leprosy patients.—The reactions obtained in the eleven patients tested—seven of the lepromatous type, two macular ("tuberculoid") and two neural—are given in Table 3. It is seen that, as is usual in the lepromatous form of the disease, the Mitsuda reaction was negative in all cases of that type. The lesions produced by the injection never persisted after the 8th day and usually disappeared before that. The reactions caused by the U-vaccine usually cleared up somewhat earlier. In the neural-type cases and healthy persons the U-vaccine reactions became reduced later, when the Mitsuda reaction was becoming stronger. The last neural case, patient S.F., showed a remarkably strong initial reddening in the U-vaccine reaction.

TABLE 3.—*Reactions produced in eleven leprosy patients by the Mitsuda vaccine and by one treated by ultra-supersonic waves without acid-fast forms.*

Patient	Antigen [a]	Readings (in mm.) on days indicated						
		2	4	6	7	8	14	22
Lepromatous cases								
F.M.	M-V	4	4	2	2	0	0	0
	U-V	4	3	0	0	0	0	0
F.D.	M-V	4	3	2	2	1	0	0
	U-V	3	4	2	0	0	0	0
Y.K.	M-V	4	3	3	3	2	0	0
	U-V	6	3	3	3	2	0	0
K.M.	M-V	4	4	2	0	0	0	0
	U-V	4	4	0	0	0	0	0
Y.E.	M-V	1	1	0	0	0	0	0
	U-V	3	1	0	0	0	0	0
S.T.	M-V	3	0	0	0	0	0	0
	U-V	3	0	0	0	0	0	0
Y.T.	M-V	3	0	0	0	0	0	0
	U-V	4	1	0	0	0	0	0
Macular cases								
K.C.	M-V	2	2	3	3	3	7	7
	U-V	4	3	3	3	3	2	0
O.Y.	M-V	1	1	2	3	3	5	4
	U-V	2	1	1	1	1	1	0
Neural cases								
K.K.	M-V	4	4	3	4	4	4	4
	U-V	7	4	5	6	3	3	3
S.F.	M-V	4	4	4	4	4	4	6
	U-V	25	20	20	20	15	3	3

[a] M-V = Mitsuda vaccine; U-V = vaccine treated with ultra-supersonic waves.

CONTROL EXPERIMENTS

Comparison of raw and boiled ultra-supersonic wave vaccines.—In comparative tests with raw and boiled Mitsuda vaccines, F. Hayashi found no difference in the skin reactions produced. A similar comparative experiment has been made with raw and boiled U-vaccines, though it is to be said that these preparations reach relatively high temperatures during treatment with the ultra-supersonic wave. This experiment was made on the healthy person F.H. The vaccines were of the incompletely destroyed type. Though there were slight differences at the beginning, the final reactions were almost equal.

Reactions with a filtrate of the ultra-supersonic wave vaccine.—Because filtrates of the Mitsuda vaccine had been found by F. Hayashi to have no ability to cause the Mitsuda reaction,

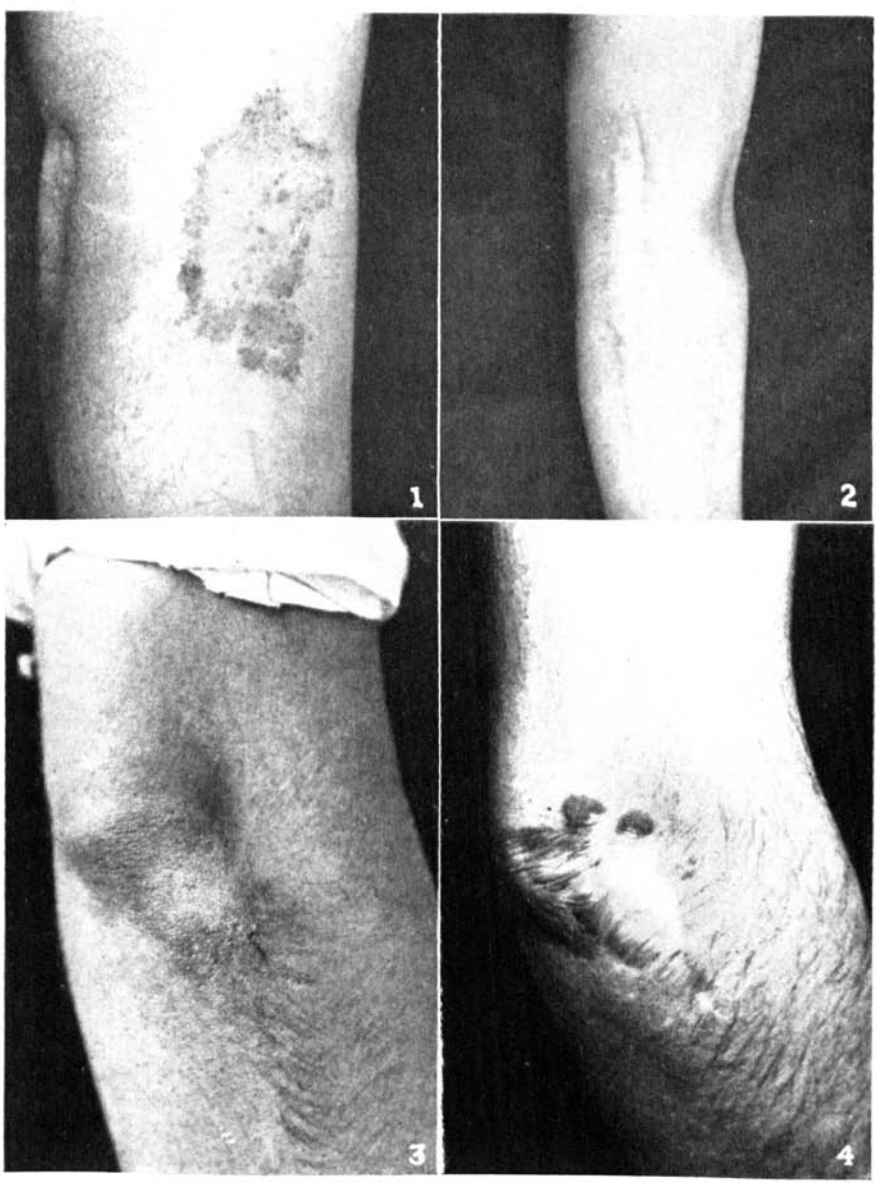

Plate 2

PLATE 3

FIG. 5. Large tuberculoid patch on the back near the shoulder, Case 19.

FIG. 6. The scar in Case 19, resulting from electrocoagulation, as seen fourteen months afterward. No lesion elsewhere on the body.

FIG. 7. Tuberculoid lesion of the forearm, Case 20. It is possible to see the enlarged nerve branch, in relief, in the interior part of the region (see Fig. 8).

FIG. 8. The appearance 2 years and 5 months after electrocoagulation of the patch and surgical extirpation of the affected nerve. No lesion elsewhere on the body.

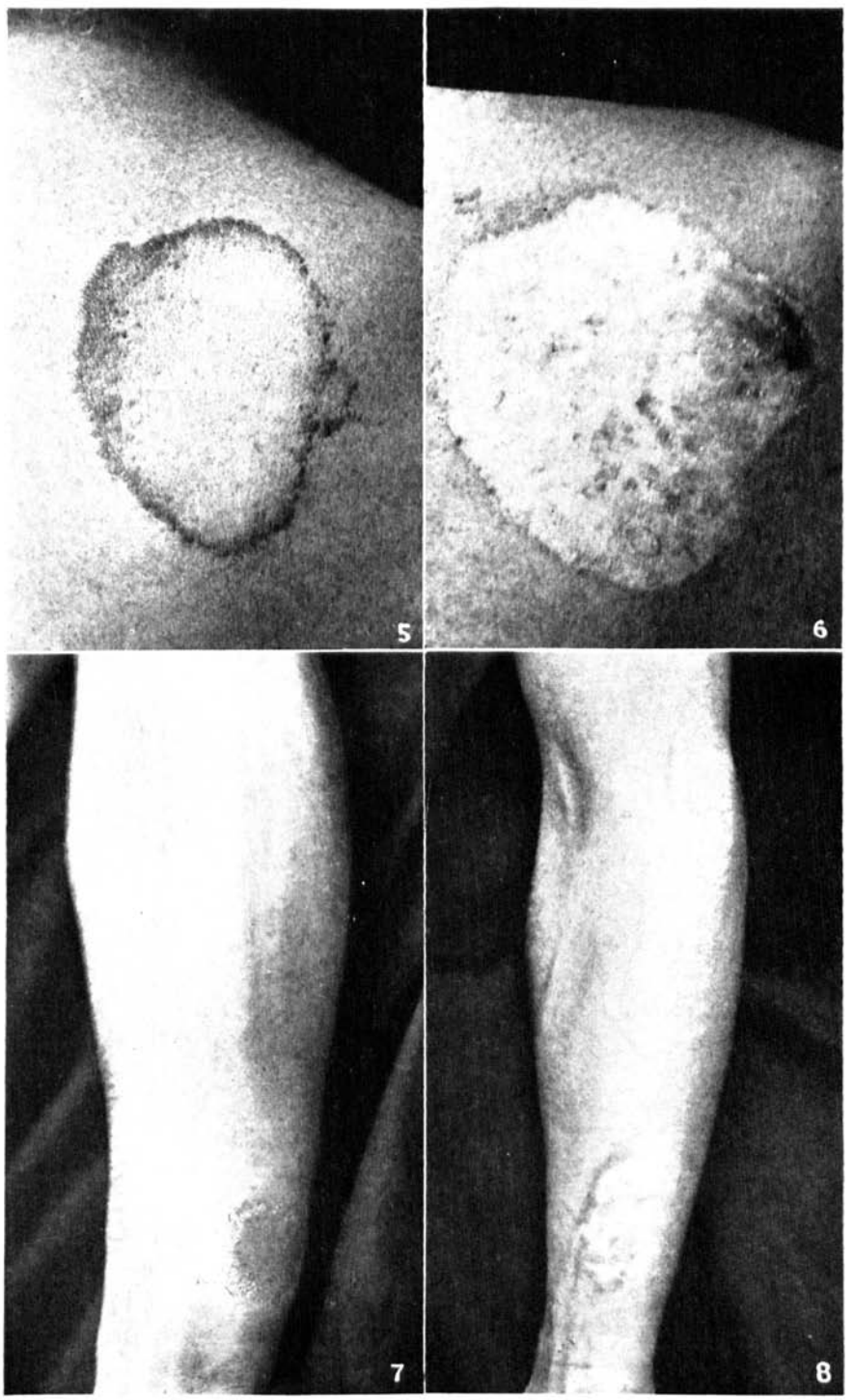

Plate 3

an experiment was made to see if that held true of a U-vaccine filtrate. Healthy persons H.K. and F.H. were used. There was not much difference between the two, except with regard to the degree of reddening and infiltration within 30 minutes after injection. On the third day no sign of a reaction remained. This shows that though the whole U-vaccine may cause positive, if somewhat weakened, Mitsuda reactions, its soluble elements alone are unable to do so. The same results were obtained with filtrates of raw and boiled U-vaccines. It is absolutely necessary for the production of the reaction that the antigen contain solid bacilli elements, even though they may be nonacid-fast.

U-VACCINE OF RAT LEPROSY

Hayashi found, in 1930, that vaccines made of other acid-fast bacilli differed from those made of leprosy bacilli in that they cause positive reactions in lepromatous as well as neural cases. Though sometimes in advanced lepromatous cases these reactions are relatively weak, they are not actually negative. Kawamura later found that a vaccine made from rat lepromas also gave positive reactions in both types of leprosy.

We have experimented with a U-vaccine made from a rat leproma, in comparison with the original rat leprosy vaccine not so treated. Six cases were tested, three lepromatous and three macular. The results are shown in Table 4.

TABLE 4.—*Reactions produced by a rat leproma vaccine and by one treated by ultra-supersonic waves, in six patients.*

Patient	Antigen [a]	Readings (in mm.) on days indicated							
		2	4	6	8	15	22	29	36
Lepromatous cases									
Y.H....	R-V	3	3	3	3	6	5	5	5
	UR-V	3	3	2	2	1	1	0	0
S.T.....	R-V	2	4	5	6	5	5	3	3
	UR-V	4	0	0	0	0	0	0	0
Y.T.....	R-V	6	10	11	12	15[b]	10	8	7
	UR-V	4	3	3	3	2	1	1	1
Macular cases									
O.Y.....	R-V	2	2	3	4	6	4	4	4
	UR-V	4	1	2	3	2	2	1	1
K.C.....	R-V	5	3	4	5	10	7	6	3
	UR-V	7	5	5	4	4	3	2	2
O.K.....	R-V	2	2	4	8	10	12[b]	7	7
	UR-V	2	2	0	0	0	0	0	0

[a] R-V = Rat leproma vaccine; UR-V = vaccine treated with ultra-supersonic waves.
[b] Suppuration.

The first thing to be noticed is that there were no marked differences in the reactions in the two classes of cases tested. With regard to the comparison of the two antigens, the U-vaccine, like similar preparations of Hansen's bacilli, is still capable of producing reactions despite the loss of acid-fastness, but these reactions become weaker at the time that those due to the original vaccine are becoming stronger.

ERYTHEMA NODOSUM LEPROSUM AND THE U-VACCINE

One of the most distressing complications in leprosy is erythema nodosum leprosum, the so-called lepra reaction in the lepromatous type. In this condition the bacilli in the tissues are undergoing destruction, and it is generally agreed that the destroyed bacillary element has some direct or indirect relation to the disturbance. A question, suggested by Mitsuda, is whether or not the U-vaccine has the capacity of inducing this condition.

To investigate this matter we made skin tests with a U-vaccine (one that contained five or six bacilli per oil-immersion field) in cases of erythema nodosum leprosum and, for comparison, in resolved lepromatous cases which had not experienced that condition. Irregular reactions were obtained in the first week in the erythema nodosum cases, evidently dependent upon that condition and its fever, but no disturbance was caused in the control cases.

As a further experiment in this connection, sera from lepromatous patients were added to a U-vaccine to see if the mixture would induce erythema nodosum leprosum. As a control, salt solution was added in the same proportion to another lot of the same material. Both preparations gave negative results.

Later, T. Maeda applied the U-vaccine in the treatment of leprosy, injecting it locally and subcutaneously in 30 patients representing both the neural and lepromatous types. During a period of some months no sign of erythema nodosum leprosum was seen.

SUMMARY AND DISCUSSION

The purpose of the work here reported was to determine whether or not the Mitsuda reaction can be produced by leproma emulsions in which the bacillary elements have been destroyed, at least to the point of loss of acid-fastness, by application of ultra-supersonic waves. Vaccines so prepared, it has been found, retain their antigenic capacity, though it is much weak-

ened. They are similar to the standard Mitsuda antigen in that they fail to cause positive reactions in lepromatous cases, while neural-type cases and healthy persons react positively. The quality of the U-vaccine is not affected by heating.

In view of the fact that all of the components of the bacilli remain in the U-vaccine, the weakening of the reaction indicates that it depends upon the presence of the formed leprosy bacilli themselves. This is further indicated by the fact that a filtrate of the U-vaccine is incapable of producing positive reactions, though it must contain all of the soluble bacterial substances.

On the other hand, it is also a fact that the small numbers of bacilli present in the U-vaccine can, in some healthy persons and maculo-neural cases, cause reactions approximately as strong as those induced by the Mitsuda antigen itself. The fact that few bacilli can cause strong reactions has already been proved with diluted Mitsuda vaccine in cases of "akuter Schub." The same results have been obtained with the U-vaccine containing small numbers of bacilli that are losing their acid-fastness.

Why can the U-vaccine cause the Mitsuda reaction, despite the fact that it may contain nothing which can be stained by methylene blue or by the Ziehl-Neelsen method? It is a fact that this preparation is turbid, though of course much less so than the original Mitsuda vaccine. This turbidity is due to a mass of fine bacillary particles that are not acid-fast. These particles still retain the capability of causing the Mitsuda reaction, though of much weakened grade. This agrees with Hayashi's findings with leprosy bacilli treated with hydrochloric acid.

The fact that, contrary to our expectations, the filtrate of this vaccine does not cause the same reaction as the unfiltered substance is further evidence of the essential nature of the Mitsuda reaction. Comparing this filtrate and one of the Mitsuda vaccine, the former, in the first several days, caused stronger reactions than the latter in healthy and neural persons, which fact we ascribe to the presence of dissolved components of the bacilli. This character of the filtrate is not influenced by heating.

Rat leprosy bacilli from rat lepromata are influenced by the ultra-supersonic wave in the same way as are human leprosy bacilli. The destroyed bacillary elements, which have lost their acid-fastness, have the original capacity of producing positive reactions in all types of leprosy, though as before this property is weakened.

Erythema nodosum leprosum, which appears in the lepromatous type of the disease when the bacilli are destroyed, chiefly by chaulmoogra oil, is not induced by the U-vaccine, whether given intracutaneously or subcutaneously.

CONCLUSIONS

1. This study represents an effort to learn more exactly the nature of the Mitsuda reaction, using a leproma vaccine treated by ultra-supersonic waves to dissolve the bacilli. This vaccine, without acid-fast particles, has the same capacity as the whole leprosy bacilli to produce that reaction.

2. It is a fact, however, that the vaccine so treated, though it contains all of the components of the original preparation, gives weaker reactions than the latter.

3. On the other hand a filtrate of the U-vaccine is not capable of causing the Mitsuda reaction.

4. In order to produce that reaction it is absolutely necessary that the antigen shall contain the solid components of the leprosy bacilli, even though they are destroyed to the point of losing their acid-fast character.

5. Rat leprosy vaccine is influenced in the same way as the human material by treatment with the ultra-supersonic wave.

6. No direct relationship has been found between erythema nodosum leprosum and the injection of the ultra-supersonic vaccine, with or without the addition of sera of lepromatous patients.

REFERENCE

(1) HAYASHI, F. Mitsuda's skin reaction in leprosy. Internat. Jour. Lep. I (1933) 31-38.

DESCRIPTION OF PLATE

PLATE 4

FIG. 1. Reactions in healthy person F.H. (see Table 1). The upper, more marked reaction is due to the usual Mitsuda vaccine, the lower one (S.S.) to ultra-supersonic vaccine. Note that the latter reaction is itself a strong one. Photograph made on the 37th day after injection.

FIG. 2. Reactions in healthy person H.K. The two upper lesions were caused by the Mitsuda vaccine, the lowest one (S.S.) by the ultra-supersonic vaccine. Note that the last is weak, in comparison with that produced in the first person. Photographed on the 37th day after injection.

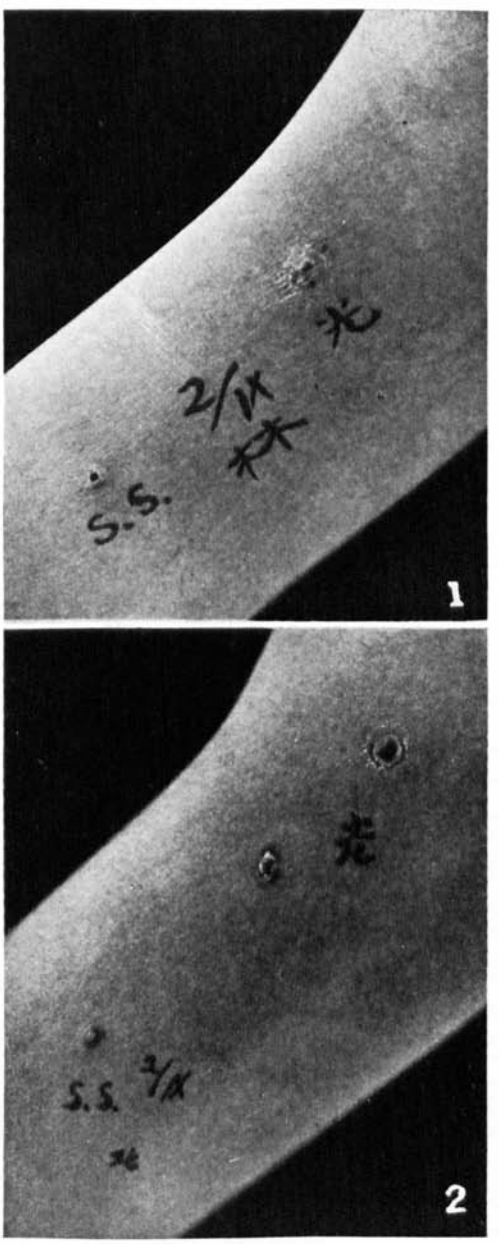

PLATE 4