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ON THE VALUE OF A SKIN REACTION TO A SUSPENSION OF LEPROUS NODULES¹

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Attempts have been made to make and apply diagnostic or therapeutic preparations from leprous nodules. Babes believed that he had obtained a tuberculin-like product, leprin, by glycerin-bouillon extraction of leprosy material obtained at autopsy. However, he himself, Klingmüller and Scholtz made check tests and denied its specific nature. Sugai reported failure to obtain, by means of a suspension of leprous nodules, specific reactions which would be equivalent to Pirquet's reaction to tuberculin in tuberculosis. Meanwhile, Murata reported that the injection of a lepra-bacillus suspension sterilized by boiling revealed some value in the diagnosis of leprosy.

Lately, Sakagami prepared a diagnostic suspension of cultured lepra bacilli. He injected this preparation intracutaneously in *L. tuberosa* and *L. nervosa* cases and obtained luetin-like reactions with reddening, swelling and pustule formation. He insisted on the diagnostic value of his preparation, because the reactions in nonleprosy cases were negative.

Y. Hayashi of our institution, Zensho-en, also prepared an inoculation suspension of cultured and probably multiplied lepra bacilli and tried it for a skin test on lepers. The results were positive, especially in the *L. nervosa* cases.

It is a very important matter to determine the true value of the materials made by these investigators. If such a preparation should have a definite value in diagnosis, it would give us an easy way to identify uncertain clinical cases in the diagnosis of leprosy.

In the preparation of a diagnostic material it would perhaps be best to use artificially cultured lepra bacilli. At present,

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however, it is quite difficult to culture large numbers of pure lepra bacilli. Therefore, I employed the following procedures, using leprous nodules.

Preparation No. 1.—After grinding the leproma, physiological saline solution was added in the proportion of 10 cc. per gram; the upper part of the suspension was taken and heated at 60°C. for 30 minutes; and carbolic acid, 0.5 per cent, was added.

Preparation No. 2.—The nodule, in saline solution in the proportion of 20 cc. per gram, was boiled for 2 hours; the nodule was then ground, and suspended in ten times as much saline solution; 0.5 per cent carbolic acid was added.

Preparation No. 3.—This was prepared from the boiled solution remaining after preparation No. 2 was made, 0.5 per cent carbolic acid being added to it.

Control solution.—For this I used physiological saline solution to which 0.5 per cent carbolic acid was added.

There were many lepra bacilli in preparations 1 and 2. In No. 3 there were very few of them. All of the preparations were set aside for more than 24 hours, after which contamination by other bacteria was carefully checked before using them for tests.

After disinfection by alcohol of the skin on the inner surface of the left arm, free from leprous infiltration, 0.05 to 0.07 cc. of each preparation was injected with a luetin syringe, so that each injection produced a pale, flat, rounded elevation. The injections were spaced from 3 to 4 cm. apart.

The total number of cases investigated was 403. By form of the disease, 279 of them were *L. tuberosa* cases, of which 225 were males and 54 were females; and 124 cases were *L. nervosa* and *L. maculosa*, of which 79 were males and 45 were females.

The reactions were observed 24 hours after inoculation. They were classified by degrees into three classes, strong, moderate and weak. Strong meant a reaction eruption more than 2 cm. in diameter; moderate meant 1 to 2 cm.; and weak, less than 1 cm.

FREQUENCY AND DEGREE OF REACTION IN *L. TUBEROSA*

In the *L. tuberosa* cases, observation of the inoculation

TABLE 1.—*L. tuberosa*, 279 cases, both sexes.

Degree of reaction	Preparation No. 1		Preparation No. 2		Preparation No. 3	
	Cases	P. c.	Cases	P. c.	Cases	P. c.
Strong	3	1.1	1	0.4	0	0
Moderate	25	9.0	6	2.1	1	0.3
Weak	208	74.5	89	31.9	7	2.5
Negative	43	15.4	183	65.6	271	97.2
Total	279	100.0	279	100.0	279	100.0

(Note: Control injections all negative.—EDITOR.)

lesions after 24 hours revealed that preparation No. 1 always gave slightly stronger reactions than the others, causing slightly elevated eruptions with slight reddening, and sometimes with pustule formation (several cases).

As shown in Table 1, preparation No. 1 caused the strongest reactions and only 15 per cent were negative, while with preparation No. 2 more than 65 per cent were negative and with preparation No. 3 more than 96 per cent. The last one seemed to have no value as a diagnostic material. Incidentally, the male group showed a slightly lower positive rate than the female group, but practically they were much the same.²

The reactions in these *L. tuberosa* cases were almost all absorbed after 2 to 3 days, without traces. However, in a few cases the reactions continued more than one week and left brown pigmentations. Table 4 shows that 5 per cent of the inoculations with preparation No. 1 and 3 per cent of those with preparation No. 2 caused residual or late reactions. In these cases there were no progressive symptoms of leprosy, and most of them were subsided cases. On the contrary, in progressive cases in which there were large numbers of new nodules no reactions occurred, or if they occurred they were very weak and disappeared in a short time. That was quite different from what occurred in the *L. nervosa* cases, as will be seen.

TABLE 4.—*L. tuberosa*, 279 cases, both sexes, after 20 to 24 days.

Degree of reaction	Preparation No. 1		Preparation No. 2		Preparation No. 3	
	Cases	P. c.	Cases	P. c.	Cases	P. c.
Strong	0	0	0	0	0	0
Moderate	2	0.7	3	1.1	0	0
Weak	13	4.7	6	2.1	1	0.4
Negative	264	94.6	270	96.8	278	99.6
Total	279	100.0	279	100.0	279	100.0

(Note: Control injections all negative.—EDITOR.)

² The data for the males and the females were given in Tables 2 and 3, which are omitted for consideration of space, the statement in the text being adequate.—EDITOR.

FREQUENCY AND DEGREE OF REACTION IN *L. NERVOSA*
AND *L. MACULOSA*

As compared with the results obtained in *L. tuberosa*, those obtained in *L. nervosa* and *L. maculosa* were very marked and the reactions remained for longer periods. Some of the reactions occurred directly after inoculation and produced urticaria-like lesions. In one case the diameter of the reaction to preparation No. 2 reached 3 cm. and became hemorrhagic. However, no case showed pustulation within 24 hours, or had fever. In some of the cases the eruptions caused by the three preparations conglomerated together, with subcutaneous infiltration, appearing as phlegmons. But there were no cases of pustule formation within several days after inoculation.

While in *L. tuberosa* the reactions to preparation No. 3 were very weak, giving only a little more than 3 per cent positives, on the contrary in *L. nervosa* 29 per cent of positive reactions were obtained. [Table 5.] Reactions to preparations Nos. 1 and 2 were also very strong and marked, especially with the latter. With No. 2 four-fifths of the cases were positive, while in *L. tuberosa* only one-third were positive.

TABLE 5.—*L. nervosa* and *L. maculosa*, 124 cases, both sexes.

Degree of reaction	Preparation No. 1		Preparation No. 2		Preparation No. 3	
	Cases	P. c.	Cases	P. c.	Cases	P. c.
Strong	28	22.6	12	9.7	4	3.2
Moderate	37	29.8	35	28.2	12	9.7
Weak	49	39.5	52	41.9	19	15.3
Negative	10	8.1	25	20.2	89	71.8
Total	124	100.0	124	100.0	124	100.0

(Note: Control injections all negative.—EDITOR.)

With No. 1 the positive reaction rate was the highest, as it was in *L. tuberosa*. While in *L. tuberosa* only about 10 per cent of the reactions to it were strong or moderate, in *L. nervosa* 52 per cent were strong or moderate. With No. 2 there were only 2.5 per cent strong or moderate reactions in *L. tuberosa*, while *L. nervosa* gave 38 per cent such positives.

As for the comparison of the male and female groups, it has been said that among the *L. tuberosa* cases the female group had a slightly higher rate of positive reactions, and in

L. nervosa that difference was more marked. With No. 1, the rates of nonreaction were 11.4 per cent in the male group and 2.2 per cent among the females; the rates of strong and moderate reactions were 44.3 per cent in the male group and 62.6 per cent in the females. In the case of No. 2, the subtotal rate of strong and moderate reactions was only 26.3 per cent in the males and 57.7 per cent in the females.³

In consideration of these results, it is clear that reactions were milder in *L. tuberosa* than in *L. nervosa*, and were stronger in the female group than in the male group.

It should be noted that reactions in *L. nervosa* lasted for weeks or even months. In the case of *L. nervosa*, in which the injections were made on 17th March, reddening, swelling and itching remained even in the middle of April, and squamous epithelium desquamated repeatedly. In May one of the eruptions became pustular, while the others became indurated and still remained as small, red eruptions the size of a rice grain.

In eight cases the reactions did not appear until several weeks after injection, except for tiny eruptions, and then the reactions became gradually stronger and finally pustules were formed. Meanwhile, of course, there were cases which developed rapid and strong reactions with reddening and swelling directly after inoculation, and within one week the initial eruption became gradually dark red and absorbed, leaving dark brown pigmented lesions.

The results observed after 20 to 24 days in *L. nervosa* are shown in Table 8/9.⁴ It shows that there were a great many cases which showed late reactions and continuance of reactions. Most of the cases showed the strongest reaction on the fourteenth day after inoculation. Sometimes, the top of the eruption lesion became softened, the epidermis thinned, and the lesion broke down to leave an ulcer with a pus-like substance in which could be found leucocytes, epithelioid cells and lepra bacilli.

In *L. nervosa* the frequency of late reactions and continuance of existing lesions was very high; while only 3 to 5 per cent of cases in *L. tuberosa* revealed late reactions, in *L. ner-*

³ The data for males and females were given in Tables 6 and 7, omitted here for reasons previously stated.—EDITOR.

⁴ In this instance the original article had two tables, for the males and females, with none for the total group. The original data are given in this combined table, and a section with the total figures has been added.—EDITOR.

TABLE 8/9.—*L. nervosa* and *L. maculosa*, after 20 to 24 days;
79 male cases, 45 female cases, and totals.

Degree of reaction	Preparation No. 1		Preparation No. 2		Preparation No. 3	
	Cases	P. c.	Cases	P. c.	Cases	P. c.
<i>Males, 79 cases</i>						
Strong	2	2.5	9	11.4	0	0
Moderate	25	31.7	31	39.2	0	0
Weak	21	26.6	15	19.0	11	13.9
Negative	31	39.2	24	30.4	68	86.1
Total	79	100.0	79	100.0	79	100.0
<i>Females, 45 cases</i>						
Strong	2	4.4	10	22.2	0	0
Moderate	14	31.1	17	37.8	0	0
Weak	17	37.8	8	17.8	5	11.1
Negative	12	26.7	10	22.2	40	88.9
Total	45	100.0	45	100.0	45	100.0
<i>Total, 124 cases</i>						
Strong	4	3.2	19	15.3	0	0
Moderate	39	31.5	48	38.7	0	0
Weak	38	30.6	23	18.6	16	12.9
Negative	43	34.7	34	27.4	108	87.1
Total	124	100.0	124	100.0	124	100.0

vosa I found late reactions in 60 to 77 per cent. Preparation No. 2 gave more severe reactions than No. 1; for instance, No. 1 gave eruptions 1 cm. in diameter and No. 2, 1.5 to 2 cm. In consideration of the general run of the positive reactions, this fact should be understood as a proper characteristic of the No. 2 preparation.

From the results obtained in many leprosy cases, it may be concluded that, in the case of *L. nervosa*, preparation No. 2—a suspension of boiled leprosy nodules—gives stronger and more numerous slow or prolonged reactions than the other preparations used.

HISTOLOGICAL FINDINGS IN THE LESIONS
DEVELOPED AFTER INOCULATION

Since dead tubercle bacilli cause histological reactions similar to the tuberculous process, we may well forecast the histological findings in the lesions caused by the inoculation of leprosy material. In order to investigate the histological picture, examinations were made of paraffin sections of inoculation lesions removed from three cases of *L. nervosa*.

CASE 1.—K. Onoma. *L. nervosa*; age 27; 14 years after onset of leprosy. On the 28th day after inoculation the reactions to Nos. 1 and 3 were weak, that to No. 2 moderate positive. The inoculation lesion of No. 2 was biopsied. The stratum corneum was proliferated, the papillae had disappeared, there was diffuse infiltration in the subepidermal stratum, and in the deeper, intermediate levels there was multiplication of epithelioid cells with foreign-body giant cells, and here individual lepra bacilli were found within the epithelioid cells. That part of the multiplied internal strata corresponded with the softened area of the nodule, around which infiltration was manifest extending down to the subcutaneous fatty tissue, where cell-nuclei were mostly atrophied and destroyed. No plasma cells were found in the infiltrate.

CASE 2.—K. Hirose. *L. nervosa*; age 22; 13 years after onset. On the 27th day after inoculation the reaction to No. 1 was moderate, that to No. 2 was strong, and that to No. 3 was weak. The reaction lesion of No. 2 was investigated. Multiplication of leucocytes, lymphocytes, star-shaped cells and histiocytes was manifest, from the subepidermal stratum to the subcutaneous fatty tissue. Although there was multiplication of mast cells, there were no plasma cells. A few lepra bacilli were found within the epithelioid cells in the intermediate levels. Several giant cells were found among the epithelioid cells. No lepra bacilli were found within them.

CASE 3.—T. Uno. *L. nervosa*; age 28; 10 years after onset. On the 28th day after inoculation the reactions to Nos. 1 and 2 were strong, and that to No. 3 was negative. The reaction lesion of the No. 2 inoculation was studied microscopically. Multiplication of epithelioid cells and giant cells in the intermediate levels of the skin. Both foreign-body and Langhans' giant cells were found. Lepra bacilli were rare, and stained very weakly. The infiltration in the cutis was diffuse and very marked; the cell-nuclei were mostly in degenerated forms.

A second specimen was taken from this case about six months later. It was a nodule the size of a rice grain which had been a strong reaction caused by the No. 1 preparation and had gradually been absorbed. There was no longer any infiltration in the superficial stratum of the skin, and the elastic fibers were reproduced. In the intermediate stratum there was a group of epithelioid cells and giant cells which seemed to be atrophied. No lepra bacilli were identified. In the subcutaneous tissue and around the blood vessels there were slight infiltrations.

In consideration of the findings in these four specimens taken from three cases, it was concluded that preparations Nos. 1 and 2 caused the same tissue reaction. The reaction process was at first an acute inflammation which gradually trans-

formed into a chronic inflammation, with the production of epithelioid and giant cells. The relationship of the inoculated lepra bacilli and the cells was quite similar to what is seen in the macular lesion of *L. maculosa*, which shows tuberculoid tissue with few lepra bacilli and much production of epithelioid cells.

Whether such tuberculoid tissue is peculiar to *L. nervosa*, or if such a reaction can be caused in animals by suspensions of lepra bacilli, should be determined by further investigation. From the results here reported it would seem that the skin reaction induced by a suspension prepared from the leprous nodule causes a quite specific reaction. However, if such a reaction is observed in nonleprous persons, its diagnostic value will be almost lost.

TABLE 10.—Results of inoculations in nonleprous children.

Name	Age	Period of isolation, years	Results of inoculation					
			24 hours			33 days		
			No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
N. K.	8	4	+	+	—	+	<i>P</i>	—
T. S.	8	4	+	+	—	+	<i>P</i>	—
F. A.	7	4	—	—	—	+	+	—
M. H.	6	4	+	+	—	—	+	—
T. I.	4	2	+	—	—	+	+	—
S. O.	5	2	—	—	—	—	—	—
K. O.	4	2	+	+	—	—	+	—
K. T.	4	2	+	—	—	—	+	—

Remarks: + is positive, — is negative, and *P* means pustule formation.

(Note: The control injections were negative throughout.—EDITOR.)

REACTIONS IN NONLEPROUS CHILDREN

To investigate this point, and also in search of a method to detect latent leprosy as is done in the case of congenital syphilis, I tested eight children who were born of leprous parents and who clinically had not yet shown evidence of suffering from leprosy. These children had lived among lepers for from 2 to 4 years, and had been separated or isolated for from 2 to 4 years. They were inoculated with all four test preparations.

Making allowance for their age only a very small amount of the materials, 0.03 cc., was injected into the skin.

As is shown in Table 10, only one case was absolutely negative. The results may indicate diagnostic value of the suspension of leprous nodules, but the reactions were generally weak, only tiny eruptions without much reddening around them; even when there was pustule formation, the infiltration around the tiny pustular eruption was slight. Persistence of the eruption was observed, as in the cases of *L. nervosa*. It is too early, however, to conclude that a diagnosis can be made on the basis of the presence or absence of this eruption, because there was one negative case out of the eight.

According to the observations of these untainted children, the degree of reaction seems to be proportional to the constitution. The single negative case is of very weak constitution, and is suffering from chronic intestinal catarrh.

REACTIONS IN NONLEPROUS ADULTS

Since I had reached the belief that inoculation of such preparations is harmless for nonleprous people, I applied this test to ten persons in the Tokyo Youikuin to study its specific nature.

TABLE 11.—Result of inoculations in nonleprous adults.

Name	Age	Diagnosis	Drug	Results of inoculations					
				24 hours			16 days		
				A	B	C	A	B	C
W.	57	Apoplexy	Potass. iodide	+	—	—	+	—	—
O.	84	Lumbago	Sod. bromide	—	—	—	—	—	—
S. Z.	48	Epilepsy	Potass. bromide	+	—	+	++	+	+
K.	57	Epilepsy	Potass. bromide	—	—	—	D
N.	67	Apoplexy	Cardiac tonics	+	—	+	—	—	—
A.	41	Apoplexy	Antidiarrhea	—	—	—	—	—	—
H.	75	Pulmonary emphysema	Potass. bromide	++	+	+	++	—	—
S. R.	65	Apoplexy	Potass. bromide	—	—	+	+	—	—
Y.	21	Spina bifida	Potass. iodide	++	—	+	+	+	+
T.	66	Ischias	(?)	+	—	+	D

Remarks: A = preparation No. 2; B = preparation No. 3; C was prepared as follows: Nodules were dried and heated at 130°C. for 40 minutes and ground, and a 10 per cent suspension was made with 0.5 per cent carbolic acid added. ++ means strongly positive; + means positive; — means negative; and D means that the patient had died.

(*Note:* The controls were all negative, as always.—EDITOR.)

As shown in Table 11, preparation C (the description of which is given with the table) gave the most numerous 24-hour reactions, but most of these reactions gradually disappeared. In the case of preparation No. 2, the reactions became weak after a few days but were again strong by the end of two weeks, and then after the third week they gradually weakened. The reactions in these people were in general less strong than in cases of *L. nervosa*.

Since three of these cases (H., S.Z., and Y.) showed moderately strong reactions, one should be careful about making the diagnosis of leprosy on the basis of the result of this test.

DISCUSSION

Skin reactions induced by means of suspensions prepared from leprous nodules were positive in a large proportion of lepers. However, it was negative in some proportions—15 to 65 per cent in *L. nodosa*, and 8 to 21 per cent in *L. nervosa* and *L. maculosa*. Since it was sometimes positive in nonleprous cases, it has no absolute value for the diagnosis of leprosy.

It should be noted, nevertheless, that while in many cases of *L. tuberosa* the reaction was a temporary elevated eruption which gradually went into complete resolution in a few days or a few weeks, the reaction in *L. nervosa* was a prolonged one, and that the inoculation lesion sometimes became pustular. In nonleprous persons there were some positive cases which showed the chronic reaction, but they were milder than those in *L. nervosa*; the reaction was a chronic process without the severe manifestations seen in *L. nervosa*. It should therefore be noted, from the point of view of pathology, that a suspension of leprous nodule causes, especially in *L. nervosa*, a sensitivity reaction which shows a specific prolonged, chronic inflammation.

After all, with the exception of a few cases, the process of infection by the lepra bacillus in the human body will not develop nodules in the initial stage, and lepra bacilli which have invaded their favorite nerve tissues and skin will be encountered with reactionary inflammation. It has been acknowledged by many students of leprosy that macular lesions and initial thickenings of nerves show under the microscope only few bacilli and multiplication of histiocytes, lymphocytes and leucocytes together with giant cells, the appearance almost the same as in tuberculosis. Such an initial process will gradually, in weeks or months, go into the next stage in which the inflammation regresses, macules subside, and thickenings of nerve undergo

reduction. There have been many careless workers who have exaggerated and propagated such findings as signifying success of their antileprosy therapy measures. However, after some period of time, macules and thickening of nerve tissues will appear again. These symptoms will regress again within weeks or months. After such repetitions lepra bacilli will gradually multiply in the tissues and the initial tuberculoid changes can no longer be seen, and there will be symbiosis of both tissues and lepra bacilli. In that stage, the case may be considered as transformed to *L. tuberosa*, and as is usual in that form many lepra bacilli will be present.

It is therefore obvious that the reaction of such a case against injected lepra bacilli will be weak. On the contrary, in the case of *L. nervosa*, in which the occurrence of macules and nerve thickenings is repeated and in which the lepra bacilli are destroyed and the leprosy process is comparatively mild, the sensitivity against lepra bacilli will be strong, not only for a few living bacilli but also for dead bacilli, and even for the minimum number of bacilli introduced into the skin in our preparation No. 3.

Some of these cases developed even hemorrhagic inflammation as a result of the inoculation. Most of them gave chronic reactions, and, two or three weeks after the inoculation, proliferation of the body tissues occurred; infiltration of epithelioid cells, giant cells, lymphocytes were observed on microscopic examination, as in macules of *L. maculosa*. This is the reason why the reaction is much stronger than that in *L. tuberosa*. Especially in old cases of *L. nervosa*, in which the disease had been arrested, the macules had disappeared for more than ten years and it was clinically difficult to diagnose the disease, the reactions were the strongest.

Since we may identify lepra bacilli from the nasal cavity, the skin and other locations in *L. tuberosa*, we can diagnose it without difficulty. However, in many cases of *L. nervosa* we cannot find bacilli, and when we cannot find any thickening of the nerves or macules we have difficulty in making the diagnosis.

The Wasserman test and the cuorin-lecithin test are positive in *L. tuberosa*, but negative in *L. nervosa*. Clinically, lymphocytosis has been considered as a characteristic of *L. nervosa*, but that symptom is not absolutely specific. Therefore, one should employ every possible method in the diagnosis of *L. nervosa*. It should be borne in mind that the suspension

of leprous nodules generally gives strong reactions in *L. nervosa*, and this test is therefore regarded as one of the diagnostic methods.

It is still to be noted, however, that the test has no absolute value in diagnosis, for nonlepers may show positive reactions. The reason for the positive reaction in nonlepers may be that the inoculation acts as a foreign body, stimulating the sensitive histiocytes and leucocytes surrounding the network of small blood vessels in the cutis. As the luetin reaction is exaggerated by the oral administration of bromide and iodide preparations, I suppose that these drugs may have some influence upon this reaction.

Since nonleprous cases will give positive reactions, the test has little value in the determination of infection in untainted children, as we diagnose congenital syphilis by means of luetin test. However, it will be necessary to record all results of inoculations in such children, so that we may be able to observe their future course.

Whether or not it is harmless to inject the family members of lepers for the purpose of prevention of leprosy, is to be considered later. We must first observe whether or not repeated inoculations of such a sterilized bacillus suspension into cases of *L. nervosa* may cause an increase in the number of bacilli. If the procedure is harmless, then we may proceed with the step just indicated.