

## THE GOMEZ COMPLEMENT-FIXATION REACTION IN LEPROSY

BY J. M. GOMEZ, M.D.

*Instituto de Higiene de S. Paulo, Brasil*

Much has been done, but there is still much to be accomplished in the matter of tests for infection and immunity in leprosy. To be of help to the clinicians it is not sufficient for a test to be positive among the inmates of a leper asylum; it is necessary that it should work also in free surroundings where are met the doubtful cases, toward the detection of which the greatest vigilance should be exerted.

Since 1926 we have devoted our efforts to obtain a serological reaction which (1) would help in the diagnosis of incipient leprosy; and which (2) as a further help in judging the value of treatment, would give information as to the patient's state of immunity. If the first part of the problem seems to have been nearly solved, the second part deserves a little more consideration.

### PREPARATION OF ANTIGEN

Immunity reactions have shown the close biological relationships between different acid-fast bacilli. For this reason we chose for our studies the streptothrix of Deycke, a microorganism cultivated by him from leprous nodules. There is really no proof that this germ is a culture of the *Mycobacterium leprae*, but we adopted it for use on account of its original habitat and its morphology, lack of toxicity and ease of cultivation.

*Cultivation of the organism.*—The streptothrix is cultivated on 6 per cent glycerin broth, in flasks of 500 or 1,000 cc. capacity at 37°C. The culture can be used after 20 days incubation. During its growth care is taken not to shake the flask, for the bacilli which have fallen to the bottom cannot be used in the fat-freeing process, and thus they spoil the specificity of the reaction.

*Removal of fat.*—There are several ways of removing the waxy-fatty covering of the bacilli, but we follow McJunkin's method, using olive oil and acetone. The technic is as follows:

The culture flask is emptied of the broth by means of a pipette, leaving only about 10 cc. Acetone, 100 cc., is then poured in and left on the culture

for about 1 minute; after that time, when it should have a milky color, it is removed with a pipette. The same amount is again added and left for 2 minutes. After the removal of this, 10 to 20 cc. of sterile olive oil is added; this has previously been shaken with sterile water, 1 drop of water to 10 cc. of oil.

The culture is placed in the incubator for 24 hours, then filtered on paper and carefully washed with acetone. The material is transferred as it is, on the filter paper, to a Petri dish and placed over the incubator. More acetone is added from time to time, in accordance with the results of several microscopic examinations after staining by the Ziehl-Neelsen method. In one or two days the bacilli are fat-free.

The fat-freeing process is the most important point in the process. To avoid group reactions it should be carried to the point where no more than 6 to 10 acid-fast bacilli are left in each microscopic field. The material is then ground in a glass mortar, giving a fine, whitish powder which must be kept in a sterile flask.

#### THE TEST

The amount of antigen solution needed for the day's work should be prepared fresh each day. An amount of the bacterial powder sufficient to give a 9 per cent suspension is weighed out and emulsified in 1 per cent physiological solution. It is triturated for 5 minutes in a glass tube and centrifuged for 10 minutes. The supernatant fluid is removed with a pipette, diluted to half strength, and heated to 100°C. for 5 minutes to destroy every trace of anti-complementary action.

The set-up of the reaction is as follows:

Tubes	No. 1	No. 2	No. 3	No. 4
Serum	0.05 cc.	0.1 cc.	0.2 cc.	0.2 cc.
Antigen	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.
Complement	(As determined by titration)			
Incubation, first	Incubator or water-bath, 1 hour			
Red cells, sensitized with 2 units	1.0 cc.	1.0 cc.	1.0 cc.	1.0 cc.
Incubation, second	Half hour.			

Readings are made at the end of the second incubation, and again twenty-four hours later, the tubes having been kept in the ice-chest in the meantime.

#### RESULTS OF TESTS

Over 2,000 reactions have been done at the Instituto de Higiene. After we had established the sensitivity of the antigen to leper blood serum, the Leper Inspection Department only called for our help when necessary. Among a total of 1,431 cases whose clinical histories we have at hand there were 559 lepers of the various clinical

types, and 713 suspicious cases, carriers and contacts. A total of 154 cases were of other diseases, and 5 were normal.

*Cases of leprosy.*—The results obtained in the cases of leprosy are shown in Table 1, both separately according to type of the disease and in total.

TABLE 1.—*Results with sera from 559 cases of leprosy, according to type of the disease.*

Type of the disease	Number tested	Positive reactions	Negative reactions	Percent positive
Mixed	119	115	4	96.7
Nodular	44	42	2	95.4
Maculo-anesthetic	305	200	105	65.6
Nervous	91	59	32	64.8
	559	416	143	74.5

The percentages of positive results in the active types (nodular and mixed) were over 95 per cent in both, the two groups together giving 96.3 per cent. On the other hand the other two groups (maculo-anesthetic and mixed) both gave just under 66 per cent, the combined figure being 65.5 per cent. Of the entire lot a total of 416 cases, or 74.5 per cent, were positive.

*Suspected group.*—The reactions given by the 713 persons in this group, which includes suspicious cases, carriers and contacts, are shown in Table 2, together with the 5 normal controls. In considering this group, and the next one, it is of interest that the five normal controls were all negative.

TABLE 2.—*Results with sera from 713 suspicious cases, carriers and contacts.*

Condition	Number tested	Positive reactions	Negative reactions	Percent positive
Suspicious cases	521	284	237	54.5
Carriers	44	14	30	31.8
Contacts	144	63	85	42.6
Normal controls	5	0	5	0

*Other conditions.*—Among the 154 cases of other conditions, mostly skin diseases, 30 gave positive reactions. The conditions in which these were obtained most often were tuberculosis and acne. It is quite possible, however, that some of these may have been cases

of latent leprosy, since they were from highly infected surroundings and came to the Leper Inspection Department to clear up the diagnosis. In a region of less leprosy density (State of Rio Grande do Sul) Maya Faillace found only six slightly positive cases among 300 control tests, while of the lepers tested 96 per cent were positive.

#### FURTHER OBSERVATIONS

It is to be emphasized that the more completely fat-free the bacillus is, the greater will be the specificity of the reaction. Fleury da Silveira and M. Mesquita have made experiments with a methylic antigen, but they found that the method had lost thereby both in sensibility and in specificity.

In collaboration with Azevedo Antunes, from the Instituto de Higiene, we introduced an activating process which greatly increased the sensitivity of the reaction. This consisted of the administration of 2 grains of potassium iodide by mouth daily for a week. In 88 cases (lepers, suspicious cases and contacts) there was an increase in the intensity of the reaction, and 31 previously negative cases became positive.

At the same time a paradoxical fact was observed. Certain of the patients who originally had had a positive reaction gave negative results after taking the potassium iodide. At the time no conclusions could be drawn in relation to this fact, but it has to some extent been explained by later observations. About 4 years ago one of these cases, still under observation, who had a disease focus at home (her brother) was married and had a son, now about 3 years old. If a complement reaction in leprosy, as in syphilis or tuberculosis, means the presence of the respective germ in tissues, a scattering of these germs should have taken place under the energizing shock of pregnancy, thus transforming the condition into the active stage. If this did not happen, it is because the germs were not in condition to set up the disease process, or rather that the organism was in such a state of allergy that it inhibited the dissemination of the germs. Thus its explanation must be immunity.

Several other individuals who had lived for quite a while in contact with lepers (usually wives of lepers) gave the same result. Furthermore, cases of seborrhea, acne, etc., which without any indications of leprosy had given a positive complement reaction, became negative after the administration of potassium iodide. If this phenomenon has a general application it will decrease the disad-

vantages of the few non-specific fixations, and it will open up a field of research to obtain a definite proof of cure in leprosy.

#### GENERAL CONCLUSIONS

The following conclusions may be drawn from the various papers we have published on this subject:

1. The serologically negative cases are "frustrated" or incipient cases.

2. When treatment is begun there is nearly always a breaking up of leprosy cells and a dissemination of the germs. A previously negative reaction becomes positive.

3. When there is a dissemination of germs the previously positive cases become more markedly so.

4. In latent or incipient cases with a positive reaction a later negative reaction can be explained by the bacillemia which is always observed, intermittently, in leprosy.

5. A weak reaction indicates a slight impregnation with the germs. There were women with slightly positive reactions who became pregnant. The reaction continued to be of the same intensity during pregnancy and labor, and there was observed no aggravation of the condition of the patients thereby, as is the rule.

6. Not only recent cases become negative on being cured. We have three old cases which were cured and gave absolutely negative complement reactions. This does not mean, however, that such a negative reaction should be awaited before giving patients a conditional discharge.

7. In contacts who give a repeated positive reaction, especially when the administration of potassium iodide has increased the same, there is a great probability that they are latent cases. In many contacts who gave positive reactions it was possible to obtain acid-fast bacteria by means of lymph node puncture.

8. The cases in which there is loss of intensity or a negative complement reaction after the administration of potassium iodide, or in which positive reactions become negative, seem to indicate a state of immunity.