STUDIES ON THE SEROLOGY OF LEPROSY, II

NITRIC ACID PRECIPITATION (BRUCK, MODIFIED)[1]

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INTRODUCTION

Investigations on the serology of leprosy have, until recently, been conducted along two lines. One involves attempts to develop a specific reaction with leprosy antigens; the other has to do with the relation of the Wassermann reaction to the disease.

The former problem is a most difficult one. The serology of infection with acid-fast bacteria in general involves peculiar difficulties, in that the antigens so far obtained have not permitted differentiation of members of the group. As regards leprosy there is, besides the close relation of the organism to that of tuberculosis, the handicap of the apparent non-cultivability of the causative agent, at least in its tissue form. A practical specific diagnostic test for leprosy seems not yet in sight.

The Wassermann reaction has not proved useful in this infection; for, though it has been generally understood that it is frequently positive, the reports have from the first been very discordant as regards the frequency. As the reaction has now been refined by syphilologists to increase its specificity or, rather, particularly for their purpose, positive reactions in leprosy uncomplicated by syphilis or yaws are at most infrequent. Mathis and Baujean,[2] using the technic of Calmette and Mossol, and recently Kolmer and Denney,[3] with the new technic of the former, find it regularly negative, as Yagley and Kolmer [4] have reported the Kahn precipitation reaction to be. Pineda,[5] applying

[1] Published with the approval of the Director of Health on recommendation of the Philippine Leprosy Research Board.

[2] Bull. Soc. Path. Exot. 8 (1915) 252.

[3] Arch. Dermat. & Syph. 8 (1923) 63.

[4] Arch. Dermat. & Syph. 8 (1923) 183–185.

[5] Antea, pp. 39–57.

Kolmer's technic in the Culion laboratory, has obtained results that agree with these reports in the main, though a small percentage of weakly positive reactions have been given by cases apparently free from syphilis or yaws, especially in "lepra reaction." It now seems clear that this question is of importance with reference to the treponematous infections rather than to the study of leprosy.

On the other hand, from the often reported occurrence of these—from the viewpoint of the syphilologist—false reactions in many cases of leprosy, particularly those with marked cutaneous involvement, it would seem that there is some peculiar serum change which, under some conditions, tends to bind complement in the Wassermann reaction. That the element involved is identical with the syphilitic reagin one would hardly suggest. It may be some factor or element irregularly present, or it may be something constantly present but only infrequently so to a sufficient degree to be demonstrable by this method.

That this general problem should be worked out need not be argued. There is urgent need of a test that will diagnose, or at least give presumptive evidence of, leprous infection in suspected cases, and also in contacts of known cases in order that by treatment latent infections may be prevented from evolving to the clinically positive stage. There is need of a test that may, by repeated application in cases under treatment, serve as a gauge of improvement. Finally, a test that would differentiate latency and actual cure in cases that have become clinically and bacteriologically "negative" would afford a far better basis of discharge than a fixed "negative period," which may be unnecessarily long in some cases and insufficient in others.

The problem is one that seems unlikely of ready solution, in spite of the advances that have been made. It would seem to call for much intensive work by a group of highly specialized investigators. From results obtained in the past it seems highly improbable that any established procedure or simple modification of such will suffice. It has, therefore, seemed profitable to approach the problem from another angle, to investigate certain of the physical and chemical peculiarities of the serum in leprosy.

The work to be reported in this and subsequent papers was begun along lines suggested by two recent reports. One is that

of Turkhud and Avari,[6] who found the formalin coagulation reaction, discovered by Gaté and Papacostas, to be positive in all cases of leprosy tested. The other is that of Schöbl and Basaca,[7] who found a distilled water globulin precipitation reaction, a modification of that of Klausner,[8] to be regularly positive. In line with these simple nonspecific reactions is the nitric acid precipitation test of Bruck, which has apparently not been applied in leprosy. The present report deals with the findings, with a slight modification, of this reaction in one hundred cases of leprosy.

THE NITRIC ACID REACTION

This reaction was described during the World War by Bruck[9] as possibly of value in diagnosing syphilis under conditions that would not permit the use of the Wassermann reaction. It is simple in principle, consisting of a rough determination of excessive (globulin) precipitate formed by nitric acid in dilute serum. It is not surprising that, as indicated by the several reports available to us, so nonspecific a reaction has met with disfavor as a means of diagnosing the presence of syphilitic infection.

Smith and Solomon[10] found disagreement with the Wassermann in 25 per cent of four hundred cases. In three hundred two nonsyphilitics 28 per cent gave doubtful or positive reactions. Stillians[11] had even poorer results, for in ninety-seven syphilitics in all stages there was 35 per cent disagreement with the Wassermann reaction. Of seventy-four nonsyphilitics 24 per cent gave positive reactions. Toyama and Kolmer[12] found that the reaction yielded 8 per cent false positives and was often negative when the Wassermann reaction was positive. Terada[13] found it to be somewhat less frequent than the Wassermann reaction in clinical syphilis (76 per cent of fifty-nine cases as compared

[6] Ind. Journ. Med. Res. 9 (1921–1922) 850.

[7] Philip. Journ. Sci. 25 (1924) 1.

[8] Wien. Klin. Wchnschr. (1908) 21, 214, 363, and Biochem. Ztschr. 47 (1912) 36 [cited by Kolmer, J. A., Infection, Immunity and Biologic Therapy. Philadelphia and London, 3d ed. (1923) 520].

[9] Münch. Med. Wochenschr. 64 (1917) 25 (cited).

[10] Boston Med. & Surg. Journ. 177 (1917) 321 (cited by Stillians and others).

[11] Journ. Am. Med. Assoc. 69 (1917) 2014.

[12] Journ. Cut. Dis. 36 (1918) 429.

[13] Kitasato Arch. Exp. Med. 3 (1919) 123.

with 86 per cent), and more frequent in nonsyphilitics (25 per cent of forty cases as compared with 12.5 per cent), but considers it of value when a more complicated test cannot be carried out.

Of interest in the present connection is the report of Corper and Fiala,[14] who tested the sera of two hundred five questionably or positively tuberculous and twenty-four nontuberculous persons. Of two hundred thirteen Wassermann-negative sera, one hundred thirteen (53 per cent) gave a positive Bruck reaction, most of them strong or fairly strong, a much higher percentage than obtained by others in nonsyphilitics. As for the relation to the stage of the tuberculous infection, it was more frequently positive among the moderately and far advanced (63 and 70 per cent, respectively) than among the nontuberculous (33 per cent), questionably tuberculous (46 per cent), and incipient (36 per cent) cases. No relation to the condition of the patient was to be seen. The authors could not see that the reaction gives any data of value.

A report by Mauchat, van Nitsen, and Walravens,[15] from tropical Africa, is also of interest. In thirty-two Europeans it was positive sixteen times; all of these were either syphilitic, with positive Wassermann reaction, or malarial or suspected malarial individuals. These positive reactions were all read as 1-plus. Of fifty-six Africans only two were negative, one with a phagedenic ulcer and the other with leprosy. In many the reaction was read as 2-plus, and in a few as 3-plus. They remark that the reaction is positive in syphilis and yaws when the Wassermann is positive, and generally in malaria though the Wassermann is negative. It is pointed out that practically all of the natives have chronic malaria.

TECHNIC

In the original technic 0.5 mil of serum is diluted with 2 mils of distilled water, and to this is carefully added 0.3 mil of a nitric acid solution of 1.149 specific gravity (approximately 25 per cent). In exactly ten minutes this is diluted with 16 mils of distilled water, and the tube is inverted three times to mix; ten minutes later the agitation is repeated. The test is read on the basis of the amount of undissolved precipitate, at the earliest a half hour later. As this is difficult to do before sedi-

[14] Am. Rev. Tuberc. 2 (1918–1919) 290.
[15] Compt. Rend. de la Soc. de Biol. 85 (1921) 720.

mentation has occurred, the tests are usually allowed to stand overnight.

At the beginning of this work efforts were made to make the test more precisely quantitative. However, it has become apparent that it has certain inherent weaknesses that, in view of the as yet indefinite significance of the results, do not recommend it for serious consideration; besides the differences in reaction of sera with similar globulin increases that follow from varying total protein contents, it is not free from technical error.

The formalin-coagulation reaction, though perhaps no more valuable or reliable, gives results that on the whole are similar, and it has the advantage of extreme simplicity and freedom from technical error. For this reason the technic used will be stated but briefly.

The sera were clear, or with at most but the faintest trace of hæmolysis, and were fresh and unheated. Some difficulty was met in determining the proper amount of acid solution, as the usual description of the standard solution is obviously incorrect. A solution was prepared [16] of which the standard quantity, determined with two known normal sera, was 0.25 mil. As each serum was tested by a titration series of four or five tubes, necessitating the use of one-half the usual amount of serum per tube, the acid solution was diluted one-half; this was found not to affect the results.

Three titration methods were tried, in which the variant was: (*a*) serum, (*b*) acid, or (*c*) final dilution. Of these, the first is probably the best, though the tests to be reported were done by the second. Better than either is a rough nephelometric determination of opacity. The sediments were examined after eighteen hours and read negative to 3-plus; the final record varied from negative to very strong, according to the number of tubes in the series showing precipitate and the amount of the precipitate.

FINDINGS

The results of the reaction with one hundred consecutive sera from lepers are given in Table 1 and, for comparison, those obtained with sixteen nonleprous controls. The cases were not selected as to type or extent of disease; as to complications, some of the sera were from hospital and clinic cases suspected of having syphilis or yaws.

[16] By Dr. G. A. Perkins, chief chemist of the Culion Leper Colony.

TABLE 1.—*Results of nitric acid precipitation reaction in sera of lepers and nonlepers.*

Case group.	Cases.	Degree of reaction.								
		Very strong.		Strong.		Moderate.		Weak.		Negative.
		Cases.	*P. ct.*	*Cases.*	*P. ct.*	*Cases.*	*P. ct.*	*Cases.*	*P. ct.*	
Untreated new cases	48	21	44	16	33	8	17	3	8	0
Treated clinic cases	41	3	7	20	49	14	34	4	10	0
Hospital cases [a]	11	3	27	8	73	0	------	0	------	0
Total	100	27	------	44	------	22	------	7	------	0
Negative lepers	10	1	------	6	------	1	------	2	------	0
Wassermann positive	21	8	38	11	52	1	5	1	5	0
Non-lepers:										
Professional staff	8	0	------	0	------	1	------	4	------	3
Laborers	8	0	------	2	------	4	------	2	------	0
Total	16	0	------	2	------	5	------	6	------	3

[a] Not suitable for antileprotic treatment.

Taking the lepers' specimens in total, seventy-one gave strong or very strong reactions, and twenty-nine were moderate or weak; none was negative. Considering the groups, there are decided differences in the distribution as regards degree of reaction. Of the newly arrived cases, not yet under antileprosy treatment, a relatively large number (44 per cent) gave very strong reactions, and only eleven (23 per cent) were moderate or weak. Of the cases under treatment only three (7 per cent) were in the very strong category, and eighteen (44 per cent) were moderately or weakly positive. The few reactions on lepers in the general hospital (not suitable for antileprosy treatment) were all strongly or very strongly positive.

Thirteen of the one hundred cases were on the "negative list;" that is, they showed no clinical signs of active leprosy and were bacteriologically negative. Such patients remain under observation and treatment for a further two years. The degree of reaction in the ten with negative Wassermann was fairly similar to that in the treated cases that are still positive. Evidently, the reaction does not tend to become negative rapidly in such patients.

The Wassermann reaction was performed (by Dr. E. V. Pineda) on all but two of the sera. In twenty-one inmates it was positive in some degree, apparently because of yaws or syphilis as a rule. Of these, all but two gave strong or very strong pre-

cipitation reactions; one, though from a probably syphilitic patient, with a 4-plus Kolmer Wassermann, was very weakly positive. On the whole, coincidence of these infections with leprosy apparently tends to increase the amount of precipitate, but this is so marked in most lepers that the difference is not great.

These results must be considered in comparison with the nonlepers. Comparatively few of these were available for examination. Of the eight specimens from the professional staff, only three were actually negative; four of the eight were weakly positive, and one was moderately strong. Of the eight laborers none was negative, and only two were weakly positive. In none of these nonlepers was the Wassermann reaction positive.

DISCUSSION

The results given by this reaction with the sera of nonlepers are of interest, because of the infrequency of negative findings. Even among the professional staff (most of them physicians), there was usually some excess of precipitate. As these individuals live in good circumstances, were apparently perfectly healthy and have remained so for nearly a year, one may doubt that a weakly positive reaction necessarily signifies the existence of a pathological condition. It would of course be difficult to determine clinically whether any particular individual is absolutely normal, but it may at least be said that if a positive reaction does depend upon an abnormality this may be very slight indeed.

It is not surprising that the laborers, whose grade of intelligence and mode of life are such as to make them more liable to infections of one kind or another, should give more frequent and stronger reactions. Still, the results seem excessive. It is to be remarked that the formol reaction has given decidedly fewer positive reactions in this nonleper group. I cannot ascribe this apparent oversensitiveness of the reaction to technic.

However this may be, it is evident that the reaction is strongly positive in the great majority of cases of leprosy uncomplicated by yaws or syphilis, indicating that there is as a rule a marked change in the serum in leprosy. The difference in the figures for treated and untreated cases are of some interest in the gross, indicating that treatment tends to reduce the abnormality on which this reaction depends. However, even in the negative cases tested such reduction had not gone very far.

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Bruck [17] classes the significance of the reaction among those that demonstrate globulin excess. Whether this change is very slight in some cases, as indicated by the weak reactions, cannot be said, without data as to the total protein content of the sera. That the conditions may vary otherwise than quantitatively in different sera is indicated by certain other observations; it does not seem profitable to discuss these in detail.

The findings herein reported are, so far as leprosy work is concerned, of interest as a further indication that there is usually a decided protein abnormality in lepers' serum. Because of the findings in nonlepers, particularly those of the laboring class, it is doubtful whether or not the test would be of value in diagnosis of suspicious cases or contacts, unless a marked degree of serum change occurs very early. As for prognosis, it would seem from the results in the small group of negatives examined that the reaction does not decrease sufficiently in accord with symptoms, at least in patients under continued treatment, to serve as a gauge of clinical improvement. The frequent positive findings in nonlepers indicates that it cannot serve as a basis for discharge.

SUMMARY AND CONCLUSIONS

The problem of the development of serological methods for establishing the diagnosis, prognosis, or cure of leprosy demands attention. That there are decided serological changes in this condition is evidenced by frequent positive Wassermann reactions as this test is usually done, and by the recent findings of Turkhud and Avari with the Gaté and Papacostas formalin coagulation reaction and of Schöbl and Basaca with the distilled water globulin precipitation test. No satisfactory specific reaction has been developed, and this is particularly difficult of realization because of the noncultivability of the organism and its evidently close relation to that of tuberculosis, which infection would have to be differentiated. The Wassermann reaction, at least in the forms most suitable for the diagnosis of syphilis or yaws, is of little if any value in leprosy, except for the diagnosis of these complications.

A study of the serum of leprosy has been undertaken by means of certain nonspecific tests and by other means, in the hope of throwing more light on the changes occurring in this disease. Results with the nitric acid reaction of Bruck, somewhat modified as to technic, are here reported.

[17] Deutsche Med. Wchnschr. 48 (1922).

Of one hundred lepers' sera tested, ninety-three were read as moderately to very strongly positive, and none was negative. Antileprosy treatment apparently reduces the degree of reaction, it being very strong in a much smaller proportion (7 per cent.) of the cases that have been under treatment than in new untreated cases (51 per cent.). The results in ten cases clinically and bacteriologically negative were essentially the same as in the treated cases, the explanation for which fact is not apparent. The reactions in twenty-one sera giving some degree of the Wassermann reaction was essentially the same as in the untreated group.

Of a group of sixteen nonlepers, only three gave negative reactions, these among the professional staff, while two (Chinese) were strongly positive. The frequency of weak reactions in apparently healthy individuals leads to speculation as to whether these weaker reactions are due to any abnormality or not.

That this reaction is of no value in the diagnosis of a particular disease is obvious; that it does not depend upon the presence of syphilis (or yaws) alone, and does not have the significance of the Wassermann reaction, is equally shown.