Clinical Laboratory Aspects of Leprosy

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ALTHOUGH the origins of leprosy probably are rooted in antiquity and the disease has been a dreaded affliction since the beginning of historic time, the etiological agent has been neither successfully cultivated in vitro nor propagated in experimental animals. As a consequence, unequivocal confirmatory laboratory tests are not available in leprosy as they are in other bacterial diseases of man. Nevertheless, numerous observations concerning the results of laboratory studies in leprosy may be found in the world literature.

In this paper, these observations, as well as our own observations based on work done at the Public Health Service Hospital in Carville, La. (the National Leprosarium), and the Public Health Service Hospital in New Orleans, will be presented and discussed in the light of present-day knowledge. It will be seen that the diagnostic laboratory procedures are limited to examination of tissues and that the abnormal clinical laboratory findings reflect, in actuality, the widespread systemic complications and sequelae of leprosy.

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Bacteriology

The human leprosy bacillus, *Mycobacterium leprae*, was the first organism reported as the etiological agent of a specific disease in man. Despite this fact, it is the only pathogenic bacterium that has not been cultured on artificial media, transmitted to animals, or even transmitted experimentally within the natural host. Attempts to cultivate the leprosy bacillus on artificial media have been innumerable, and positive, as well as negative, results have been reported. The claims of successful cultivation, however, have never been substantiated by other investigators.

Although chick embryos are susceptible to a remarkable variety of bacteria, viruses, and rickettsiae, attempts to infect chick embryos and chick tissue cultures with leprosy bacilli have also met with failure (*1*).

In 1947 Hanks studied the effect of adding *Myco. leprae* to actively growing fibrocytes obtained from the skin of patients with tuberculoid (*2*) and with lepromatous (*3*) leprosy. He observed rapid reduction of the organisms to acid-fast debris in the tissue cultures of fibroblasts from the patients with tuberculoid leprosy; the degeneration of the *Myco. leprae* was less rapid with fibroblasts derived from lepromatous lesions. Cultivation from 2 to 7 months did not result in growth of the leprosy bacillus within the fibrocytes.

Chemistry

Serum Proteins

Most investigators agree that there are profound changes in the serum proteins in leprosy. For the determination of protein values, many

workers have used chemical methods (*4–6*). The quantitative and qualitative aspects of these methods compare favorably with the more recent electrophoretic methods of fractionating the complex mixture of serum proteins, but the latter methods are more sensitive and may detect abnormal constituents of the protein mixture.

The majority of the workers who have studied leprosy serums by electrophoretic methods have used the moving-boundary type apparatus (*7–12*). Miguel and his co-workers (*13*) made use of the Kern apparatus and paper electrophoresis. No specific references were found in the literature pertaining to the examination of serum from leprosy patients by paper chromatography, column chromatography, or ultracentrifugation.

According to most of the references cited, the total proteins are normal or increased in the great majority of leprosy serums. The quantity of albumin is usually moderately reduced, and, in our experience, in one case complicated by amyloidosis, albumin was undetectable by ordinary chemical methods and only poorly demonstrated by paper electrophoresis. There is a moderate to marked hyperglobulinemia in the majority of cases, and, obviously, reversal of the albumin-globulin ratio occurs frequently in leprosy.

There is less unanimity of opinion in regard to the quantity of the various globulin fractions in leprosy. However, it seems that alpha$_2$ is the most frequently elevated fraction. Miguel and his associates reported that this fraction was above normal in 69.76 percent of the patients studied (*13*). Alpha$_1$ and beta globulin are elevated less frequently, although a majority of the reports specify an increase. All observers noted an abnormal increase of gamma globulin, and in Miguel's study the gamma globulin was above normal in 68.82 percent of the patients.

C-Reactive Protein

Serum from 100 patients at the National Leprosarium was tested recently for C-reactive protein. This abnormal protein was found in 79 percent of the patients with clinically active lepromatous leprosy and in 30 percent with clinically inactive lepromatous leprosy. It is believed that serial determinations of C-reactive protein may have some value in following the progress of the inflammatory response to *Myco. leprae* infection.

Liver Function Tests

Many of the reports of liver function studies in leprosy are based on methods now considered obsolescent or are no longer significant in the light of our better, though still imperfect, understanding of the mechanism of the reactions involved in these procedures (*14*). As should be expected, the incidence of abnormally high cephalin flocculation and thymol turbidity tests closely parallels the number of serums in which the proteins deviate from normal (*15*).

Normal values for urobilin and bilirubin in blood and urine were reported by Molinelli and Royer (*16*). It is important to note that the bilirubin test employing Ehrlich's diazo reagent diazotizes the two amino groups of the sulfone drugs. A positive test for bilirubin, therefore, would be indistinguishable from the reaction produced by the sulfones, a fact which makes this procedure as unreliable in leprosy as other liver function tests.

In our review of the literature, no reports regarding the results of the bromsulfalein excretion test in leprosy were found. Probably, bromsulfalein is excreted by the parenchymal cells in a manner similar to that in which bilirubin is excreted. Therefore, in the absence of jaundice and with normal renal excretion, this test should be one of the more useful procedures in leprosy.

Hopkins and others (*17*) reported that cholesterol was high in 14 and normal in 27 leprosy patients and that cholesterol esters were increased in the majority. Gomes and his colleagues (*18*) observed that reduction of serum cholesterol levels was proportional to the gravity of the disease, especially in febrile reactions. Lippi (*19*) noted that the serum cholesterol fell in patients with the lepromatous type and in those in serious condition. Ross (*20*) found serum alkaline phosphatase to be within normal limits in 87 percent of 102 patients. There is little information regarding the zinc sulfate turbidity test or the hippuric acid test in leprosy.

At the National Leprosarium laboratory, we have studied a small number of liver biopsy

specimens obtained percutaneously by the Vim-Silverman needle method from patients with active lepromatous leprosy. Hepatic miliary lepromata were easily identified, and *Myco. leprae* organisms were demonstrated by acid-fast staining. Hepatic amyloidosis was demonstrable histologically by crystal-violet stain in the instances in which there was 100-percent absorption of congo red. Our experience with needle biopsies of the liver has been too limited to draw definite conclusions, but the technique seems to be a very promising one for evaluation of hepatic involvement by leprosy and by amyloid. The procedure is not without hazards, however, and all established safeguards should be observed.

Calcium and Phosphorus

There is disagreement in the observations of investigators regarding serum calcium levels in leprosy. Reports of normal levels (*21–23*), of hypocalcemia (*24*) and of hypercalcemia (*25*) may be found. Wooley and Ross (*26*) found diffusible calcium to be lower than normal in leprosy patients and that it increased with clinical improvement of the disease. They reported that inorganic phosphorus was normal in uncomplicated leprosy.

Lemann and others (*27*) found no relation between serum calcium levels and the bone absorption in leprosy; however, Wooley and Ross reported that in the aforementioned series bone atrophy was shown by radiography in 44 of 53 patients whose serum was studied.

Glucose

Very little has been reported on carbohydrate metabolism in leprosy since Villela's review in 1938 (*24*). Certain of the older reports, such as those of Lai (*28*) and Otsuka (*29*), mentioned lowering of glucose tolerance. Wayson, Badger, and Dewar (*30*) stressed the high frequency of disturbance of carbohydrate metabolism in Hawaiian patients. Glycosuria was noted in 23.0 percent of 175 patients, and 80 percent had abnormal glucose tolerance curves.

More recently, Lancepleine (*31*) reported the frequency of hypoglycemia to be greater in patients with neural leprosy than in those with lepromatous leprosy, but the number of patients studied was too small to give significance to the observation. Tanioku (*32*) found some increase in blood sugar in both macular and lepromatous leprosy, but he did not consider it to be specific for leprosy. Sakakibara (*33*), however, could detect no consistency in occurrence of either hyperglycemia or hypoglycemia.

The sugar content of the blood is determined routinely on admission of patients at the National Leprosarium, and, if it is above normal, a glucose tolerance test is performed. A urinalysis is made at 3-month intervals on every patient. There are usually 20 to 30 patients in the hospital, or about 5.0 to 7.5 percent of the total, who have glycosuria.

Hematology

In 1949 Kiang and Choa (*34*) reported the results of the hemograms for 36 leprosy patients and reviewed a few pertinent references dealing with the blood picture in leprosy. Anemia was found to be frequent but never severe, and no morphologic abnormalities of the erythrocytes were noted. The reticulocytes were low, averaging 0.2 percent. For all except 4 patients, the erythrocyte sedimentation rate was increased. Leukocytosis was inconstant, and when present it was only of moderate degree and only in patients with lepromatous leprosy. The differential counts indicated that leukocytosis was due to increased numbers of mononuclear cells; however, in the untreated cases there was a definite neutrophilic shift to the left. Eosinophilia was observed in 36.1 percent of the patients, and the condition was believed not to be due to parasitism.

From the literature and from our own observations it can be stated that anemia in leprosy is seen mainly in the lepromatous type, especially in patients in whom the disease is advanced, is in a reactional crisis, or is complicated by nephrosis. The majority of workers emphasize leukopenia, but at the Carville hospital leukocytosis has been maintained occasionally over a period of years even in uncomplicated cases. Eosinophilia has been mentioned frequently; however, findings at Carville do not substantiate the opinion that leprosy

per se causes an increase in the number of eosinophils.

The erythrocyte sedimentation rate is accelerated in leprosy, especially in the lepromatous type and in mixed types (*35*). In our experience, there are no characteristic alterations in the bleeding time, coagulation time, or clot retraction.

In bone marrow from patients with leprosy, *Myco. leprae* bacilli are occasionally demonstrated in the smears as well as in the marrow particles. In a series of 32 bone marrows obtained at autopsy, we found bone marrow involvement in 6. Although there is a relatively high incidence of amyloidosis among patients at the National Leprosarium, amyloid has not been convincingly demonstrated in aspiration material from the sternal or iliac crest marrow or from autopsy material. Cytologically, the marrow usually is not remarkable.

The demonstration of *Myco. leprae* in the cytoplasm of leukocytes has been reported. Namba (*36*) observed the presence of bacilli in both neutrophils and monocytes removed from the buffy layer of sedimented citrated blood. Montel (*37*) also found bacilli by this method. He nearly always obtained positive results in generalized lepromatous cases, especially during febrile reactions, but never in tuberculoid cases.

Myco. leprae bacilli have been observed in dehemoglobinized blood. By examining thick drops of blood dehemoglobinized with water, Clouston (*38*) obtained 77.7 percent positive results in nodular leprosy. The results were negative in 21 early cases, however, and he concluded that the procedure was of no practical use in detecting early or latent leprosy. Baru (*39*) laked and centrifuged venous blood and found bacilli concentrated in the sediment. At the National Leprosarium, a similar technique revealed the presence of bacilli in approximately 50 percent of the newly admitted patients with lepromatous leprosy.

Immunology

Serologic Tests for Syphilis

It is generally agreed that there is a high incidence of biologically false positive serologic tests for syphilis in leprosy patients (*40*). A clinico-serologic study of 224 patients at the National Leprosarium was made in 1952 (*41*). The serologic tests for syphilis used were (*a*) the Kahn standard quantitative; (*b*) the Kolmer (cardiolipin) quantitative; (*c*) the VDRL slide flocculation quantitative; (*d*) the Rein-Bossak slide flocculation quantitative; and (*e*) the *Treponema pallidum* immobilization (TPI). The reactivity rates were as follows:

	Reactivity rate (percent)
Kolmer	63.4
Kahn	52.7
VDRL	46.9
Rein-Bossak	31.8
TPI	11.2

It was concluded that the reactivity rate for the *Treponema pallidum* immobilization test appeared to be consistent with the syphilis rate that might be expected in a comparable population group without leprosy.

The majority of the patients considered to have false positive tests for syphilis had lepromatous leprosy. This seroreactivity seemed to diminish with the prolongation of the disease, but there did not appear to be any correlation with the age of the patients. There was no significant relation to seroreactivity in amyloidosis of leprosy, diabetes, or carcinoma.

Universal Serologic Reaction

In his discussion of the universal serologic reaction with lipid antigen, Kahn (*42*) states that the reaction between tissue lipids and serum upon which serodiagnostic tests for syphilis are based is not primarily a syphilitic reaction but a biologically universal reaction. A special method known as the universal serologic technique seemed to distinguish specific serologic patterns in all human beings and animals. Concerning such patterns, Kahn states, "In lepromatous leprosy, the serologic pattern of the universal reaction is also apparently distinctive; yet, it is entirely different from the serologic patterns noted in either syphilis or yaws." In addition, he indicates that this test should assist in differentiation of the various clinical forms of leprosy.

Ross and Gemar (*43*) investigated the universal serologic reaction in 130 leprosy patients, 20 of whom had tuberculoid leprosy and 110

of whom had lepromatous leprosy. From analysis of the data, they concluded that there was no indication of any distinctive serologic pattern in lepromatous leprosy. In addition, the data suggested that treatment may alter the type of pattern in this form of the disease. Pinto and Zeo (*44*) also were unable to confirm a distinctive universal serologic reaction in lepromatous leprosy, and their conclusions were in agreement with those of Ross and Gemar.

Human Leprosy Antigen

Microflocculation tests employing antigens made from human leprosy material, according to the method of Castro (*45*), have been investigated recently at the National Leprosarium. Serums from 144 normal persons were all negative. Serums from 50 patients with tuberculosis were negative except for one doubtful and one positive test, and serums from 32 patients with syphilis were all negative. The overall results of the microfloccuation tests on serums from 219 leprosy patients were as follows:

Tuberculoid, inactive_____ 13 negative; 0 positive.
Lepromatous, inactive_____ 4 negative; 2 positive.
Lepromatous, active_____ 23 negative; 177 positive.

These results, which are in close agreement with the original investigations by Castro, indicate that the use of antigens made from human leprosy material may be a worthwhile laboratory procedure in leprosy.

Middlebrook-Dubos Hemagglutination Test

The erythrocyte agglutination test of Middlebrook and Dubos has also been studied at the National Leprosarium (*46*). In a study of 261 cases of leprosy, it was found that the proportion of high titers was significantly higher in clinically active lepromatous cases with large numbers of bacilli than in quiescent lepromatous or tuberculoid cases. Results in a small number of cases studied repeatedly over a 1-year period suggest that there may be some correlation between the clinical course of the disease and alterations in titer.

Spinal Fluid

In 32 National Leprosarium patients, no characteristic changes were observed in the spinal fluid. This study included cell count; sugar, chloride, protein, and colloidal gold tests; and a Wassermann complement fixation test. The negative finding is in agreement with the results of other studies (*47*, *48*).

Tissue Diagnosis

From the preceding discussion, it is obvious that the reviewed clinical laboratory procedures offer no pathognomonic diagnostic information in leprosy. At this time, the laboratory procedures which are most likely to be diagnostic of leprosy are limited to (*a*) microscopic examination of biopsy material and (*b*) microscopic examination of smears from skin and nasal mucous membrane scrapings (*49*).

Examination Techniques

It is of considerable importance to select the site of biopsy or smear specimen with care. The margins of macules and areas of infiltration offer the best material.

We prefer that the biopsy specimen be obtained with a sharp scalpel and that it be sufficiently deep to include subcutaneous tissue. In this country, 10 percent formalin solution is preferred as the fixative, and the sections are stained with hematoxylin and eosin. We have found that the Fite-Cambre-Turner modification of the Ziehl-Neelsen stain to be the most satisfactory acid-fast stain for tissue sections.

In obtaining material for a smear, the skin is compressed between the fingers until it blanches, and a very small incision is made with a single edge razor or a scalpel blade. The blade is scraped through the incision at right angles to collect tissue fragments, which are spread on a glass slide. The slide is stained by the Ziehl-Neelsen acid-fast method.

Demonstration of *Myco. leprae* in leproma is not difficult; however, it is usually a laborious task to obtain bacilli in acceptable numbers from the macules of tuberculoid leprosy. Recent reports from India describe simple methods of concentrating the bacilli by tissue homogenization which are superior to the usual skin smears in such cases (*51*, *52*). These methods are being studied at the Carville hospital, and the results thus far tend to confirm the observations of the Indian researchers.

Characteristics of Three Leprosy Types

Lepromatous leprosy, one of the two polar types, is the one usually illustrated in textbooks and found in class slide sets. Except for a characteristic uninvolved zone immediately beneath the basal layer of the epidermis, the dermis is infiltrated by large, pale, mononuclear phagocytes with a slightly granular cytoplasm, the so-called lepra or Virchow cell. In the early stages of the disease, collections of these cells are oriented around capillaries and lymphatics, and, in later stages, they coalesce into homogeneous masses of lepra cells. Acid-fast stains reveal astounding numbers of leprosy bacilli in the cytoplasm and in cystlike structures called globi. Bacilli are also present in walls of vessels. No caseation necrosis or giant cells are seen unless complicating factors have altered the picture. On rare occasions, foreign-body type giant cells are present around the globi.

Tuberculoid leprosy is the other of the two polar types. Its most obvious feature is the presence of epithelioid tubercles with Langhan's type giant cells. No uninvolved sub-basal zone is present, and the picture is closely mimicked by some cases of sarcoid of the skin, tuberculosis, beryllium granulomas, and other granulomatous cutaneous lesions. In a study of the histological differentiation of nonleprous and leprous cutaneous tuberculoid granulomas at the Armed Forces Institute of Pathology, one of the authors (Swan) and Dr. Chapman H. Binford concluded that nerve involvement is seen almost exclusively in the leprosy cases. *Myco. leprae* bacilli are infrequently seen in tuberculoid leprosy, although a few may be found in most cases if a determined and vigorous search is made.

What is known as the indeterminate type of leprosy is histologically nonspecific. In this type the presence of acid-fast bacilli is rather infrequent. The dermal reaction consists of perivascular and perineural lymphocytic infiltration.

Summary and Conclusions

The cultivation of *Mycobacterium leprae* is probably the one most important goal sought by leprologists. Conclusive proof of the presence of leprosy, exact determination of activity, precise evaluation of therapeutic agents, and development of specific diagnostic tests await the cultivation of this organism. The leprosy bacillus appears to be an intracellular parasite, and it is possible that with present-day tissue culture techniques the organism will be successfully grown in vitro.

The abnormal values of serum proteins generally are considered to be due to the response of the reticuloendothelial system to leprosy bacilli. It is important to evaluate the protein values in leprosy according to (*a*) the type of leprosy from which the serum is obtained and (*b*) the activity of the disease and complicating conditions, such as amyloidosis and renal disease. It is apparent that in almost all cases of lepromatous leprosy there are abnormalities of serum proteins. The globulins are increased during acute lepra reactions and erythema nodosum leprosum, and they subside as the erythema disappears. Abnormalities of the serum proteins are rare in tuberculoid leprosy. In view of the frequency of complicating amyloidosis in leprosy, the evaluations of serum proteins are significant, especially in the light of recent evidence suggesting that amyloidosis seems to be related to the appearance of beta and alpha$_2$ globulins in the blood of experimental animals (*53*).

The cephalin flocculation and thymol turbidity tests are abnormal in leprosy because of the quantitative, and possibly the qualitative, variations in the serum proteins. The reduction of serum cholesterol levels, as reported by most observers, is more in keeping with what is known concerning cholesterol metabolism and excretion. The relationship between free and esterified cholesterol seems not to have been adequately studied in leprosy. The low serum cholesterol levels in active lepromatous leprosy suggest that there is hepatic involvement, a condition which the finding of lepromata and organisms in needle biopsies of the liver seems to confirm.

Other clinical chemistry tests reviewed do not appear to be altered by leprosy per se.

Hematology offers little in the diagnosis of leprosy. The presence of organisms in the cytoplasm of circulating leukocytes or in smears from laked blood is usually seen only in cases

in which organisms can be demonstrated more easily from smears of cutaneous lesions. Bone marrow examination likewise contributes little to the diagnosis of the disease.

False serologic tests for syphilis in leprosy are well known and have made the diagnosis of concurrent syphilis difficult unless spirochetes are demonstrated. The recent results of the *Treponema pallidum* immobilization test in patients with leprosy suggest that the false biological positive reactions have been eliminated.

The Middlebrook-Dubos hemagglutination test reveals that leprosy probably produces antibodies which cause agglutination of red blood cells that have been sensitized with tuberculin. The microflocculation test of Castro, from the serologic point of view, may be assumed to be a diagnostic test for leprosy. We believe that this test may be of value as a routine laboratory procedure in the detection of overlooked cases of lepromatous leprosy in endemic areas.

It is our opinion that at the present time examination of tissues is the best method of making a laboratory diagnosis of leprosy and of classifying the disease as to type. The concentration of organisms by homogenization of tissue is perhaps the method most likely to demonstrate organisms in all types of leprosy. Meticulous technique and cautious interpretation of results are essential for proper utilization of this procedure.

REFERENCES

(*1*) Hanks, J. H.: Attempts to infect chick embryos and chick tissue cultures with bacilli from human lepromatous lesions. Internat. J. Leprosy 15: 70–77 (1947).

(*2*) Hanks, J. H.: The fate of leprosy bacilli in fibroblasts cultivated from macular and tuberculoid lesions. Internat. J. Leprosy 15: 31–47 (1947).

(*3*) Hanks, J. H.: The fate of leprosy bacilli in fibroblasts cultivated from lepromatous lesions. Internat. J. Leprosy 15: 48–64 (1947).

(*4*) Ross, Sister H.: Studies on the serum proteins in leprosy. The alpha, beta and gamma globulin fractions. Internat. J. Leprosy 19: 445–452 (1951).

(*5*) Stevenson, W. D. H.: Complement deviation and globulin content in the blood of lepers. Indian J. M. Research 12: 583 (1925).

(*6*) Wooley, J. G., and Ross, Sister H.: Calcium, phosphorus and protein metabolism in leprosy. A study of the total calcium, diffusible and nondiffusible calcium, phosphorus, total proteins, albumin, and globulin in the blood serum. Pub. Health Rep. 47: 380–389 (1932).

(*7*) Hoxter, G., Batista, L., and Vellini, L. L.: Estudos electroforeticos nas diversas formas clinicas da lepra (with English summary). Rev. Brasil de Leprol. 19: 27–40 (1951).

(*8*) Kono, M., Tsugami, H., and Sakurai, H.: On serum protein components and leucocyte percentage in erythema nodosum leprosum. La Lepro 21: 98–102 (1952); Abstract in Internat. J. Leprosy 21: 411 (1953).

(*9*) Mauzé, J., and Arnaud, G.: L'electrophorèse du serum de lepreux (with English summary). Internat. J. Leprosy 22: 55–60 (1954).

(*10*) Mayama, A.: Electrophoretic studies on the protein distribution in the serum of leprosy patients. Sc. Rep. Res. Inst. Tohoku Univ. Ser. C. 5: 273–282 (1954).

(*11*) Namba, M., and Fujiwara, H.: Studies on the electrophoresis of leprous serum. La Lepro 21: 5–10 (1952); Abstract in Internat. J. Leprosy 21: 5 (1953).

(*12*) Seibert, F. B., and Nelson, J. W.: Electrophoresis of serum; Serum proteins in tuberculosis and other chronic diseases. Am. Rev. Tuberc. 47: 66–77 (1943).

(*13*) Miguel, S., Roldan, A., Guillen, J., Teremio, J., y Ponciani, J.: Proteinas plasmaticas en la lepra. Internat. J. Leprosy 22: 47–54 (1954).

(*14*) Hanger, F. M.: The meaning of liver function tests. Am. J. Med. 16: 565–573 (1954).

(*15*) Ross, Sister H.: The thymol turbidity and cephalin-cholesterol tests, euglobulin, albumin-globulin ratio and serology in leprosy. Internat. J. Leprosy 17: 237–245 (1949).

(*16*) Molinelli, E. A,, and Royer, M.: La urobilina y bilirubina sanguinea y urinaria en la lepra. Semana méd. 3: 1799–1801 (1929).

(*17*) Hopkins, R., Black, S., and Ross, Sister H.: Xanthelasma and leprosy. Arch. Dermat. & Syph. 39: 239–252 (1939).

(*18*) Gomes, J. M., Pereira Leitao, C. A., and Wancolle, A: Cholesterinemia na lepra. Rev. Biol. Hyg. 1: 39–52 (1927).

(*19*) Lippi, M.: La colesterinemia nella lepra. Arch. ital. sc. med. trop. 31: 266–273 (1950); Abstract in Internat. J. Leprosy 19: 257 (1951).

(*20*) Ross, Sister H.: Serum phosphatase in leprosy. Internat. J. Leprosy 9: 57–61 (1941).

(*21*) Concepcion, I., and Salcedo, J. F.: The calcium content of blood in leprosy. J. Philippine M. A. 6: 154–157 (1926).

(*22*) Cruz, M. C., Lara, C. B., and Paras, E. M.: Blood calcium in leprosy. J. Philippine M. A. 8: 216–221 (1928).

(*23*) Morales-Otero, P., and Hernandez, L. G.: II Studies of the blood chemistry of leprosy. Analysis of findings of fifty cases. Puerto Rico J. Pub. Health & Trop. Med. 5: 443–451 (1930).

(24) Villela, G.: The biochemistry of leprosy. A review. Internat. J. Leprosy 6: 61–72; 223–232 (1938).
(25) Herrera, R. M.: La calcemia en los leprosos. Siglo med. 95: 394–396 (1935); Abstract in Internat. J. Leprosy 4: 544 (1936).
(26) Wooley, J. G., and Ross, Sister H.: Phosphorus, total calcium and diffusible calcium content of the blood sera of lepers and their relation to bone changes. Pub. Health Rep. 46: 641–658 (1931).
(27) Lemann, I. I., Liles, R. T., and Johansen, F. A.: Blood serum calcium in leprosy. Am. J. Trop. Med. 7: 61–70 (1927).
(28) Lai, D.: The dextrose tolerance test in leprosy. Am. J. Trop. Med. 14: 575–584 (1934).
(29) Otsuka, H.: On the sedimentation phenomena of red blood-cells and the amount of total nitrogen, non-protein nitrogen, fibrin nitrogen and sugar in the blood of lepers (with English summary). Japanese J. Dermat. & Urol. 33: 127–169 (1933).
(30) Wayson, N. E., Badger, L. F., and Dewar, M.: Leprosy with evidence of abnormalities in carbohydrate metabolism. Pub. Health Rep. 44: 2971–2983 (1929).
(31) Lancepleine, J.: Cholesterinemie, calcemie, and glycemie, chez les lepreux de l'A.O.F. Méd. trop. 9: 502–518 (1949); Abstract in Internat. J. Leprosy 19: 257 (1951).
(32) Tanioku, K.: The chemical views of the blood in leprosy (with English summary). La Lepro 19: 24–28 (1950).
(33) Sakakibara, I: Blutzuckergehalt des leprasserums. La Lepro 7: 29 (1936); Abstract in Internat. J. Leprosy 5: 541 (1937).
(34) Kiang, S., and Choa, G. H.: The blood picture in leprosy. Am. J. Med. Sc. 217: 269–276 (1949).
(35) Faget, G. H.: Erythrocyte sedimentation tests in leprosy. Statistical study of more than 2000 tests in more than 500 patients. Ann. Int. Med. 22: 212–224 (1945).
(36) Namba, M.: A new method of demonstrating lepra bacilli in the circulating blood. Japanese J. Dermat. & Urol. 44: 192 (1938); Abstract in Internat. J. Leprosy 8: 135 (1940).
(37) Montel, R.: Bacillemia of leprosy. Staining characters of Hansen's bacillus. Bull. Acad. Med. Paris 130: 165–168 (1946); Abstract in Internat. J. Leprosy 17: 361 (1949).
(38) Clouston, T. M.: An investigation into the thick blood-drop method of diagnosis in leprosy. M. J. Australia 2: 430–432 (1936).
(39) Barua, K. C.: A new method of examining the blood in leprosy. Indian M. J. 32: 512 (1938).
(40) Parran, T., Hazen, H. H., Mahoney, J. F., and others: Preliminiary report on the Washington Serology Conference. Ven. Dis. Inform. 23: 161–194 (1942).
(41) Edmundson, W. F., Wolcott, R. R., Olansky, S., and Ross, Sister H.: A clinico-serologic study of leprosy. 1. Results of serologic tests for syphilis, including *Treponema pallidum* immobilization test. Internat. J. Leprosy 22: 440–449 (1954).
(42) Kahn, R. L.: Serology with lipid antigen with special reference to Kahn and universal reactions. Baltimore, Md., Williams and Wilkins, 1950, p. 39.
(43) Ross, Sister H., and Gemar, F.: The Kahn universal serological reaction in leprosy. Internat. J. Leprosy 21: 27–33 (1953).
(44) Pinto, T. J., and Zeo, A.: Universal serologic reaction in leprosy. Ann. New York Acad. Sc. 54: 48–52 (1951).
(45) Olmos Castro, N.: Contribution to the study of the serology of leprosy. Internat. J. Leprosy 19: 309–321 (1951).
(46) Ross, Sister H.: The results of a modified Middlebrook-Dubos hemagglutination test in leprosy. Internat. J. Leprosy 22: 174–180 (1954).
(47) Koenigstein, R. P., and Chang, S. T.: Colloidal gold reaction in the spinal fluid in leprosy. Internat. J. Leprosy 19: 323–326 (1951).
(48) Molinelli, E. A., and Vaccarezza, A. J.: The cerebrospinal fluid in leprosy. Rev. Soc. argent. de biol. 5: 428–434 (1929).
(49) Swan, L. L.: The laboratory diagnosis of leprosy. J. Louisiana Med. Soc. 106: 411–413 (1954).
(50) Fite, G. L., Cambre, P. J., and Turner, M. H.: Procedure for demonstrating lepra bacilli in paraffin sections. Arch. Path. 43: 624 (1947).
(51) Dharmendra and Mukherjee, N.: A simple method of concentration of leprosy bacilli from "closed" cases of leprosy. Leprosy in India 24: 169 (1952).
(52) Khanolkar, V. R., and Rajalakshmi, K.: A method for concentration of acid-fast bacilli in skin biopsies from leprosy patients. Leprosy in India 24: 47–61 (1952).
(53) Richter, G. W.: Amyloidosis and increased beta globulins of the serum in rabbits given ribonucleotide. Am. J. Path. 29: 583–584 (1953).