

THE BIOCHEMISTRY OF LEPROSY

A REVIEW

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Introduction

Biochemistry of the blood

Calcium and inorganic phosphorus

Alkali reserve

Serum proteins

Immuno-chemistry

Blood and tissue lipase

Cholestesterol and cholesterol esters

Total lipids, fatty acids and iodine number

Glucose

Other blood constituents

Metabolism and nutrition

Chemistry of *Mycobacterium leprae*

References

INTRODUCTION

Biochemical investigation in leprosy has not been carried out as extensively as in other infectious diseases, and it is only within the last twenty years that any important chemical studies have been made. The nutritional problem in leprosy has but recently received the attention of biochemists, though it is of interest to associate chemical tests with clinical examinations in order to obtain a more exact understanding of the evolution and prognosis of the disease. Information concerning the chemistry of *Mycobacterium leprae* is based largely on examinations of cultures supposed to be of that organism, conducted along the same lines as those on the tubercle bacillus.

A review on the biochemistry of leprosy has not heretofore been undertaken. Articles are scattered throughout the literature, making it difficult to obtain a general view of the subject. It is the purpose to assemble here as completely as possible, all the work done in this field. For clarity of understanding the subject is divided into three parts: (a) the chemical picture of the blood, (b) metabolism and nutrition in its relation to leprosy, and (c) the chemistry of cultivated organisms supposed to be the leprosy bacillus.

BIOCHEMISTRY OF THE BLOOD IN LEPROSY

During the past few years the composition of the blood of lepers has been investigated and some interesting results have been obtained. Analyses of the blood before and during lepra fever have shown results of practical value.

CALCIUM AND INORGANIC PHOSPHORUS

The question of calcium metabolism in leprosy is of particular interest because of the bone changes that occur. The first research dealing with the matter was published by Underhill, Honeij and Bogert (94), who reported an apparent retention of calcium in the more advanced cases. Boulay and Leger (15) tried to determine whether the retention was continuous or whether there were intermittent losses. These authors studied two lepers submitted to a diet containing from 1.50 to 1.60 gm. of CaO and observed a daily excretion of 0.597 gm. and 0.735 gm. of calcium, respectively. In two other cases with hyperexcretion they found also an excess elimination of phosphorus, which is not lost uniformly. In a later report (15) they stated that calcium retention is observed in the less advanced cases and a loss in the advanced ones. It is to be noted that these observations were made on only a few patients. Rajewski (79) reported some experiments on the metabolism of calcium, sodium and potassium in 11 cases, some mixed, some in lepra reaction, and some with bone changes, and concluded that in progressive leprosy there is a loss of calcium and hypocalcemia accompanied with high potassium values.

In the blood serum calcium exists in three forms: (a) ionized, diffusible, (b) nonionized, diffusible, and (c) nonionized, nondiffusible, probably combined with proteins. The diffusible fraction represents 45 to 60 percent of the total and is dialyzable through collodion membranes (ultrafilters). This fraction is partly nonionized, according to Rona and Takahashi (84), Neuhausen and Pincus (61), and Cantarow (21). The normal average for total blood serum calcium is 10.5 mg. per 100 cc., varying from 9.0 to 12 mg. (21, 74).

Concepcion and Salcedo (25), Leman, Liles and Johansen (51), Cruz, Lara and Paras (27) and Villela (97) have studied this element in leprosy. Concepcion and Salcedo, who published the first report on the subject, found that the calcium content of the blood in 37 nodular and mixed cases varied from 5.76 to 13.3 mg. per 100 cc.; normal values varying between 7.4 and 9.8 mg. The technique employed was that of Halverson and

Bergeim, using whole blood, and the results should therefore be recalculated to be comparable with those of other investigators, because of the variability of the plasma content of whole blood in leprosy. Apart from that consideration their figures are generally lower than normal.

Leman, Liles and Johansen in 54 lepers did not find variations in calcemia, and concluded that there is no relation between the serum calcium and the bone changes. Chaulmoogra oil also has no influence on calcemia of lepers, according to Cruz, Lara and Paras. These authors determined the serum-calcium values in 70 lepers and found that they are usually within normal limits, and that neither type, advancement and duration of the disease, nor treatment with chaulmoogra oil, have any influence. In cases with pulmonary tuberculosis and nephritis, without lepra fever, the values averaged 9.34 and 9.61 mg. respectively. Lower values were found in lepra reaction cases (variations: 6.18 to 10.45 mg. per 100 cc., average 8.26).

Normal serum calcium varies within narrow limits, and values lower than 9.0 mg. must be taken as a sign of disturbed calcium metabolism. Vilella obtained lower figures than normal in 51 out of 113 patients examined. The total average for all types of the disease was 8.82 mg. The neural form, except in lepra fever and nephritis, showed hypocalcemia. Badenoch and Byron (8), having examined 54 cases, are of the opinion that the values are as a rule below normal, and that definitely lower values are observed in lepra fever. The variations in 33 cases of lepra reaction were from 7.9 to 10.6 mg. and for the nonreaction group from 8.2 to 16.0 mg. Herrera (37) observed a decrease only in reaction cases.

The serum calcium was analyzed for its diffusible fraction by Wooley and Ross (106), who found a considerably lower average in lepers than in normal young men (53 lepers and 15 controls). In patients showing marked bone atrophy they obtained an average of 4.5 mg., and for normal healthy men 5.8 mg. per 100 cc. More recently the same authors (105) have shown that the decrease in the diffusible calcium corresponds to an increase of the non-diffusible fraction, which returns to normal values when the patient improves. In the same paper they reported that hyperglobulinemia is often accompanied by a high content of diffusible calcium. Their data are shown in Table 1.

Inorganic serum phosphorus is not affected by leprosy (Wooley and Ross, 105, Nishkawa, 63) and high values are observed

only in patients suffering from renal disorders, depending therefore on the degree of renal impairment. Wooley and Ross found variations from 1.7 to 4.5 mg. per 100 cc. (average 3.42 mg.), the normal being 3.7 mg.

TABLE 1.—Calcium in mg. per 100 cc. of serum (Wooley and Ross).

Kind of case	Total calcium	Diffusible calcium	Nondiffusible calcium
Stationary.....	9.4 - 11.5	4.0 - 5.6	4.4 - 7.5
Improved.....	8.7 - 11.0	4.1 - 6.3	3.8 - 6.5
Progressive.....	9.3 - 12.1	4.2 - 6.2	4.3 - 9.9
Normal average.....	10.4	5.4	5.0

A question, not yet investigated in leprosy, which probably would yield interesting results is the study of the phosphatase content of blood plasma. Bone changes being found in advanced leprosy, the power to hydrolyze phosphate esters by plasma is possibly disturbed. An investigation in this field is suggested.

ALKALI RESERVE

The beneficial results obtained by Nicolas in lepra reaction, using an alkaline treatment previously employed by Mitsuda, suggested investigation of the plasma alkali reserve before and after the reaction. Paras (70), studied the matter in 100 patients comprising various groups as follows: (a) leprosy without complication, (b) lepra reaction without alkali treatment, (c) lepra reaction with alkali treatment, (d) leprosy with tuberculosis, (e) leprosy with nephritis, and (f) leprosy with miscellaneous complications. Uncomplicated cases and those with tuberculosis showed normal values. When leprosy was accompanied by renal injury low values were found, indicating mild or marked acidosis. In lepra reaction without alkaline treatment there was slight decrease of the alkali reserve. Paras' figures are given in Table 2.

TABLE 2.—Alkali reserve, expressed in volume percent CO₂ combined in 100 cc. of plasma (Paras).

Condition of cases	Range	Average
Nonlepers.....	60 - 78	70
Lepers without complications.....	60 - 83	67.9
Lepers with lepra reaction, without alkali treatment..	52 - 78	66.5
Lepers with lepra reaction, with alkali treatment.....	64 - 98	75.7
Lepers with tuberculosis.....	68 - 78	72
Lepers with nephritis.....	26 - 78	61
Lepers with miscellaneous complications.....	50 - 84	59.6

Confirmatory results were reported by Roxas-Pineda, Ni-

colas and Lara (85) in 44 cases of lepra reaction and 18 of uncomplicated leprosy. The average for lepra reaction was 59 (range 48 to 69.6). However, no clinical symptoms of acidosis were present. These authors supposed that the decrease in the alkali reserve in the febrile cases is due to an excessive decomposition of tissue proteins. Treatment with sodium bicarbonate gives good results and the administration of ammonium chloride, which increases the acidosis, is harmful. The association of calcium treatment with alkaline therapy is advantageous in severe cases. Other authors, as Bejarano and Medina (12), have studied the alkali reserve in leprosy and reported low values. Herrera (37) found only a slight decrease in the neural type of the disease. He observed that a high protein diet is harmful to the patients because it produces an acid condition.

SERUM PROTEINS

Stevenson (91), Frazier and Wu (32) and Neill and Dewar (60) have shown that there is a marked increase in total serum proteins in leprosy, which is due to the high serum globulin values. The averages reported by Frazier and Wu are given in Table 3.

TABLE 3.—*Serum proteins in leprosy, in grams per 100 cc. of serum (Frazier and Wu).*

Type of case	Number of cases	Total proteins	Albumins	Globulins
Nodular.....	8	8.72	4.08	4.68
Mixed.....	7	8.67	4.15	4.52
Maculo-anesthetic.....	16	8.66	5.01	3.65
Normal.....	5	6.94	4.85	2.09

The high degree of hyperglobulinemia that is found in leprosy sera is equalled only in kala-azar and schistosomiasis (Sia and Wu, Meleney and Wu, cited by Frazier and Wu). The tests used for the diagnosis of kala-azar (formol-reaction, Ray's test, Sia's test), which depend on an increase in the globulin fraction of the serum, give positive reactions in leprosy, particularly in the nodular and mixed types.

Schlossmann (88) also obtained high figures for globulins (average of 4.53 gm. percent). He believes that the condition is related to several factors, as follows: erythrocyte destruction, fever, local injuries (resorption of bacterial products), auto-immunization, allergy and malnutrition. This author calls attention to the correlation existing between hyperglobulinemia

and the reticulo-endothelial system, which is frequently disturbed in leprosy (Peschkowski, 73, Herxheimer).

In the opinion of Wooley and Ross (106) the serum globulin decreases in improved cases and may be an index of good prognosis. Globulinemia varied inversely with the calcium content of serum. Rao (80) observed high values for globulin, principally in the cutaneous type of the disease. Treatment with chaulmoogra derivatives produces a fall in the globulin fraction of blood serum, followed by a slight increase. He observed that the sedimentation index varied inversely as the globulinemia. Paras, Lagrosa and Ignacio (72) showed that in leprosy the index is not related to the lipid content of the plasma (72).

The sedimentation index is a very useful test for following the evolution of an infectious disease and is related to the plasma protein content. Many authors agree that the red-cell sedimentation rate is correlated with the albumin-globulin ratio and the fibrinogen content of the blood plasma. The relation is so close that some authors (Theorell, Westergren, Widstrom, cited by Reichel, 82) have proposed formulae by which, the protein content of the plasma and the cell volume being known, it is easy to calculate the sedimentation index. A marked increase in sedimentation velocity is generally demonstrable in diseases in which hyperglobulinemia is frequently found, as kala-azar, schistosomiasis and lymphogranulomatosis, or in those with a relative increase in globulins (low albumin-globulin ratio), as tuberculosis.

The physical properties of serum (viscosity, specific gravity, refractive index) have been investigated in leprosy patients but the results are not concordant (Kusaka, 43). The increased refractive index found in some cases (Meineri, 56) may be due to the hyperproteinemia and has the same significance as variations in the serum globulins. The amino-acid content was studied in a few cases by Molinari and Ré (57) and is generally low, except in lepra fever. These variations are related to liver damage.

Lafrou and Bonnet (49, 48) are of the opinion that a probable diagnosis of leprosy may be confirmed when there is hyperglobulinemia, inverted albumin-globulin ratio and a normal Vernes index, syphilis being absent. When the Vernes index is abnormal and the other factors are present the diagnosis is less conclusive. With a normal Vernes index accompanied with normal values for globulinemia, the presence of leprosy is doubtful. As these authors pointed out, the Vernes index is not related to the globulin content

of blood serum, as was observed in other diseases (malaria, leishmaniasis and tuberculosis) by Chorine and Prudhomme (24).

IMMUNO-CHEMISTRY

Chemical studies in the field of the immunology of leprosy have been made by Schreus (89), Schlossmann (88), Marras (55) and more recently by Hayata (36) and Rhee (83). Some relationship between the serological reactions (Wassermann, Kahn, Vernes) and the protein and lipid content of blood serum have been reported.

BLOOD AND TISSUE LIPASE

The existence of a lipolytic enzyme which can destroy the external layer of the leprosy bacillus has not yet been demonstrated. However, many authors have tried to show that in active leprosy there is a marked decrease in the lipolytic power of blood serum (Rogers and Muir). Both Rogers and Pomaret believe that an increase of blood lipase is a good index of improvement, and that this increase permits the bacilli to be easily taken up by the white blood cells. Recently Shen (90), working with bacilli of the same group as the leprosy bacillus, was able to demonstrate the defensive value of the lipid layer against chemical substances because such organisms when cultivated in nonlipid-forming media are rapidly destroyed.

In tuberculosis Sparmann observed a decrease in serum lipase. Syphilis cases show low lipase values, which return to normal under treatment (Somogyi, Mietling, Shapiro, Bachkewitch). Blood lipase studies in leprosy are not in agreement. Aoki (6), using tributyrin, could not find any corresponding enzyme in the blood serum of lepers (in vitro) although with "gynocardic" (chaulmoogra?) oil an enzyme was produced.

Prichodcenko and Kruglijak (77) investigated lipase in serum treated by heat. The thermostable lipase increased when the infection was progressive and bacilli abundant. A fall was noted when improvement began. The lipase content, according to these authors, is proportional to the amount of bacilli and lymphocytes present. Quinine-resistant lipase not being found, they concluded that only pancreatic lipase is present. It will be borne in mind that they employed Hanriot's technique, which is liable to error (instability of monobutyryl, pH not considered). According to Neill and Dewar (59) low values occur only during lepra fever. Antileprosy treatment has no influence on the serum lipase. These results, therefore, do not confirm Roger's hypothesis. In 10 cases of leprosy (one neural, two nodular and seven mixed) Leigheb (50) found the serum

to have normal lipolytic activity. Zaslavski also reported normal values (28 cases). Henderson, in old cases, observed low lipase figures. Other authors (Sannicandro, Shaw and MacKenzie, Pooman, Paldrock) confirm these results, while Klingmüller cites Gollezkeri and Charpure as obtaining values four to six times higher than normal. Paldrock believes that substances active and effective in the treatment of leprosy induce an increase of blood lipase, and Pooman (75) studied the lipolytic activity of serum against monobutyryl, castor oil and lecithin. He observed an increase of lipase after treatment with chaulmoogra derivatives.

The contradictions existing in the literature may be attributed to the inexactitude and the lack of standardization of the techniques employed. In the opinion of the writer uniform results cannot be obtained by determining either the quantity of the acids liberated by the enzyme or the fall of surface tension (Rona's technique).

Skin lipase shows normal values, according to Sannicandro. Stearase (i.e., the enzyme which hydrolyzes esters) is possibly an important factor in the mobilization of skin tissue lipids. Porter (76), in 1913 and later, showed that tissues rich in stearase are especially resistant to infection. The lung, being poor in that enzyme, is susceptible to tuberculous infection. The tissues of the cat are rich in it, and that animal is very resistant to the tubercle bacillus. Recently, Emerson, Anderson and Leake (29) found low values for tissue lipase in rats infected experimentally with rat leprosy. They concluded (31, 30) that if the chaulmoogrates exert any indirect action in leprosy therapy, besides their direct bactericidal action, it is improbable that the fat-splitting ferment is involved.

CHOLESTEROL AND CHOLESTEROL ESTERS

Blood plasma cholesterol varies normally between 160 and 200 mg. per 100 cc. (Myers, Bloor, Boyd, Man and Gildea, Villela and Silva). Cholesterolemia in leprosy has been studied by several investigators. Marchand (54), the first author to publish on the subject, found in three mixed and one nodular case that there was a slight decrease in the older cases (120 to 130 mg. percent) and normal values in the earlier ones. Boulay and Leger (17), employing Grigaut's technique (normal value 160 mg. percent) found low figures in four cases, two old and two new ones. Chaulmoogra treatment produced an increase when the patient was in good condition and was recently infected. Girard and Woltz (33) found only low values for cholesterol in the nodular type of the disease.

Ushida (95), studying 126 cases, found an average of 121.9 mg. per 100 cc., which is slightly below the normal range established by him (150.6 mg.). Borgatti (14), using the Autenrieth and Funk method, found hypocholesterolemia in a few cases, except in one in which psoriasis was associated (213 mg. percent). Boyd (18) also found a decrease of plasma cholesterol, principally in old cases. According to this author antileprosy treatment has no influence on this substance. Many other authors, as Otero and Hernandez in Puerto Rico (64) and Gomes, Leitao and Wancolle in S. Paulo (34), have reported low values. The latter authors observed that cholesterolemia is inversely proportional to the severity of disease, and that chaulmoogra treatment causes an increase. Balbi (9) found hypocholesterolemia with, however, no relation to the degree of the infection.

The hypocholesterolemia observed in leprosy is due to a decrease in both fractions of cholesterol (free and bound as esters) according to Vilella and Castro (98), who recently studied 55 cases. Black and Ross (13), found in patients with stationary lesions that the ester fraction of the plasma increases. However, these cases gave a positive van den Bergh test for bilirubin, and therefore the increase was related to liver damage.

Paras (71) obtained the following results: In untreated cases plasm cholesterol is slightly reduced; in cases treated with chaulmoogra oil derivatives but still bacteriologically positive the values are somewhat higher than in untreated cases; in treated cases that have become negative cholesterol is within normal limits. Vilella, Castro and Anderson (99) have more recently shown that hypocholesterolemia appears principally in recent cases and pertains to the mixed type of the disease. In those in which the duration of the disease is more than ten years they observed higher figures (100). Only slight differences were found between treated and untreated lepers, which is in accordance with Boyd's findings.

Variations that occur in relation to sex, age and race are of the same order as those encountered in normal people (higher values in older and in women). White people show higher values than Negroes (Vilella, Castro and Anderson). In experimental rat leprosy Chorine and Grougue (23) reported irregular low values.

TOTAL LIPIDS, FATTY ACIDS AND IODINE NUMBER

Normal values for total lipids in blood plasma average 600 mg. per 100 cc. (Boyd 589 mg., Wilson and Hansen 657 mg., Vilella, Castro and Anderson 603 mg.), and for total fatty acids

320 mg. (Man and Gildea 53), 350 mg. (Villela and Silva 101), 353 mg. (Boyd 18). Normal plasma values for the iodine number vary around 88 (Boyd, Csonka, and Villela, Castro and Anderson).

Blood plasma lipids in leprosy were studied for the first time by Paras (71), who found low figures for total fatty acids in untreated or insufficiently treated cases. On the other hand Ito (39), observed low cholesterol and high fatty acids in almost all of 33 cases. Phospholipids of the blood plasma in lepers vary within normal limits (Paras). Anderson and Anderson (1) determined the iodine number for the fatty acids in leprosy blood serum ("cuti-neural," or mixed cases) and concluded that in improving cases lower values are observed (average 54.5) than in advanced ones (average 77.8). One case of lepra fever showed marked decrease (31.2).

Recently Villela, Castro and Anderson (99), studying 100 cases of all forms of the disease, found low iodine numbers in the plasma, specially in untreated lepers. The average total lipids were increased, while cholesterol was decreased. Iodine number values were within the lower normal range (Table 4).

TABLE 4.—Blood lipids in 100 cases of leprosy, in mg. per 100 cc. of blood plasma (Villela, Castro and Anderson).

Kind of case	Total lipids	Fatty acids	Iodine values	Cholesterol
Total cases.....	857	386	62.8	129
Cutaneous type.....	856	390	62.9	131
Neural type.....	861	387	60.5	136
Mixed cases.....	851	364	63.5	127
Treated cases.....	819	391	58.4	130
Untreated cases.....	873	384	64.7	128

GLUCOSE

Paras (69) reported a slight hyperglycemia in leprosy without relationship to the duration of type of the disease. Other investigators (Wayson, Wooley) have confirmed these findings, but Lai (44) found normal values in 59 cases, with a low glucose excretion level. Hypoglycemia was observed by Rajewski (79) in 31 cases, but he claims that there exists a disturbance in the carbohydrate metabolism which is detected by the glucose tolerance test. He found progressive as well as stationary cases to present hyperglycemic curves possibly related to liver, adrenals and pancreas damage. According to Wayson, Badger and Dewar (103) glycosuria frequently appears three to twelve hours after meals (23.4 percent positive tests in 175 cases). A summary of the data published on glycemia of lepers is given in Table 5.

In rat leprosy Emerson (28) encountered abnormally high, prolonged glucose-tolerance curves and these were interpreted as evidence of liver damage. Prolonged curves are present in rats in the more advanced stages of the disease, which show extensive histological lesions of the liver. In early rat leprosy the microscopic appearance and glycogen content of the liver are normal.

TABLE 5.—*Glycemia in leprosy. (Glucose in mg. per 100 cc. of blood).*

Author	Averages	Number of cases	Method used
Paras.....	119	17	Folin-Wu
Wayson, Badger and Dewar.....	108	32	Benedict
Wooley.....	109	80	Folin-Wu
Lai.....	88.5	59	Folin-Wu
Rajewski.....	54-120	31	—

OTHER BLOOD CONSTITUENTS

Nonprotein nitrogenous substances of the blood show significant quantitative alterations in leprosy only when the cases are complicated by renal impairment. Nephritis being found in high percentages among lepers, these nitrogenous constituents (urea, creatinine, uric acid, non-protein nitrogen) must be considered (Paras, 69). When kidney disturbances are not present nitrogenous non-protein substances are within normal limits. No studies have been made in the field of blood biochemistry in relation to antileprosy treatment and the pathology of kidney damage. The estimation of chaulmoogra derivatives in the urine also would give important information with regard to the drugs to be employed.

Recently Lee (47) studied sodium chloride metabolism in 40 cases of leprosy. Blood and urine were investigated and normal values were obtained (480 to 497 mg. percent). He concludes that there is no evident disturbance of sodium chloride metabolism in this disease. The observation of Keil (40) that a diet poor in sodium chloride is favorable to the improvement of lepers suggests that an accurate investigation in this field should be made. The free acid content of the gastric secretion of lepers is somewhat lower than normal, as observed by Cerqueira and Anderson in 25 cases (22, 2). Lee (46) also found decreased free acidity of gastric juice in some instances, and even achylia was found. A possible relationship between hypoacidity and sodium chloride metabolism may exist but investigations in this direction are lacking.

As in animal tuberculosis, the blood and tissue glutathion was found decreased in rat leprosy by Prudhomme (78) and in human

leprosy by Takashima (93). Blood and urine urobilin and bilirubin were investigated by Molinelli and Royer (55) who observed normal values in 23 cases.

(To be concluded)