

THE BIOCHEMISTRY OF LEPROSY

A REVIEW

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(Conclusion)

METABOLISM AND NUTRITION

Recent biochemical investigations have been directed toward establishing close relationships between nutrition and infection. A few observations have already been published that show a possible influence of a diet rich in vitamins on the frequency of leprosy infection. Brown (20) in Nigeria administered vitamin B to lepers submitted to a poor diet. The results obtained were not very convincing but a gain in weight of the patients was observed. Pellagra is found in regions in which there is an abundance of leprosy and the diet is poor in vitamin G (B₂). With this fact in mind Basu (10, 11) administered a diet rich in vitamin B and G and in proteins. Neural cases improved while nodular ones remained stationary. Atkey (7), from his experience in the Anglo-Egyptian Sudan, is of the opinion that in that country a low intake of milk is the cause of a lack of vitamins in the diet and consequently of a high leprosy incidence.

Faulty nutrition is noteworthy as a real factor which permits the dissemination of the disease, because of decrease in the defense against it. The development of the leprosy bacillus being related to the nutritional state of the animal or human organism, studies in the field of nutrition are of paramount interest to the leprologist. Rat leprosy is very similar to human leprosy, and nutritional factors (vitamins, protein intake, etc.) influence the former as they do the latter.

Kobashi (42) found that in experimental rat leprosy a diet rich in vitamins tended to attenuate the infection. Vitamin B was more active than vitamin A; vitamin C had no influence. It is to be noted that the animals employed by Kobashi to produce avitaminosis C were guinea pigs, which are not susceptible to the leprosy infection.

Lambe (45) called attention to the presence of beriberi in regions where leprosy is endemic. In experimental rat leprosy he found that the animals are more sensitive to infection when kept on a diet poor in vitamins of the B complex (Lamb, 45). Rats infected with rat leprosy and with avitaminosis present more extensive lesions of the liver than those on a normal diet.

Avitaminosis A is related to leprosy in the opinion of Nicolls (62), who made an epidemiologic study of the occurrence of this condition in Ceylon. As in tuberculosis, leprosy is more frequently found when nutritional conditions are bad. Keil (40) recalls the fact that both infections attack the human organism in childhood, i.e., when nutrition plays a particularly important part. Applying to leprosy the dietetic rules formerly used by Gerson, Hermannsdorff and Sauerbruch in the treatment of skin tuberculosis, Keil obtained satisfactory results. He observed that in Suriman (Dutch Guiana) the diet of the inhabitants is poor, consisting of unpolished rice and salted fish, which is very injurious to lepers. Abolishing from the diet the sodium chloride, and introducing foods rich in vitamins and fat and poor in carbohydrate (glucides) and animal proteins, good results were quickly obtained. Such a diet is more effective in patients presenting edema and hypersensitivity than in others.

CHEMISTRY OF MYCOBACTERIUM LEPRAE

The first information concerning the chemistry of the Hansen bacillus (*Mycobacterium leprae*) was based on histo-chemical studies of tissues rich in bacilli because of the difficulty in obtaining pure cultures of this microbe. Later the staining properties of the fatty substances obtained from a supposed culture of the leprosy organism by extraction with various solvents, and also the acid-fastness of the extracted bacilli, were studied by microchemical methods (Gurd and Denis). Unna's histochemical technic has been applied by some authors (Herxheimer, Paldrock, Mitsuda) but has given only qualitative information.

Paldrock (66), using tissues rich in bacilli, showed that their granules contain lipid and lipoproteins, which can be stained with fuchsin when previously acidified with 10 percent nitric acid. The lipoprotein fraction he called "plasteoprotein." Upon addition of a hot alcohol-hydrochloric acid mixture a basic albumin was separated by dissociation (66, 67). The albumin is present in the form of a nucleoprotein and a "caryoprotein." The granules, like those of the tubercle bacillus, contain nucleic acids (caryonic acid)

and are gram positive, the fatty acids being gram negative (66, 68). According to Paldrock, therefore, leprosy bacilli consist of free and bound nucleic acid, free lipoids and basic proteins. He showed that bacilli from cultures (strains isolated by Kedrowsky and Schlossberger and by C. Martin) gave microchemical reactions identical to those obtained with bacilli in leprosy tissues (67). On the basis of this observation he concluded that these cultures are of the true leprosy bacillus (68).

Mitsuda employing the polarimeter and staining with Sudan III, found lipid substances in the bacillary bodies, but no cholesterol. According to Cedercreutz, leprosy tissues contain doubly-refracting corpuscles and therefore cholesterol esters. Herxheimer was not able to confirm this finding. The writer and Portugal studying tissue sections of lepromata with a polarizing microscope and microchemical technique, were unable to find cholesterol esters.

Uyei and Anderson (96) studied extensively the chemistry of the tubercle bacillus. They showed that the waxy substance contained in the lipid fraction of that organism is saponified with great difficulty, but that after complete saponification it yields higher alcohols and fatty acids. Tamura, in 1913, studying the alcohols of the waxy substance, obtained one of high molecular weight ($C_{29}H_{58}O$) which he named "mykol." This alcohol is acid-fast and is probably responsible for the acid-fastness of the tubercle bacillus. Mykol gives none of the sterol reactions, but according to Wells, De Witt and Long (104) it belongs nevertheless to the cholesterol group. The wax purified by Anderson is not acid-fast, but the unsaponifiable fraction has acid-fast properties because of the presence of mykol.

The fatty acids isolated from the tubercle bacillus are of different types—cerotic, palmitic, oleic, stearic. Liquid fatty acids constitute 45 percent of the total and represent the biologically active fraction. The acids isolated from the acetone-soluble fraction by hydrolysis of phosphatides were phthioic acid and tuberculostearic acid (Anderson and Chargaff, 4). Sabin, Doan and Forkner (86) found that phthioic acid, when injected in the peritoneal cavity of guinea pigs, stimulates the proliferation of monocytes and epithelioid cells and causes the formation of massive artificial tubercular tissues.

The first attempt at chemical analysis of cultures of leprosy bacilli was made by Gurd and Denis, in 1911 (35); the organism studied was one that had been isolated by Duval. These authors

were able to extract from the fatty fraction a waxy substance containing phosphorus that they supposed to be a lecithin. The pigment they identified as a lipochrome. The fat upon hydrolysis yielded unsaturated fatty acids. Unsaponifiable substances separated after saponification gave a cholesterol reaction.

Long and Campbell (52) have reported a series of analyses of acid-fast organisms for total and non-saponifiable lipids. Table 6 shows that high lipid content for acid-fast as compared with non-fast micro-organisms.

TABLE 6.—*Lipid content of acid-fast bacilli (Long and Campbell).*

Organism examined	Total lipids in percent of dry weight	Saponification number	Percent total lipid, non-saponifiable
H-37, Human type tubercle bacillus.....	22.7	131	77.1
B-1, Bovine tubercle bacillus.....	22.3	139	60.0
A-1, Avian tubercle bacillus.....	11.0	194	35.7
Lepra bacillus, Duval.....	9.7	188	27.2
B. subtilis.....	4.4	—	—
Staphylococcus albus.....	2.8	—	—

Another cultured organism was more recently investigated by Uyei and Anderson (96) who used a technique similar to that employed in their study of the tubercle bacillus. The culture used by these authors is known as the Hygienic Laboratory Strain No. 370 (Apa case), isolated from a human leprosy case in Honolulu in 1909; the material that they examined was obtained from 3,000 cultures of this organism. Their results are given in Table 7, taken from their report.

TABLE 7.—*Substances isolated from a culture of M. leprae, and one of M. tuberculosis hominis (Uyei and Anderson).*

	<i>M. leprae</i>		<i>M. tuberculosis</i>	
	Grams	Percent	Grams	Percent
Phosphatide.....	100.5	2.25	253.1	6.54
Acetone-soluble fat.....	289.5	6.47	240.0	6.20
Chloroform-soluble wax.....	444.8	9.98	427.0	11.02
Total lipids.....	834.6	18.70	920.1	23.78
Polysaccharide.....	41.2	0.92	33.9	0.87
Dry bacillary residue.....	3,389.8	80.38	2,902.0	75.01
Dry bacterial matter per culture....	1.488	—	1.928	—

The most apparent differences between this organism and the tubercle bacillus found by these authors are in the phosphatide and wax content. The phosphatide fraction after purification yielded

a neutral waxy substance which Anderson and his coworkers (5) designated "leprosin." On saponification leprosin liberated some fatty acids, glycerol and an aliphatic secondary alcohol. Among the fatty acids (myristic, palmitic, stearic, tetracosanic) isolated by these investigators was a new hydroxy acid which they called "leprosinic acid."

Leprosin, like mykol of the tubercle bacillus, is acid-fast and according to Sabin is probably responsible for the acid-fastness of the bacilli. It does not give the color reactions for cholesterol. The antigenic properties of the bacilli are supposed to be associated with the higher fatty acids and possibly even with the water-soluble fraction (polysaccharide).

This brief review is intended to indicate what has already been accomplished in the field of the biochemistry of leprosy and may help investigators who are interested in continuing the work. Certain possibilities for further research in the metabolism and biochemistry of human leprosy are suggested.

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