THE BIOCHEMISTRY OF BACILLUS LEPRÆ.*

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Since the cultivation, about two years ago, of the leprosy bacillus, increased attention has been directed to the study of immunity processes in infection caused by this microörganism. Experiments which aim at the development of some method for the specific treatment of the disease have already been undertaken by Dr. Duval and his associates in this laboratory, and by Clegg in the Philippines. The present study of the chemical components of *Bacillus leprœ* has been made in order that a more analytic examination of the immunity-inducing substances present in the microörganism may be carried out. We believe that even though the results obtained in therapy with injections of killed bacillary suspensions prove efficacious, it will be necessary to test the relative value of extracts prepared in different ways. The present work will indicate the constituents of the organism found in such extracts as are now employed in the treatment of human leprosy.

Our investigations have been confined chiefly to the quantitative determination of water, fats, lipoids, and proteins. The components of certain of these groups have been identified and estimated, and their relative toxicity has been tested. Our studies serve further to differentiate from *Bacillus tuberculosis* the acid-fast bacillus isolated by Dr. Duval from human leprous lesions.

Contrary to a preconceived notion, we have found that notwithstanding the moist appearance of fresh leprosy cultures as compared with those of the tubercle bacillus, the constant dry weight of the former is relatively higher than that of the latter. According to Hammerschlag,[1] whose findings have been corroborated by many other observers, the average water content of the tubercle

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[1] *Centralbl. f. klin. Med.*, 1891, xii, 9.

bacillus is 85.9 per cent. The constant weight of *Bacillus lepræ* varies considerably according to the moisture present in the medium upon which it is grown, and is found to range from 21.2 to 34.6 per cent. of the total moist weight.

Method.—The cultures tested were grown upon alkaline fish agar[2] containing 3 per cent. glycerin agar, and were scraped from the surface by means of a heavy platinum loop. They have been under cultivation for more than fourteen months, so that a moderately profuse growth is obtained in five or six days. Ordinary culture tubes in which the medium is solidified in the sloped position and poured into Petri dishes have been used in cultivating the organisms.

Upon removal of the growth material from the surface of the agar, it was placed in glass dishes in very thin layers, weighed, and subsequently dried in an air bath for forty-eight hours at a temperature of 62° C. Heating at this temperature for forty-eight hours has been found to bring about constant weight of the growth and does not interfere with the morphology and staining properties. Moreover, although this treatment suffices to kill the bacilli, their toxicity and sensitizing properties are not markedly lessened, as has been shown by animal experiments. When dried in this manner, the material consists of an almost transparent golden brown substance resembling toffee in appearance and being of a similar brittle consistence.

Fat.—In the determination of fat, the dry material was extracted twenty times with cold, and twenty times with boiling acetone. The various extracts were then united, the acetone distilled off, and the residue dried over sulphuric acid to constant weight. The final product had a characteristic acrid odor and consisted of a deep brown, viscous, semi-fluid mass, which, on microscopic examination, was found to contain a few slender, prismatic crystals. 3.95 grams of the dry bacilli yielded 1.37 grams, or 34.7 per cent. of extract.

The presence of free fatty acids could be demonstrated, while an immediate blackening with osmic acid indicated the presence of the glycerids of oleic acid. On saponifying 1.2 grams of the bac-

[2] *Jour. Exper. Med.*, 1911, xiii, 365.

terial fat with alcoholic potash and subsequently extracting the alkaline solution with ether, 0.001 of a gram of a fatty material was obtained, which, on the application of the usual qualitative color tests, gave positive reactions for cholesterol.

The residue left after extraction with acetone was treated fourteen times with boiling chloroform, which, upon distilling off the solvent, permitted the recovery of a small amount of material. This was dissolved in choloroform and reprecipitated by the addition of an excess of acetone. The precipitate, which consisted of a pale yellow, waxy solid, weighed, after drying over sulphuric acid, 0.07 of a gram (equivalent to 1.1 per cent.), and was found to contain phosphorus and to possess the properties of lecithin.

Protein.—After treatment with chloroform, there remained 2.5 grams of a pale yellow material which was almost insoluble in water and in cold dilute acids, but readily soluble in a solution of dilute potassium hydroxid from which it was not precipitated by the addition of acetic acid. This material reacted positively with the biuret, molish, and xanthoproteic tests, with the Hopkins and Cole tryptophan test, and with Millon's reagent, thus proving the presence of a carbohydrate radical, and of tryptophan, tyrosin, and other members of the aromatic series. Sulphur and phosphorus are both demonstrable, the latter in considerable amounts. Nitrogen is present to the extent of 8.20 per cent.

A small quantity of the fat-free portion of the bacilli was boiled with hydrochloric acid until dissolved. The solution was then saturated with ammonia, and formed, on the addition of an ammoniacal solution of silver nitrate, a flocculent precipitate of the silver compounds of the nuclein bases.

Carbohydrates.—Watery extracts of *Bacillus leprœ* give no reduction with Fehling's solutions. Levene[3] has found in the tubercle bacillus a glycogen-like body soluble in water and in dilute salt solutions; but an attempt to isolate a similar substance from *Bacillus leprœ* was unsuccessful.

Coloring Matter.—The greater part of the material which gives to cultures of *Bacillus leprœ* their bright yellow tint belongs to the group of lipochromes. The substance is soluble in all the ordi-

[3] *Med. Rec.*, 1898, liv, 873.

nary fat solvents, such as ether, alcohol, acetone, chloroform, etc., and gives with concentrated sulphuric acid and with nitric acid the color changes characteristic of this class of dyes. There is also present another yellow coloring matter which is insoluble in the solvents used for fat extraction, and which may be extracted with water from the residue left after treatment with chloroform.

A summary of the composition of moisture-free bacilli is given in the following table.

Acetone-soluble extract	34.7 per cent. (neutral fats, free fatty acids, cholesterol.)
Acetone-insoluble, chloroform-soluble extract	1.7 per cent. (lecithin.[4])
Residue	63.6 per cent. (nucleo-proteid, nucleic acid, ash.[5])

Two strains of the leprosy bacillus have been tested for lecithin by methods similar to those described above. In general, the variations obtained are not more marked than can be readily explained by differences in media and errors of experiment.

The fatty content of the tubercle bacillus has been differently estimated by several observers. The variations noted have usually been from 22 to 39.6 per cent., the latter figure representing the benzol-soluble material. Hammerschlag, using alcohol and ether as extractives, recovered 26.2 per cent. of the total dry weight of the bacillus. Klebs,[6] using the same solvents, found but 22 per cent. De Schweinitz and Dorset[7] alone have been successful in isolating as much as 39.6 per cent.

By most investigators, cholesterol is said to be absent, and, as far as we have been able to discover, it has been identified by none.

[4] Preliminary estimations of lecithin resulted in a large percentage of this material, which may be accounted for by differences in the media used in growing the organisms.

[5] Although owing to scarcity of material, actual quantitative estimations of the ash have been impossible, it is evident that a very small portion consists of this substance.

[6] *Centralbl. f. Bakteriol., 1te Abt.,* 1896, xx, 488.

[7] *Jour. Am. Chem. Soc.,* 1895, xvii, 605.

Hammerschlag has found minute quantities of a phosphatid fat, but his observations have not been confirmed.

TABLE I.

Protocols of Mice Inoculated with Leprous Protein and Dead Bacilli.

Animal.	Dose.	Material.	Result.
Full grown mouse	1.00 c.c.	4 per cent. L.P.*	Died in 6 hours.
Full grown mouse	0.50 c.c.	4 per cent. L.P.	Died in 6 hours.
Full grown mouse	0.25 c.c.	4 per cent. L.P.	Died in 6 hours.
Full grown mouse	0.25 c.c.	4 per cent. L.P.	Died in 6 hours.
Full grown mouse	0.10 c.c.	4 per cent. L.P.	Very sick for 36 hours; recovery.
Full grown mouse	0.05 c.c.	4 per cent. L.P.	No apparent symptoms.
Full grown mouse	0.05 c.c.	4 per cent. L.P.	No apparent symptoms.
Full grown mouse	0.05 c.c.	4 per cent. L.P.	No apparent symptoms.
Full grown mouse	0.50 c.c.	4 per cent. D.L.*	Sick 24 hours; recovery.
Quarter grown white mouse	0.50 c.c.	4 per cent. D.L.	Died in 30 hours.
Full grown white mouse	0.50 c.c.	4 per cent. D.L.	No apparent symptoms.
Full grown white mouse	0.25 c.c.	4 per cent. D.L.	No apparent symptoms.
Full grown white mouse	0.10 c.c.	4 per cent. D.L.	No apparent symptoms.
Ful grown white mouse	0.05 c.c.	4 per cent. D.L.	No apparent symptoms.

* L.P. = leprous protein; D.L. = dead bacilli.

The essential chemical differences between the leprosy bacillus and the tubercle bacillus are the relatively higher water content of the latter and the presence of comparatively large quantities of lecithin and cholesterol in the former.

Toxicity.—Experiments have been performed with a view to determining the relative toxicity of the bacillary constituents as compared with the whole organism. It should be stated that like the tubercle bacillus the essential toxicity of the leprosy bacillus is very low. The observation of human cases harboring enormous numbers of the bacilli without showing symptoms of specific intoxication proves this fact. That the bacillus does, however, possess a certain degree of toxicity, and that the protein content is the source of poison, is shown by the following experiments.

Into the peritoneal cavity of fifteen white mice were inoculated simultaneously a suspension of dead bacilli, killed by heating to 62° C. for forty-eight hours, and a suspension of leprous protein. Both suspensions contained 4 per cent. by weight of the dried material.

The effect produced by the inoculation of both dead bacilli and leprous protein appears to be of the nature of collapse. The animals huddle together in the corner of the cage and can not be induced to move. Respiration becomes accelerated and the animal falls into a stuporous condition and dies.

At autopsy, with the exception of a slight peritoneal change, no lesions of any kind were seen in any of the animals injected with leprous protein. However, in the peritoneal cavity of each animal there was found, about the site of inoculation, a localized area of slightly brownish discoloration, which was apparently the remains of injected material. On microscopical examination, the small amount of material present in these areas was seen to contain no fibrin.

The mice dying as the result of inoculation with dead bacilli showed a more marked lesion of the peritoneum, as was evidenced by the presence of a moderate amount of exudate. Microscopically, the exudate contained fibrin and cells chiefly of the polymorphonuclear type, together with a few large mononucleated cells. The former as well as many of the latter were filled with leprosy bacilli.

From these experiments we conclude that the protein portion of the organism represents practically the whole of the toxic body. Indeed, it appears that instead of destroying the potency of the toxic property of the bacillus, the freeing of the organism from its fatty covering renders the toxin more absorbable so that it acts more quickly and effectively.