

Städeler¹ by treating fibroin with 5 per cent NaOH and subsequently neutralizing with HCl. The material so obtained can be finely ground, and by using a large needle a suspension of this substance can readily be injected intraperitoneally. Two rabbits received 7 doses of 100 mg. each over a period of two weeks, and two others were given 8 injections of 25 mg. each over a period of two months. Attempts to demonstrate the presence in the blood stream of fixation antibodies were entirely negative.

169 (2692)

Studies in tuberculosis. V. Phosphatids of tuberculin with diagnostic and sensitizing properties.

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In a series of earlier publications¹ an alcohol-soluble and ether-precipitable substance, "Ether-insoluble X," was described in connection with experiments on sensitization and diagnosis in animals and in tuberculous patients. Preliminary qualitative procedures pointed toward identifying this derivative with a phosphatid, probably lecithin, which represented a small fraction of the original old tuberculin. Typical tuberculin reactions were elicited, notwithstanding the exceedingly high dilutions that were used in the tests. It was suggested that the antigenic radical of *B. tuberculosis* might reside in such a fraction, and that the isolation of this derivative from tuberculin might offer a lead in the direction of explaining the mechanism of tuberculin reactions and the phenomena of sensitization which I have reported previously.

The present report is a chemical study of some lecithin-like fractions which have been obtained from old tuberculin (human)

¹ Städeler, G., *Ann. d. Chem. and Pharm.*, 1859, iii, 12.

¹ Ebersson, F., *PROC. SOC. EXP. BIOL. AND MED.*, 1924, xxi, 539; *Am. J. Dis. Child.*, 1925, xxix, 29; *Am. Rev. Tuberc.*, 1925, March (in press).

prepared from beef-extract media,* and from two different synthetic substrates which were described by Löwenstein and Pick,² and by Gessard and Vaudremer.³ Quantitative analyses of the derivatives from these protein-free media will be published shortly. Qualitative studies have indicated that all of these derivatives are identical, and give typical skin tests regardless of the kind of media used in the preparation of tuberculin.

Tuberculin, in weighed 50 gram portions (49 cubic centimeters), was added drop by drop from a burette to pure methyl alcohol until complete precipitation was effected. Nineteen volumes of alcohol were required for each volume of tuberculin. To the clear filtrate which yielded no further precipitate, was added pure ethyl ether until complete precipitation occurred. This procedure required 3.4 volumes of ether for each volume of alcohol filtrate. The yield by alcohol precipitation from 50 grams of tuberculin was 7.45 grams dry weight, representing 14.9 per cent of the original tuberculin. Ether precipitation from 980 cubic centimeters of the alcoholic filtrate yielded 6.88 grams of substance, or 13.8 per cent of the original tuberculin. The ether residue obtained by evaporation and drying yielded 1.62 grams from 3,000 cubic centimeters of ether filtrate.

The alcohol precipitate is chocolate-brown in color, is freely soluble in water and in physiologic salt solution, from which it can be re-precipitated upon addition of alcohol. It contains 11.3 per cent Kjeldahl Nitrogen.

The ether-insoluble fraction obtained from the alcoholic filtrate is pink in color, browns on exposure to air, and the precipitate is gummy and hygroscopic. Repeated washing of the substance with ether causes a loss of hygroscopic properties with an approximate reduction of 0.03 per cent by weight. (The hygroscopic character of the material is due probably to the union of the ether-soluble fraction to the ether-insoluble portion.) The Kjeldahl Nitrogen present was 4.8 per cent.

The ether residue is gray in color, sticky, hygroscopic, fatty or waxy, gives none of the tests for protein, is soluble in acetone

* The tuberculin used in this work was prepared and donated by Eli Lilly and Company, Indianapolis, Indiana, through the courtesy and kind co-operation of the Biological Department.

² Löwenstein, E., and Pick, E. P., *Biochem. Z.*, 1911, xxxi, 145.

³ Gessard, C., and Vaudremer, A., *Compt. rend. Soc. de Biol.*, 1922, lxxxvii, 1012.

and in benzol, and is insoluble or only slightly soluble in chloroform. The nature of this substance and more detailed description of its properties are deferred for the present, pending completion of further studies. This derivative represents approximately 3.2 per cent of the original tuberculin by weight, and contains 0.0045 per cent Kjeldahl Nitrogen (not checked).

Positive skin tests were obtained by both Pirquet (cutaneous) and Manteau (intracutaneous) methods in dilutions ranging as high as 1:10,000,000. In view of the fact that these derivatives represent small fractions of the original tuberculin, their effective potency appears to be very great. The actual range of activity, therefore, was calculated between the limits of 1:72,000,000 and 1:333,000,000.

TABULATION OF QUALITATIVE TESTS.

	Alcohol Precipitate	Lecithin Derivative
Coagulability	Positive	Negative
Heller Test	"	"
Amm. Sulphate	"	"
Potass. Ferrocyamide	"	"
Picric Acid	"	"
Phosphotungstic Acid	"	"
Tannic Acid	"	"
Copper Sulphate	"	"
Mercuric Chloride	"	"
Silver Nitrate	"	Positive
Iodin Potass. Iodide	Negative	Negative
Mercuric Iodide	"	"
Xanthoproteic Test	Positive	"
Millon Test	"	"
Biuret Test	"	"
Adamkiewicz Test	"	"
Liebermann Test	"	"
Lead Acetate	—	Positive
Molybdic Test	Positive	"
Lassaigne Test	"	"

The studies up to the present suggest that the antigenic radical of *B. tuberculosis* is contained probably in tuberculin derivatives such as these which have been described. That a specific substance is associated with the lipoids of *B. tuberculosis* has been shown by Boquet and Nègre,⁴ whose methyl alcohol extract of the organism conforms to the tuberculin derivative which we have described previously. Additional observations which appear to confirm our own studies may be found in those dealing with Wildbolz reaction. Von Bergen⁵ and Ichok⁶ found an alcohol-

⁴ Boquet, A., and Nègre, L., *Ibid*, 1922, lxxxvi, 717.

⁵ von Bergen, J., *Schweiz. med. Wchnschr.*, 1921, xli, 655.

⁶ Ichok, M. G., *Ann. d. Med.*, 1921, ix, 97.

soluble antigen in the urine of tuberculous patients. Recently, Enright and Rettger⁷ reported on the chemical nature of the alcohol-soluble antigen in urine from tuberculous animals and patients, and characterized the substance as a phosphatid, probably lecithin, in accordance with its chemical properties.

⁷ Enright, J. J., and Rettger, L. F., *Am. Rev. Tuberc.*, 1924, x, 104.