considerably less than the 135 kcal. supplied by one einstein at λ 2138 Å.

Summary

The photodecomposition of ammonia is found to proceed about 1.3–1.5 times as rapidly as that of deutero-ammonia in the predissociation bands at λ 2100 Å. Approximately the same ratio holds for the rate of decomposition of deutero-ammonia at λ 2138 Å. in a discrete band to that at λ 2100 Å. in a diffuse band. These differences in rates are discussed and explanations to account for them suggested.

ROCHESTER, NEW YORK RECEIVED MARCH 23, 1937

[CONTRIBUTION FROM THE HENRY PHIPPS INSTITUTE OF THE UNIVERSITY OF PENNSYLVANIA]

The Chemical Composition of the Active Principle of Tuberculin. XX. Comparative Study of the Yield, Potency, Specificity and Acid–Base-Combining Capacities of the Proteins from Five Human Tubercle Bacilli Culture Filtrates and other Acid-Fast Bacilli

BY FLORENCE B. SEIBERT[1]

During comparative studies of the chemistry of different strains of tubercle bacilli, the question has arisen whether as great differences might not occur on repeated examinations of the same strain. It is also a question whether constant differences can be established between tubercle bacilli and other members of the large group of acid fast bacteria, to which the tubercle bacillus belongs. In addition to the well-known method of differentiating the human, bovine and avian types of tubercle bacilli by means of their respective virulences for different animals, the distinctions made by Wilson[2] on the basis of agglutination and by Furth[3] on the basis of complement fixation and absorption experiments, it has recently been possible to differentiate them by means of the serological precipitin reaction,[4] using the purified proteins isolated from the bacillary culture filtrates as antigens. Furthermore, the proteins from these three types of tubercle bacilli, as well as from the acid-fast bacillus found on timothy grass, differed quantitatively in potency when equal doses were injected in the skin of tuberculous animals,[5] whereas no difference whatever could be distinguished between the potencies of proteins prepared from three different strains of the human type tubercle bacilli which differed widely in their virulence.

Chemical differences in the lipide contents of the different types of tubercle bacilli, human, bovine, avian, and the acid-fast timothy bacillus have been reported by Anderson[6] and his coworkers, and Chargaff and Dieryck.[7] Anderson noted especially the presence of an optically active liquid saturated fatty acid, phthioic acid, in the human H37 strain, whereas analogous acids from the other types of acid-fast organisms were optically inactive. He has made a further report[8] on the lipide contents of the bacilli of four different cultures of tubercle bacilli, recently isolated from human cases of tuberculosis, in comparison with results obtained on a freshly grown lot of the human type H37 bacillus.

The following paper is a comparative study of the proteins isolated from the culture filtrates of the same five batches of human tubercle bacilli used in Anderson's[8] studies. The results will be compared with similar studies on comparable proteins prepared from the bovine and avian tubercle bacilli and leprosy and timothy grass acid-fast organisms.

Experimental

History of Strains of Human Tubercle Bacilli Used.—*Strain A10*—isolated May, 1932, on guinea pig passage from retroperitoneal lymph glands of patient (25435). Pathogenic for guinea pigs and only very slightly for rabbits. Recent test showed no pathogenicity for rabbits.

Strain A12—isolated November, 1932, from the sputum of patient (38135). Pathogenic for guinea pigs; nonpathogenic for rabbits.

Strain A13—isolated December, 1932, from the sputum of

(1) Aided by a grant from the Committee on Medical Research of the National Tuberculosis Association.

(2) G. S. Wilson, *J. Path. Bact.*, **28**, 1, 69 (1925).

(3) J. Furth, *J. Immunol.*, **12**, 273 (1926).

(4) F. B. Seibert, *Am. Rev. Tuberc.*, **21**, 370 (1930).

(5) F. B. Seibert and N. Morley, *J. Immunol.*, **24**, 149 (1933).

(6) R. J. Anderson, *Physiol. Rev.*, **12**, 166 (1932).

(7) E. Chargaff and J. Dieryck, *Biochem. Z.*, **255**, 319 (1932).

(8) J. A. Crowder, F. M. Stodola, M. C. Pangborn and R. J. Anderson, THIS JOURNAL, **58**, 636 (1936).

patient (21443). Mildly pathogenic for guinea pigs; non-pathogenic for rabbits.

Strain A14—isolated January, 1933, from the sputum of patient (38502). Pathogenic for guinea pigs; non-pathogenic for rabbits.

H37—(Saranac Lake strain isolated in 1910.)[9] Non-pathogenic for rabbits when recently tested.

The first four strains were isolated at the Henry Phipps Institute by J. D. Aronson and H. J. Henderson.

Isolation of Proteins.—Approximately 300 cultures of each strain were grown in 1-liter Pyrex bottles under identical conditions on Long's synthetic medium[10] for eight weeks during 1934, by Mr. John Glenn of the Mulford Biological Laboratories, Sharp and Dohme, Glenolden, Pa. The bacterial masses were then filtered off on Büchner funnels and subsequently used by Dr. Anderson. The filtrates were preserved with 0.5% phenol, later filtered free of bacteria on Mandler filters, and sent to our laboratory in 8-liter Pyrex bottles. The quantities of raw tuberculin so prepared varied from 32 to 44 liters per strain, representing from 53 to 60 liters of original medium.

The proteins were isolated from these filtrates by a method described in 1932.[11] Briefly it was as follows. The unheated culture filtrates were concentrated by means of ultrafiltration through alundum filters impregnated with 13% guncotton, and then washed free of filtrable substances by repeated additions of 0.5% phenol. The non-filtrable residue representing approximately a 100 times concentration of the original solution, was filtered through the Seitz filter and then precipitated with 10% trichloroacetic acid, washed on the centrifuge five to six times with trichloroacetic acid, partially dried *in vacuo* at room temperature and finally thoroughly extracted and dried to a powder, by grinding with anhydrous ether.

Analyses and Potency of the Proteins.—These powders designated as TPT-H37, TPT-A10, TPT-A12, TPT-A13 and TPT-A14, were then analyzed for ash and volatile substances at 110° by the micro methods described by Pregl[12] and for nitrogen by the Parnas–Wagner modification of the micro-kjeldahl method,[12] using selenium[13] instead of copper sulfate as the oxidizing agent.

The potencies of the products were compared by making six simultaneous injections, each of 0.1-cc. saline solution containing 0.005 mg. protein, in eleven tuberculous guinea pigs inoculated six weeks previously with 0.1 mg. of H37 bacilli. The five proteins were compared with an equal dose of Purified Protein Derivative[14] which is the standard purified tuberculin adopted by the National Tuberculosis Association.

The results of the analyses and potency tests are recorded in Table I. The constancy in the yield of protein from the five strains is remarkable, when consideration is given to the fact that the A13 strain grew sparsely and was found by Anderson[8] to have yielded the lowest bacterial mass per culture. The A10 protein proved to be much less soluble than any of the other proteins and consequently there was an obvious loss in the product during its isolation. There was comparatively little difference in the potencies of the five proteins, although the A10 and H37 were slightly less potent than the other three. All were less potent than the standard Purified Protein Derivative.

TABLE I

TPT made from strain of human tubercle bacillus	Yield, g. TPT per liter original medium	Moisture, %	Ash, %	Nitrogen (ash-free dry wt.), %	Potency, av. size skin reaction with 0.005 mg. tested simultaneously on 11 tuberculous guinea pigs, 48 hrs.
H37	0.30[a]	14.6		15.9	
	.26	9.5	1.3	15.2	14 × 16 × 2.7
A10	.18	4.7	0.9	14.2	13 × 15 × 2.9
A12	.24	9.0	.5	14.0	16 × 19 × 3.6
A13	.17	10.7	.4	13.9	16 × 18 × 3.2
A14	.24	8.8	.9	14.1	17 × 19 × 3.1

[a] This product was made in 1932 from the same H37 strain.

Serological Specificity of the Proteins.—Since no significant differences between the proteins were obtained by means of the preliminary analyses reported above, the very delicate serological precipitin reaction was employed. It has been shown by many investigators, but especially by Landsteiner,[15] that very small differences in chemical structure of proteins and even polypeptides can be detected by this method. Since earlier work[4] showed that proteins made similarly from different types of tubercle bacilli, human, bovine, avian and timothy grass, could be differentiated by means of the precipitin reaction, it was advisable to determine whether serological differences could also be detected between these five strains, all of the human type tubercle bacillus.

Five per cent. solutions of the different antigens were prepared by putting the powders into solution with the minimal amount of normal sodium hydroxide, neutralizing to litmus with 0.1 normal hydrochloric acid, and diluting to standard volume with saline containing 0.5% phenol.

Two rabbits were injected intracutaneously with 10 mg. of each antigen ten times at approximately weekly intervals. The increasing degree of sensitiveness of the animals was followed by observing the Arthus reactions which occurred, and seven days after the tenth injection the rabbits were bled to death. The precipitin tests were performed by adding to 12 tubes containing 0.1 cc. each of serum 0.1 cc. of varying dilutions of 1% antigen solutions, varying from 1:200 to 1:2,000,000. They were shaken and kept in the ice box for forty-eight hours and then the amount of precipitate in each tube was noted. A control tube with saline was negative in all cases.

Table II shows the results of these tests. No differences in serological specificity could be detected between the five human strains, H37, A10, A12, A13 and A14, either in the end titer or in the amount of precipitate in any of the tubes. On the other hand, differences could be detected between these human strain proteins and the proteins of

(9) E. R. Baldwin, *J. Exptl. Med.*, **22**, 323 (1910).

(10) E. R. Long, *Am. Rev. Tuberc.*, **13**, 393 (1926).

(11) F. B. Seibert and B. Munday, *Am. Rev. Tuberc.*, **25**, 724 (1932).

(12) F. Pregl, "Quantitative Organic Microanalysis," 2d edition, Blakiston's Son and Co., 1930.

(13) J. Tennant, H. L. Harrell and A. Stull, *Ind. Eng. Chem., Anal. Ed.*, **4**, 410 (1932).

(14) F. B. Seibert, J. D. Aronson, J. Reichel, L. T. Clark and E. R. Long, *Am. Rev. Tuberc.*, supplement 30, 707 (1934).

(15) K. Landsteiner, "The Specificity of Serological Reactions," Charles C. Thomas, 1936.

TABLE II

PRECIPITIN TITERS WITH ANTIGENS

Immune anti-sera from rabbits in duplicate sensitized with	H37	A10	A12	A13	A14	Bovine	Timothy	Polysaccharide from human tubercle bacilli
TPT-H37	100 000	100 000	100 000	100 000	100 000	500 000[a]	20 000[a]	0
	100 000	100 000	100 000	100 000	100 000	500 000[a]	10 000[a]	0
TPT-A10	100 000	100 000	100 000	100 000	100 000	100 000[a]	0	0
	100 000	100 000	100 000	100 000	100 000	50 000[a]	0	0
TPT-A12	100 000	100 000	100 000	100 000	100 000	100 000	20 000[a]	0
	100 000	100 000	100 000	100 000	500 000	20 000[a]	50 000[a]	0
TPT-A13	100 000	100 000	100 000	100 000	100 000	100 000[a]	20 000[a]	0
	100 000	100 000	100 000	100 000	100 000	400[a]	5 000[a]	0
TPT-A14	100 000	100 000	100 000	100 000	100 000	50 000[a]	20 000[a]	0
	500 000	100 000	100 000	100 000	100 000	50 000[a]	20 000[a]	0
TPT-Bovine	100 000	50 000	100 000	50 000	100 000	100 000	0	0
	100 000	20 000	50 000	50 000	50 000	100 000	0	0
TPT-Timothy	50 000[a]	0	0	0	0	0	100 000	0
	20 000[a]	1 000[a]	200[a]	0	20 000[a]	0	100 000	0

[a] Precipitate light.

bovine or timothy bacilli. While the bovine antigen gave in all cases some precipitate with the five human type antisera and even in several cases, as high a titer, the amount of precipitate produced was always obviously less, and in many cases there was zoning effect. On the other hand, the bovine antisera gave as high titers and as heavy precipitates with the five human type antigens as with itself. The sparseness of precipitate was very conspicuous in the case of the timothy antisera precipitated with the human type antigens as well as with the different human type antisera precipitated with timothy antigen. Therefore, the timothy protein showed a high degree of specificity, as noted earlier,[4] the bovine protein some degree of specificity and the human proteins only a type specificity. While the similarities and differences here described were plainly seen on observing the tubes, there is no doubt that it will be possible to record such differences more nearly quantitatively when Heidelberger's[16] quantitative precipitin method is perfected so that it can be applied to bacterial proteins as well as to such proteins as have been already studied by him.

Tests were made in order to determine whether the presence of so small an amount of polysaccharide as 0.5% or less, which is the usual content of reducing substances in these TPT products, may be responsible for the precipitin reactions obtained. A polysaccharide, prepared as described in an earlier publication[17] from the culture filtrate of the H37 tubercle bacillus, was serially diluted in the same way as the protein antigens and added to all the antisera. No precipitation occurred with any of the antisera in any of the dilutions, as recorded in Table II.

When relatively small quantities of proteins are available, possibly more information concerning their chemical character and reactivity can be obtained by studying the titration capacities with acid and alkali than in almost any other way, except possibly by migration velocity determinations. Studies of the latter will be reported in the future. At present, however, only electrometric titration data will be considered. So far no significant differences had been detected between the five human strain proteins, even by means of the delicate immunological precipitin reaction. Nevertheless, it was obvious that the solubility of, for example, TPT-A10 was much less than that of any of the other proteins and, therefore, physico-chemical methods may be of value in finding differences indistinguishable in any other way.

Base Combining Capacity and Titration Curves of the Proteins.—Because of the small quantities of material available in the cases of certain fractions required for comparative analyses the following method of performing the titration was chosen. In all cases 250 mg. of the product was put into solution with the minimal amount of 0.1 *N* sodium hydroxide and the volume was made up to 25 cc. with freshly distilled water. The hydrogen ion activity of the solution was then determined by means of two hydrogen electrodes immersed in the solution, so that every reading could be checked. Hydrogen gas washed through water, potassium hydroxide and pyrogallic acid, was bubbled through the solution at a rapid rate, without discontinuing the flow of gas during the entire titration. The hydrogen electrodes, freshly platinized before each titration, were referred to a saturated calomel half cell through a saturated potassium chloride agar bridge. After the bridge was introduced into the system no difficulty with poisoning of the electrodes was experienced, and there was no difficulty in obtaining a potential which remained practically constant for an indefinite period of time. The two electrodes usually gave identical potentials. Otherwise the maximum variation between them was 0.0015 volt or a difference of 0.035 *p*H.

The foaming of the protein solutions was controlled by the addition of ether in the experiments recorded in this paper. In later experiments caprylic alcohol was used. The ether had the disadvantage of reducing the temperature to the neighborhood of 10°, but the temperature of the solution was recorded with each determination and the proper *p*H obtained with the use of Leeds and Northrup conversion tables. When the temperature was not 10° the *p*H was corrected to 10° by multiplying by a factor,

(16) M. Heidelberger and F. E. Kendall, *J. Exptl. Med.*, **59**, 519 (1934).

(17) B. Munday and F. B. Seibert, *J. Biol. Chem.*, **100**, 277 (1933).

TABLE III

TITRATION OF H37-TPT

c, kg. per liter	Vol. cc.	Cc. 0.1 N NaOH added	Cc. 0.1 N HCl added	b	a	$\sqrt{\mu}$	Voltage (electrode #2)	pH, corrected to 10°	$p$oh	h	oh	$\frac{b'}{\frac{b-a}{c}+\frac{h-oh}{c}}$
0.00905	27.6	5.2	0	0.0188	0	0.137	0.9037	11.52	2.58	Negligible	0.002605	1.79
.00889	28.1		.5	.0185	.0018	.136	.8842	11.17	2.93	Negligible	.001170	1.76
.00877	28.5		.9	.0182	.0033	.135	.8631	10.79	3.31	Negligible	.000482	1.65
.00853	29.3		1.7	.0177	.0058	.133	.8205	10.03	4.07	Negligible	.000086	1.39
.00844	29.6		2.0	.0176	.0067	.133	.8071	9.79	4.31	Negligible	.000012	1.28
.00836	29.9		2.3	.0174	.0077	.132	.7731	9.19	4.91	Negligible	Negligible	1.17
.00799	31.3		2.7	.0166	.0084	.129	.6754	7.45	6.65	Negligible	Negligible	1.03
.00788	31.7		3.1	.0164	.0098	.128	.6392	6.80	7.30	Negligible	Negligible	0.85
.00778	32.1		3.5	.0162	.0109	.127	.6132	6.34	7.76	Negligible	Negligible	.69
.00764	32.7		4.1	.0159	.0125	.126	.5816	5.78	8.32	Negligible	Negligible	.45
.00759	32.9		4.3	.0158	.0131	.126	.5612	5.42	8.68	Negligible	Negligible	.36
.00752	33.1		4.5	.0157	.0136	.125	.5499	5.22	8.78	Negligible	Negligible	.28
.00748	33.4		4.8	.0155	.0143	.125	.5218	4.71	9.29	0.000019	Negligible	.16
.00744	33.6		5.0	.0154	.0149	.124	.5000	4.32	10.32	.000047	Negligible	.07
.00740	33.8		5.2	.0153	.0154	.124	.4568	3.55	10.45	.000278	Negligible	.02
.00729	34.3		5.7	.0151	.0166	.129	.4256	3.00	11.10	.001000	Negligible	−.07
.00718	34.8		6.2	.0149	.0178	.136.	.4073	2.67	11.43	.002120	Negligible	−.10

(pKw at 10°)/(pKw at t°) = 14.375. This pKw value was obtained from data given by Michaelis.[18]

After the potential was determined aliquots of 0.1 N sodium hydroxide (0.1 to 0.5 cc.) were added, determining the potential after each addition, until a pH of 11 to 12 was reached, and then aliquots of 0.1 N hydrochloric acid were added in a similar way until a pH of 2 to 3 was reached.

Calculation of the Combining Capacities.— The system was checked before and after each titration by means of 0.1 N hydrochloric acid, made from constant boiling hydrochloric acid, and pH 1.087 was used as the standard value at 20°. Adequate corrections were made, in the same manner as by Simms,[19] for errors inherent in the entire system and for those due to variations in ionic strength of the solutions titrated, such as the activity coefficients. Accordingly, a blank titration with hydrochloric acid in water at 10° (obtained by the use of ether) was made and from the data a table was constructed of corrections to be applied to the hydrogen ion concentration at each $\sqrt{\mu}$ (μ = ionic strength = the concentration of hydrochloric acid). The limiting values of μ for base or acid in all of the experiments here reported were from 0.0001 to 0.025 M. Similarly a blank titration with sodium hydroxide in water was made at 10° (obtained by the use of ether) and a curve constructed from the data by plotting $\sqrt{\mu}$ (μ = ionic strength = the concentration of sodium hydroxide) against pH-log oh. In the equation pH-log oh = pKw + log f_{OH}, f_{OH} is the activity coefficient of the hydroxyl ion. In this way, a table was constructed of the proper values for pH-log oh at every $\sqrt{\mu}$. From these pH-log oh values the pH determined for the solution under test was subtracted, giving the pOH. In the titrations of protein solutions as here recorded, μ was equal to b until a exceeded b and then it was equal to $b + (b-a)$.

The equivalents of base minus acid combined per kilogram of substance, were then calculated by means of the equation used by Simms,[19] $b' = (b-a)/c + (h-oh)/c$ in which b = concentration of strong base, a = concentration of strong acid, h = concentration of hydrogen ions, oh = concentration of hydroxyl ions, and c = concentration of protein. Since the molecular weight of none of the proteins studied was accurately known, all concentrations were expressed as kilograms of protein per liter. This would represent the molar concentration if the molecular weight were 1000.

The volume was changing throughout the titration, due to the repeated additions of alkali or acid, and, therefore, in the final calculation proper correction was made in a, b and c for each determination. A set of calculations, typical of those used for all of the curves in the paper, is given in Table III. In the curves, pH (activities) were plotted as abscissas against the corrected equivalents of base minus acid per kilo-

(18) L. Michaelis, "Hydrogen Ion Concentration," Williams and Wilkins Co., Baltimore, Md., 1926, p. 26.

(19) H. S. Simms, *J. Gen. Physiol.*, **14**, 87 (1930).

gram of protein (b') found by the above equation.

Discussion of Results

Since all the proteins were insoluble in water and for solution required sufficient hydroxide to result in a high pH value, the curves plotted begin with the values obtained when the pH was most alkaline and extend to the most acid pH. In order that the corrections due to this procedure might not be too large, the pH was never carried beyond 11.5.

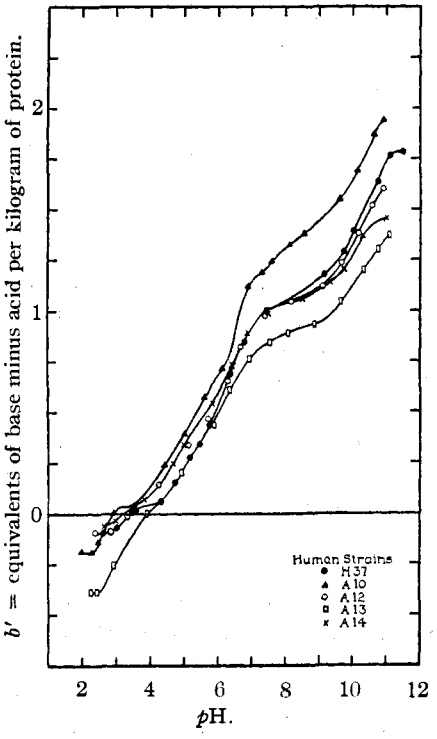

Fig. 1.

Figure 1 shows that the curves of the five human strain TPT's were similar. They were almost identical in the region between pH 2 and 6, and these similarities would exist regardless of what the real isoelectric points of the products might be. The curves of the TPT's from the bacillary strains H37, A12 and A14 are almost identical throughout; that of TPT-A13 shows a slightly less acidic substance, while that of TPT-A10 is more acidic. These similarities indicate a close correspondence in pK values for the different proteins and, therefore, presumably a similar amino acid structure. This probably explains why the precipitin tests showed identical crossing between all five proteins made from the human strain tubercle bacilli.

On the other hand, the curves of these human strains are quite different, in parts at least, from those of the TPT's made from acid-fast bacilli of other types (Fig. 2). The curves of the

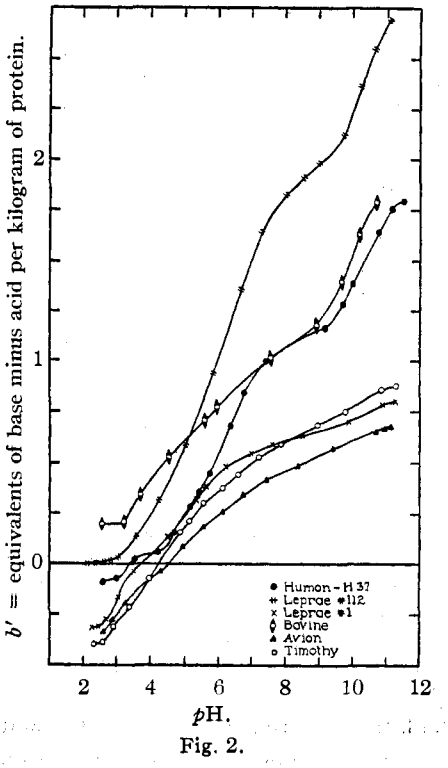

Fig. 2.

five human TPT proteins (represented by TPT-H-37 in Fig. 2) resemble that of the bovine TPT protein more than any of the others, especially in the portion of the curves more alkaline than pH 7.5, where the tyrosine, lysine and arginine groups are titrated. It is interesting to speculate on the bearing this may have on the fact that more immunological relationship was demonstrated, by means of the precipitin reaction, between these human TPT and the bovine TPT proteins than between the proteins of other type bacilli. It will be recalled that the bovine TPT antiserum crossed with the human TPT antigens almost as well as it did with itself, whereas the crossing, while real, was not as marked between the human TPT antisera and bovine TPT antigen. On the other hand, no crossing whatever occurred between the timothy and bovine TPT's. Furthermore, it has been shown by Henderson[20] that there is no immunological relationship between the *M. leprae* no. 1 TPT and the *M. leprae* no. 112 TPT, but that there is some crossing

(20) H. Henderson, in press.

between the Human-H37-TPT and the *M. leprae* no. 1 TPT and that the crossing between timothy and *M. leprae* no. 1 TPT's was very marked, facts which also correspond with the titration curves. These results indicate that further work along this line may be fruitful. Discussion as to the significance of different parts of the curves in relation to the nature of the products will be reserved for another paper.

I wish to express my appreciation to Dr. H. S. Simons for his advice in calculation of the base-binding capacities.

Summary

A comparative study of the proteins isolated from five different strains of human type tubercle bacillus culture filtrates, grown under identical conditions, yielded the following results:

The yields of protein were very much alike, *viz.*, about 0.2 g. per liter of original medium.

The nitrogen content of four of them was 14% and of the fifth 15.2%.

The tuberculin potency, determined by means of the skin test in tuberculous guinea pigs, showed some variation, but this was not marked. All five proteins were potent tuberculins.

All five proteins proved to be identical serologically, when compared by the precipitin reaction. They were type specific, in that they could be distinguished easily by this method from proteins made similarly from other acid-fast bacilli, as had been shown in earlier work. The protein from the bovine type tubercle bacillus, while distinctive, was related to some extent to the human type proteins. Polysaccharide was not responsible for the immunological reactions, since it gave no cross reactions with any of the antisera.

Acid–base-combining capacity curves throughout the range of *p*H 2 to 11, as determined by electrometric titration, using the hydrogen electrode, showed a much closer correspondence between the five proteins from human type tubercle bacilli than between them and proteins made similarly from other types of acid-fast bacilli.

PHILADELPHIA, PENNA. RECEIVED FEBRUARY 3, 1937

[CONTRIBUTION FROM INTERNATIONAL LEPROSY CENTER, RIO DE JANEIRO]

Hydnocarpic and Chaulmoogric Acids and Ethyl Esters

BY HOWARD IRVING COLE AND HUMBERTO CARDOSO

In making quantitative analyses of various chaulmoogra oils, it was necessary for us to know accurately the optical rotation and the boiling or melting points of pure hydnocarpic and chaulmoogric acids and their ethyl esters. The published data were not only incomplete but varied so widely that we found it necessary to prepare these substances in the pure state and redetermine their constants before proceeding with our analyses. Undoubtedly most of the errors found in the literature were due to impurities, since hydnocarpic acid is completely separated from chaulmoogric acid and from palmitic acid only with extreme difficulty. Hydnocarpic and chaulmoogric acids are optically active. They contain a double bond in the pentene ring and are classed theoretically as unsaturated acids as they absorb iodine or bromine in the theoretical amounts. On the other hand, being solid acids they behave physically more like the solid saturated acids, hence standard methods of separating unsaturated from saturated acids cannot be used for their isolation.

Methods of Separation

Pure chaulmoogric acid can best be obtained from the oil of *Oncoba echinata* or *Hydnocarpus alcalae* as these oils do not contain hydnocarpic acid. Analyses of chaulmoogra oils to date show no oil which contains hydnocarpic but not chaulmoogric acid, hence to obtain the former it must be separated from chaulmoogric acid. This was first accomplished qualitatively in 1905 by Power and his co-workers by fractional precipitation of their barium salts.[1] Other workers have used fractional crystallization of the free fatty acids or their salts, fractional distillation of the acids or methyl or ethyl esters, or some combination of these methods. Even so, complete purification is extremely difficult. For the preparation of pure hydnocarpic acid, *H. Wightiana* oil is recommended, as it does not contain palmitic acid whose physical properties are so close to those of hydnocarpic acid as to make complete separation of the latter next to impossible.

We have been able to separate completely hydnocarpic from chaulmoogric acids by employing a combination of

(1) F. B. Power and M. Barrowcliff, *J. Chem. Soc.*, **87**, 888, 896 (1905).