OBSERVATIONS ON MYCOBACTERIAL ESTERASES WITH A SERIES OF SYNTHETIC SUBSTRATES[1]

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The presence of enzymes in culture filtrates or suspensions of acid fast bacilli capable of catalyzing the hydrolysis of such esters as monobutyrin, tributyrin, ethyl esters of short chain fatty acids, and olive oil has been known for many years (Carrière, 1901; Wells and Corper, 1912; Kendall *et al.*, 1914; Michaelis and Nakahara, 1923; Bloch *et al.*, 1947; Sartory and Meyer, 1947). Relatively little is known of the specificity of these mycobacterial esterases. The characterization of esterases and lipases of animal sources is further advanced. In studies of animal enzymes the use of homologous series of synthetic substrates has been of value (Huggins and Lapides, 1947; Nachlas and Seligman, 1949; Ravin and Seligman, 1953). This paper indicates the applicability of a series of synthetic chromogenic substrates to the study of mycobacterial esterases. The esterolytic activities of the mycobacterial enzymes are shown to be relatively similar to those of mammalian aliesterases and dissimilar to pancreatic lipase. Certain quantitative differences in activity are noted between esterases of representative saprophytic mycobacteria and tubercle bacilli with respect to different substrates and to susceptibility to inhibitors.

MATERIALS AND METHODS

The following cultures were used in this study: *Mycobacterium tuberculosis*, strain H37RA (A.T.C.C. no. 9431); *Mycobacterium tuberculosis*, strain H37RV (A.T.C.C. no. 9360); *Mycobacterium smegmatis* (A.T.C.C. no. 10,143); *Mycobacterium lacticola* (A.T.C.C. no. 9626); *Mycobacterium tuberculosis*, strain BCG, from Henry Phipps Institute, Philadelphia, no. 858/16[2]; *Mycobacterium phlei*, from Department of Bacteriology, Harvard Medical School[3]; *Mycobacterium butyricum*, from the Public Health Research Institute of the City of New York, New York.[4]

Stock cultures of tubercle bacilli were grown on Petragnani (Difco) slants for 2 to 3 weeks, refrigerated for the remainder of the month, and transferred. Stock cultures of saprophytic mycobacteria were grown on tryptic digest of meat slants for 3 to 4 days, refrigerated for 2 to 4 weeks, and transferred. Broth cultures of tubercle bacilli were inoculated from starter cultures of the same medium, except for strain H37RV which was inoculated directly from the Petragnani slant culture. Broth cultures of the saprophytic mycobacteria were inoculated directly from the stock slants.

Tubercle bacilli were grown in Dubos broth for 2 to 3 weeks or Youmans medium (Youmans, 1946) for 3 to 5 weeks. Dubos broth was prepared by the addition of 0.5 per cent "tween 80", 0.5 per cent bovine albumin (Armour), and 0.2 per cent glucose to the basal medium prepared with enzymic hydrolyzate of casein (Dubos and Davis, 1946). Saprophytic mycobacteria were grown in beef heart infusion broth (Difco) with 1 per cent glucose or Youmans broth for 3 to 5 days. The organisms were collected by centrifugation and washed 2 times, usually with distilled water, but in some experiments with 0.9 per cent sodium chloride or 0.067 M phosphate buffer, pH 7.2. The organisms were resuspended in water or buffer by gradual addition of the appropriate medium while being rotated in a flask containing a double or triple layer of glass beads. The suspensions were diluted to an appropriate optical density measured at 450 mμ in the Coleman 6A spectrophotometer. The weight of bac-

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[4] Supplied through the courtesy of Dr. Jules Freund.

teria in these suspensions was calculated from the optical densities on the basis of calibrations obtained by determination of the dry weight of bacteria after 16 hours in an oven at 100 to 105 C. Their enzymic activity was essentially unchanged for periods of 1 to 2 weeks' storage in the refrigerator after which they were discarded.

Extracts were prepared by grinding washed packed organisms with about twice their wet weight of powdered alumina (E111 Alumina-M, Norton Co., Worcester, Mass.) in a mortar (McIlwain, 1948). The ground material was extracted with 20 ml of H_2O per gram of bacteria for 20 minutes. Centrifugation at 0 C at 20,000 × G for 1 to 2 hours yielded a pale yellowish opalescent supernatant containing from 2 to 10 mg of total solids per ml. Opalescence was much more marked in extracts of tubercle bacilli than in extracts of the saprophytic mycobacteria. Extracts not in immediate use were stored in the frozen state at −15 C. Extracts in current use were stored in a refrigerator at 0 C. Under these conditions, enzymic activity was stable for many weeks although an occasional extract showed slow diminution of activity. Dilutions were made in 0.027 M sodium phosphate buffer, pH 7.2.

Chromogenic substrates consisted of the β-naphthyl esters of acetic,[5] propionic,[6] caprylic,[6] capric,[6] lauric,[5] myristic,[6] and stearic[6] acids. They were employed according to Nachlas and Seligman (1949) with slight modifications. Five ml of a solution of the ester in acetone were pipetted with stirring below the surface of a mixture of 35 ml of water and 10 ml of 0.067 M sodium phosphate buffer of pH 6.8. Five ml of the resultant mixture (pH 7.1 to 7.2)[7] were used in each test. Under these conditions concentrations of β-naphthyl acetate of 12.4 micromoles per test, concentrations of β-naphthyl propionate of 3.1 or more micromoles per test, and all concentrations of the longer chain esters were visibly turbid.

The usual test had a total volume of 7 ml of which 1 ml was enzyme, 5 ml were substrate mixture (3.1 to 12.4 micromoles), and 1 ml was either water or solution of inhibitor. If the enzyme was a suspension of bacteria, this mixture was incubated in 50 ml glass stoppered flasks which were supported rigidly in a wire basket which was shaken in a water bath to and fro over a distance of 9 cm at a rate of 60 strokes per minute. If the enzyme was a bacterial extract, the mixture was incubated without shaking in screw capped glass tubes.

The temperature of incubation was 37 C.

Solutions of inhibitors were adjusted to pH 7.1 to 7.2 with phosphate buffer or 0.1 N NaOH and were preincubated at 37 C with the enzyme preparations for 1 hour prior to addition of substrate. In experiments with quinine as inhibitor, tris(hydroxymethyl)aminomethane was used as buffer instead of phosphate in order to avoid precipitation of quinine. Quinine HCl was dissolved in water and the solution brought to pH 7.05 with a few ml of 0.1 M tris(hydroxymethyl)aminomethane. In these experiments, the substrate solution was prepared with 0.067 M tris(hydroxymethyl)aminomethane-HCl buffer, pH 6.8.

At the end of the period of incubation, 4 mg of tetrazotized diorthoanisidine (Naphthanil Diazo Blue B[5]) in 1 ml of aqueous solution were added followed in two minutes by 1 or 2 ml of 40 per cent trichloracetic acid. The purple dye resulting from the coupling of β-naphthol and diazonium salt was extracted with 10 or 20 ml of ethyl acetate and measured at 550 mμ in round cuvettes 12 mm in diameter in the Coleman 6A spectrophotometer. The results were translated into micromoles of β-naphthol from a calibration curve made with β-naphthol which was linear in the range used.

All experiments were made in duplicate. Satisfactory checks were obtained, the standard deviation of the differences between duplicates being 2.1 per cent of the mean value.

Experiments with monobutyrin and tributyrin as substrates were conducted manometrically (Smith *et al.*, 1949). The volume of CO liberated was recorded at 10 minute intervals for a total of 30 minutes beginning 5 minutes after tipping in the substrate.

[5] Purchased from Dajac Laboratories, Leominster, Massachusetts.

[6] Kindly supplied by Dr. A. M. Seligman.

[7] This pH, although not quite optimal, was used because earlier experiments indicated that higher pH's would yield excessively high blanks. Later work did not confirm this impression, but the original pH was maintained for the sake of uniformity.

EXPERIMENTAL RESULTS

All mycobacteria which were tested, either as the intact bacterium or the extract, hydrolyzed β-naphthyl esters rapidly. β-Naphthyl acetate

TABLE 1

Esterolytic activity of mycobacterial suspensions with β-naphthyl esters

β-NAPHTHYL ESTER	MYCOBACTERIUM TUBERCULOSIS					MYCOBACTERIUM BUTYRICUM	MYCOBACTERIUM SMEGMATIS	MYCOBACTERIUM LACTICOLA	MYCOBACTERIUM PHLEI
	Strain BCG		Strain H37RA		Strain H37RV				
	Youmans medium	Dubos medium	Youmans medium	Dubos medium	Dubos medium	Meat infusion broth			
Acetate	100	100	100	100	100	100	100	100	100
Propionate	230	188	190	190	220	205	260	290	151
Caprylate	23	39	19	26	26	80	95	160	71
Caprate	4.5	19	5.1	13	17	70	61	62	45
Laurate	2.2	6.1	2.1	5.3	5.6	20	20	17	8.4
Acetate—micromoles per mg per hour	24	15	23	7.9	—	11	—	—	24

An optimal quantity of substrate, shown by preliminary tests to be attained by 12.4 micromoles, was used in each test. A constant amount (1.0 ml) of the same suspension of each organism was used with all substrates. The experimental period was 30 minutes except for tests with β-naphthyl laurate which were run for one hour with saprophytic mycobacteria, for two hours with the tubercle bacilli, and corrected to 30 minutes. The quantity of β-naphthyl acetate hydrolyzed was set equal to 100 for each organism and results with other substrates corrected proportionately.

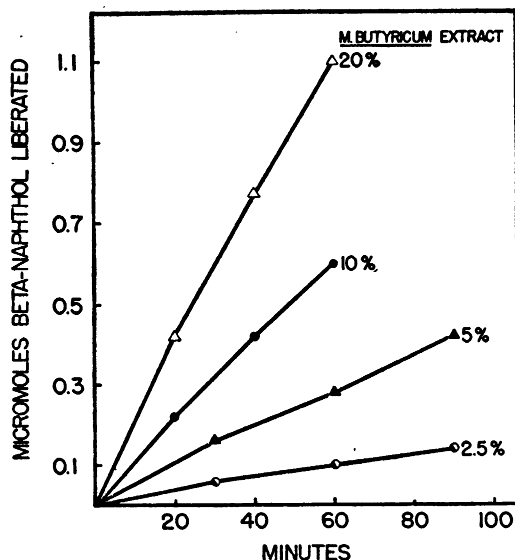

Figure 1. Rates of hydrolysis of β-naphthyl laurate (3.1 micromoles per test) by serial twofold dilutions of extracts of *Mycobacterium butyricum* (meat infusion broth).

was hydrolyzed by mycobacterial suspensions at rates of 8 to 24 micromoles per milligram of dry weight per hour (table 1). This may be compared with more conventional substrates such as tributyrin which was hydrolyzed by these bacteria at rates of 5 to 25 micromoles per milligram per hour.

Individual strains of other organisms, tested with β-naphthyl laurate only, showed much lower enzymic activity than did the mycobacteria. *Pseudomonas aeruginosa* was moderately active. *Micrococcus pyogenes* var. *aureus* and *Escherichia coli* were slightly active. Strains of *Corynebacterium diphtheriae*, *Streptococcus pyogenes*, and *Proteus vulgaris*, strain OX-19, showed no measurable activity during one hour periods of incubation.

The rate of hydrolysis of β-naphthyl esters by all mycobacterial esterases was directly proportional to the amount of enzyme provided that dilute concentrations of enzyme were used (figure 1). Deviation from linearity often appeared when the amount of enzyme was large enough to produce 20 to 30 per cent hydrolysis of the total substrate (3.1 micromoles) in one hour.

Rates of hydrolysis of β-naphthyl esters by dilute suspensions or extracts of mycobacteria were initially zero order. These rates fell below linearity with time when between 10 and 20 per cent of the substrate was hydrolyzed or, in some instances, when incubation was prolonged beyond two hours (figures 1 and 2).

The effect of pH upon enzymic activity was studied only with *M. butyricum* extracts and

phosphate buffers. The results (table 2) indicated a broad zone of optimal activity between pH 7.2 and 9.9. Rates of hydrolysis of β-naphthyl esters in phosphate or veronal buffer at pH 7.1 to 7.2 were not significantly different.

Activity profiles of mycobacterial esterases. The relative rates of hydrolysis of the series of β-naphthyl esters by suspensions and by extracts of mycobacteria are listed in tables 1 and 3, respectively. Evidently the order of decreasing susceptibility of β-naphthyl esters to hydrolysis at optimal substrate concentration is propionate, acetate, caprylate, caprate, and laurate. In other experiments with suspensions of *M. butyricum*, the myristic ester was hydrolyzed at one-fifth the rate of the laurate, and hydrolysis of the stearate was negligible.

A partial exception to this order was seen with β-naphthyl caprylate. With suspensions of the saprophytic mycobacteria grown in tryptic digest broth β-naphthyl caprylate was in most instances split almost as rapidly as the acetate, while in the case of *M. lacticola* it was hydrolyzed even more rapidly than the acetate. Furthermore, at lower, suboptimal concentrations of substrate (3.1 micromoles per test) β-naphthyl caprylate was hydrolyzed more rapidly than the acetate by *M. phlei*, and with some preparations this was also true of *M. butyricum*. This suggested that, at higher concentrations of substrate, hydrolysis of the caprylate ester fell behind the acetate only because of the greater solubility of the latter ester. With suspensions of tubercle bacilli hydrolysis of β-naphthyl caprylate did not at any significant concentration approach that of the shorter chain esters.

It is apparent from the data of tables 1 and 3 that the relative rates of hydrolysis of the C-8 to C-12 fatty acid esters were distinctly higher for the saprophytic mycobacteria than for the tubercle bacilli. The disparity was greatest with β-naphthyl caprate which was hydrolyzed by the saprophytic mycobacteria 2.5 to 15 times as rapidly as by the tubercle bacilli.

These results may be compared with those obtained with the same substrates and mammalian enzymes. The data of table 4, taken from Nachlas and Seligman (1949) and Ravin and Seligman (1953), illustrate the characteristic specificities of liver esterase, an aliesterase, and pancreatic lipase. The aliesterase gave a sharply declining rate of esterolysis with β-naphthyl esters in the C-8 to C-14 range while the rates for the

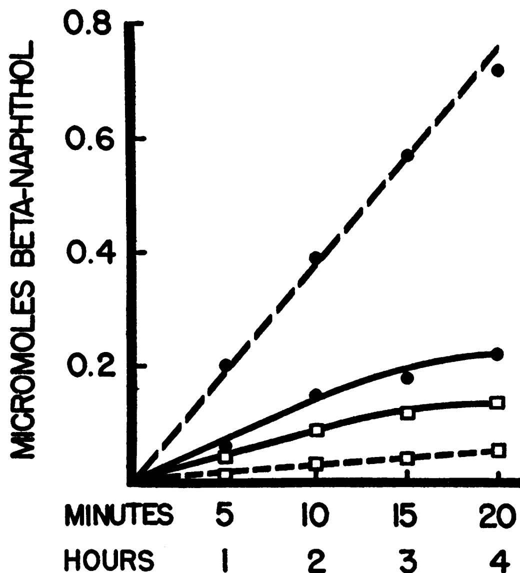

Figure 2. Inhibition of mycobacterial esterase (*Mycobacterium tuberculosis*, strain H37RA, extract-Youmans broth) by sodium fluoride 7.2 × 10^{-2} M with β-naphthyl acetate (broken lines) and β-naphthyl laurate (solid lines). ● = esterolysis in absence of inhibitor. □ = esterolysis in presence of inhibitor. The horizontal scale is in minutes for the tests with β-naphthyl acetate and in hours for those with β-naphthyl laurate.

TABLE 2

Effect of pH upon activity of Mycobacterium butyricum esterase

pH	MICROMOLES β-NAPHTHOL
5.6	0.074
6.5	0.23
7.2	0.32
8.3	0.38
9.0	0.35
9.8	0.33
11.0	0.089

The substrate was β-naphthyl laurate. The enzyme, an aqueous extract of *Mycobacterium butyricum*, was adjusted to the experimental pH with sodium phosphate buffer final concentration 0.03 M. The pH of the test sample was determined immediately after mixing enzyme and substrate. Immediately prior to addition of the tetrazonium salt, the pH of the test sample was brought to 7.1 to 7.5 by addition of 0.2 M NaH_2PO_4 or Na_2HPO_4 in amounts determined by previous titrations.

same substrates with pancreatic lipase showed very slight diminution. It is apparent that the mycobacterial esterases resembled liver esterase in showing a similarly declining rate of esterolysis with the longer chain acid esters. Even though the saprophytic mycobacteria had a relatively high rate of hydrolysis of the caprylic and capric esters, their sharp decline in activity with the lauric and myristic esters resembled more nearly aliesterase than lipase.

TABLE 3

Esterolytic activity of mycobacterial extracts with β-naphthyl esters

β-NAPHTHYL ESTER	MYCOBACTERIUM TUBERCULOSIS		MYCOBACTERIUM BUTYRICUM		MYCOBACTERIUM PHLEI	
	Strain BCG	Strain H37RA	Meat infusion broth	Youmans medium	Meat infusion broth	Youmans medium
	Youmans medium	Youmans medium				
Acetate	100	100	100	100	100	100
Propionate	169	220	250	197	—	150
Caprylate	15	22	61	36	—	49
Caprate	8	10	43	31	40	40
Laurate	7	6	16	11	18	18
Acetate—micromoles per mg per hour	4.3	8.7	4.4	3.8	10	6.2

3.1 micromoles of substrate were used in each test. The time of incubation was one hour. Different dilutions of extracts in 0.027 M phosphate buffer (pH 7.2) were used with each substrate, such that about 0.2 to 0.4 micromoles of naphthol were liberated in each test. Preliminary experiments with dilutions of these extracts demonstrated that the rate of hydrolysis of each ester was directly proportional to the amount of enzyme used. The results therefore could be corrected in each case for the dilution of enzyme used.

TABLE 4

Esterolytic activity of liver and pancreas homogenates with β-naphthyl esters

	LIVER ESTERASE	PANCREAS LIPASE
Acetate	100	100
Caprylate	23	40
Caprate	20	37
Laurate	4.6	36
Myristate	—	33

Chemical inhibition of mycobacterial esterases. The characterization of esterases has been advanced through the use of a variety of inhibitors. Thus, arsenilate (Rona and Pavlovic, 1923) and fluoride (Loevenhart and Pierce, 1906–1907; Amberg and Loevenhart, 1908) are relatively specific inhibitors of aliesterases while quinine (Rona and Pavlovic, 1923) is relatively specific for pancreatic lipase. Eserine in low concentration (10^{-6} M to 10^{-7} M) is the classical inhibitor of cholinesterases but in higher concentrations (10^{-3} to 10^{-5} M) inhibits aliesterase (Huggins and Lapides, 1947) and to a lesser extent pancreatic lipase (Nachlas and Seligman, 1949). Taurocholate potentiates the activity of pancreatic lipase but is somewhat inhibitory to aliesterases (Willstätter and Memmen, 1924). These characteristics were in general confirmed with β-naphthyl esters as substrates although considerable variation was noted among enzymes derived from different mammalian species (Nachlas and Seligman, 1949). It was of interest therefore to compare results in similar experiments with mycobacterial esterases. The findings of such experiments are summarized in table 5. The figures given for the BCG strain were almost duplicated by those for the H37RA and H37RV strains of tubercle bacilli. Strain H37RV was tested only with fluoride and arsenilate.

It is apparent that, with all mycobacterial species studied here except *M. butyricum*, hydrolysis of β-naphthyl acetate was inhibited more readily than β-naphthyl laurate. The difference in inhibition of hydrolysis of the two substrates was greatest with fluoride or arsenilate as inhibitor and extracts of one of the tubercle bacilli as enzyme. The possibility was considered that

this differential inhibition might be due to the fact that, in order to obtain a readily measurable quantity of β-naphthol, a larger concentration of enzyme was used with β-naphthyl laurate than with the acetate. The ratio of inhibitor to enzyme therefore was higher for the acetate than for the laurate. Accordingly the experiments with fluoride and arsenilate were repeated using identical concentrations of inhibitor and enzyme for each substance. The results confirm the difference in degree of inhibition of hydrolysis of the two substrates. These results may be compared with those of Nachlas and Seligman (1949) who found, with few exceptions, similar degrees of chemical inhibition of liver esterase for acetate and laurate esters. Pancreatic lipase, on the other hand, showed greater inhibition of activity for the acetate than the laurate ester except for quinine where the reverse was true. The significance of this differential chemical inhibition of activity for long and short chain esters is not clear.

The data of table 5 indicate that all the agents listed inhibited the β-naphthyl acetate esterase activity of the tubercle bacilli relatively uniformly and to a greater degree than was true of *M. phlei* and *M. butyricum*. The difference was most marked with fluoride but held to a greater or lesser degree with the other inhibitors. With β-naphthyl laurate as substrate there was no great difference in the degree of inhibition of esterases derived from the various mycobacteria.

The possibility was considered that the inhibitory action of fluoride might be due to simple binding of metal ions such as Ca^{++}. This seemed unlikely in view of the fact that the phosphate

TABLE 5

Chemical inhibition of mycobacterial esterases

INHIBITOR		MYCOBACTERIUM TUBERCULOSIS, STRAIN BCG		MYCOBACTERIUM BUTYRICUM		MYCOBACTERIUM PHLEI	
		β-Naphthyl acetate	β-Naphthyl laurate	β-Naphthyl acetate	β-Naphthyl laurate	β-Naphthyl acetate	β-Naphthyl laurate
Sodium fluoride	7.2×10^{-2} M	100	42	42	52	61	27
	1.0×10^{-2}	64	11	20	9	21	6
	1.0×10^{-3}	18	0	6	4	3	4
Sodium arsenilate	3.0×10^{-2} M	71	20	46	61	50	48
	1.4×10^{-3}	43	13	30	59	33	19
	1.4×10^{-4}	13	0	18	34	14	7
	1.4×10^{-5}	6	0	13	22	12	9
Quinine HCl	1.5×10^{-2} M	97	95	81	89	92	67
	1.5×10^{-3}	94	44	57	80	63	0
	1.5×10^{-4}	20	—	—	—	43	4
Eserine sulfate	2.9×10^{-3} M	93	59	70	76	91	45
	2.9×10^{-4}	78	23	29	31	73	26
	2.9×10^{-5}	60	13	9	16	31	8
	2.9×10^{-6}	20	—	5	—	7	—
Sodium taurocholate	1.0×10^{-2} M		71				37
	1.0×10^{-3}		5				5

All experiments were performed with extracts of organisms grown on Youmans broth. Dilutions of extracts were chosen to yield approximately 0.34 micromoles of β-naphthol from 3.1 micromoles of substrate in one hour in the absence of inhibitor. Therefore, approximately 10 times as much extract was used with β-naphthyl laurate as with β-naphthyl acetate. Final concentrations of inhibitors are given. Their concentrations when mixed with enzyme for preincubation were 3.5 times these values.

Results are averages of two or more series of duplicate determinations with different extracts except for experiments with taurocholate where a single set of duplicate tests was made.

buffer routinely used did not inhibit esterolysis. However, it was checked by testing the effect of the chelating agent, ethylenediaminotetraacetate. This chelating agent (3×10^{-3} M) failed to inhibit the hydrolysis of β-naphthyl acetate by extracts of *M. butyricum* and *M. tuberculosis*, strain H37RA.

In view of the fact that appreciable inhibition (60 per cent) of hydrolysis of β-naphthyl acetate by extracts of tubercle bacilli was obtained with 2.9×10^{-5} M eserine, the possibility of cholinesterase activity in these organisms was considered. Manometric experiments with a substrate consisting of 1.37×10^{-2} M acetylcholine chloride in 3.12×10^{-2} M $NaHCO_3$ gassed with 5 per cent CO_2-95 per cent N_2 showed no hydrolysis of the substrate. Acetylcholine itself, 3×1.0^{-2} M and tetraethylammonium bromide 3×10^{-3} M, the latter a strong inhibitor of cholinesterase but not of aliesterase (Bergmann and Shimoni, 1951), failed to inhibit the β-naphthyl acetate esterase activity of extracts of *M. butyricum* or *M. tuberculosis*, strain H37RA. Evidently cholinesterase did not occur in the extracts of these mycobacteria.

Taurocholate, which strongly potentiates the activity of pancreatic lipase, particularly with β-naphthyl laurate, was inhibitory to mycobacterial esterases. In this respect, the mycobacterial esterases resemble aliesterases.

DISCUSSION

The high content and the distinctive chemistry of lipids of the mycobacteria suggest as a reasonable corollary the presence of highly active enzymes concerned with the formation and dissolution of lipids. A high level of esterolytic activity was found in the mycobacteria studied here, in confirmation of older reports. It is interesting to compare the activity of these bacteria with a common esterase containing tissue, such as liver. Nachlas and Seligman (1949) found that liver homogenate split about 1 to 5 micromoles of β-naphthyl acetate per mg (wet weight) of liver. Assuming a water content of liver of 75 per cent it is apparent that the esterolytic activity of the more active suspensions of mycobacteria (20 to 25 micromoles per mg per hour) is of the same order of magnitude as liver. This is higher than the value of 3 micromoles per mg per hour reported for tubercle bacilli and triacetin (Bloch *et al.*, 1947).

In view of the unusual, long chain fatty acids synthesized and esterified by these bacteria, it was considered possible that their esterases might have distinctive substrate specificity, perhaps with particularly high activity with long chain acid esters.

Older work on this subject (Wells and Corper, 1912; Kendall *et al.*, 1914) contributes little useful information on this subject since it was performed prior to definition of requirements for optimal enzymic activity and with preparations of low specific activity. Sartory and Meyer (1947) noted that the supernatant fluid from a culture of tubercle bacilli split most rapidly lipids extracted from tubercle bacilli, to a lesser degree olive oil, and least ethyl butyrate. By comparison, the order of activity for pancreatic lipase was olive oil, ethyl butyrate, and, least, the lipids of the tubercle bacillus. However, these authors apparently have failed to allow for the difference in molecular weight between ethyl butyrate and olive oil. Such allowance would indicate that ester bonds were hydrolyzed about four times as rapidly with ethyl butyrate as with olive oil.

Our observations indicate that the mycobacterial esterases show no unusual substrate specificity but resemble the common aliesterases. The similarity is extended by the response to the common inhibitors of esterases. Reference to these mycobacterial enzymes as lipases does not appear justified by these observations.

Certain differences are recognizable between the enzymic activities of the saprophytic mycobacteria and the tubercle bacilli which were studied here. One is the lower esterolytic activity of the tubercle bacilli toward β-naphthyl caprylate, caprate, and laurate, relative to their activity toward β-naphthyl acetate. Another is the generally greater sensitivity of the tubercle bacilli to chemical inhibition, particularly with fluoride as inhibitor and β-naphthyl acetate as substrate. There was no significant difference in these respects between BCG, H37RA, and H37RV strains of tubercle bacilli. Accordingly, these properties do not appear to be related to virulence within tubercle bacilli.

Dubos (1950) has noted the toxicity for tubercle bacilli of free fatty acids, particularly caprylic, capric, and lauric acids. He has suggested that free fatty acids released by autolysis may play a role in the diminution of tubercle bacilli in closed caseous lesions. Esterolytic activity of the tu-

bercle bacilli may contribute also to the concentration of free fatty acids. In these circumstances, a relatively low rate of hydrolysis of long chain fatty acid esters such as we have noted may have survival value for tubercle bacilli.

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SUMMARY

The enzymic hydrolysis of a homologous series of β-naphthyl fatty acid esters by suspensions and extracts of saprophytic mycobacteria and of tubercle bacilli has been described. The pattern of activity was similar to aliesterase. Tubercle bacilli showed a lesser ability to hydrolyze β-naphthyl esters of C-8 to C-12 fatty acids relative to β-naphthyl acetate than did the saprophytic mycobacteria.

Mycobacterial esterases were inhibited by typical inhibitors of esterase, the inhibition being, in general, greater with enzymes derived from tubercle bacilli than from saprophytes.

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