Tubercle Bacillary Extracts Immunogenic for Mice. 4. Lipids.* (27393)

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The identity of tubercle bacillary immunogen is unknown, but it may be a polysaccharide bound to one or more bacillary components. Of these "carrier" components, one might be ribonucleic acid(1); another could be lipid. As part of an attempt to find a common biologic denominator for seemingly different bacillary extracts which have been reported immunogenic against tuberculosis, we have, under well standardized conditions, screened a number of lipids extracted from tubercle bacilli for potential immunogenicity. The following report shows that 2 of these lipids are protective enough to merit special attention.

Materials and methods. Our lung density technic for measuring fraction immunogenicity in mice has been described(2). It is based on the fact that since lung specific gravity in tuberculous mice rises in direct proportion to the severity of disease, immunogenicity can be measured in these animals by its suppressing effect on lung density increases due to development of challenge tuberculosis. This method was employed for the experiments reported here.

Lipids to be tested for immunogenicity were injected intraperitoneally in 0.5 mg quantities as water-in-oil emulsions, also as described previously, these lipids being dissolved or suspended in the oil phase of the emulsion before the latter was prepared.

Most of the lipids tested were those extracted from human variety tubercle bacilli, both heat-killed virulent and living avirulent, by Anderson's classic technic as described by Asselineau(3). They included, therefore, crude ether-alcohol extract, crude chloroform extract, phosphatide, acetone-soluble fats, and Waxes A, B, C, and D. Occasionally chloroform was replaced by toluene in the extraction scheme to provide a crude toluene extract.

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Pmko wax was extracted from tubercle bacilli by Choucroun's method(4), using light mineral oil U.S.P. to obtain the crude extract and *p*-dioxane to precipitate and to wash the wax. Also tested were the fractions FI, FII, FIII, FIV, FI-P, and FI-S, prepared from methanol extracts of tubercle bacilli as described by Williams and Dubos(5). These "F" fractions were tested for immunogenicity by injection both as aqueous suspension and as water-in-oil emulsions.

All of the lipids in some stage of their preparation were filtered through a sterilizing Seitz pad to remove bacilli and bacillary debris(6).

Results. Table I presents a summary of results from many experiments in which 14 different kinds of lipids were tested, some repeatedly. For brevity and clarity the fractions are listed as either immunogenic or nonimmunogenic. So distinct a classification is only partly an artificiality of simplified tabulation, for none of the fractions fell into an intermediate category when their possible protective effects were tested for statistical significance at probability values of 0.01 or

TABLE I. Immunogenicity for Mice of Various Lipids Extracted from Tubercle Bacilli.*

Immunogenic	Nonimmunogenic
Wax B (1)	Chloroform extract (2)
Pmko (3)	Toluene extract (3)
	Ether-alcohol extract (8)
	Wax A (1)
	" C (1)
	" D (1)
	FI (1)
	FII (1)
	FIII (1)
	FIV (1)
	FI-P (1)
	FI-S (1)
	Pmko (1)

* Numbers in parentheses after fractions indicate number of batches tested one or more times. To be classified as immunogenic a fraction had to induce immunity in a group of animals statistically significant at a probability level of 0.01 by comparison with a group of animals vaccinated with emulsion containing no antigen.

TABLE II. Results from a Single Experiment Testing Immunogenicity of Several Bacillary Lipids.

Vaccine*	No. of mice	Mean lung density	M_d†	P‡ value
Emulsion alone	9	.886		
Acetone-killed bacilli	9	.792	.094	<.001
Chloroform extract 104	10	.860	.026	ns
" " 172	10	.880	.006	ns
Ether-alcohol extract 172	11	.890	−.004	ns
Pmko 87	11	.784	.102	<.001
" 88a	9	.834	.048	<.05

* All antigens were administered in water-in-oil emulsion intraperitoneally.

† Difference between mean lung density of nonimmune control group ("emulsion alone") and test group.

‡ A P value of 0.001 indicates that the observed M_d could occur purely by chance less than once in a thousand experiments. The letters ns indicate that M_d was not statistically significant.

less. This table indicates that nearly all of the lipids fail to immunize mice against tuberculosis, but it also shows that 2 varieties, Wax B and Pmko, are protective. As indicated, however, one of the 4 batches of Pmko tested elicited no statistically significant protection.

Results from one of the experiments in which Pmko was tested are presented in Table II. This table illustrates the kind of data obtained from a typical experiment and both degree of effectiveness of fractions listed as "immunogenic" in Table I and the indifference of those classified as "nonimmunogenic". It shows that effective batches of Pmko are as protective as entire bacilli, but that an occasional batch (*e.g.*, batch 88a in Table II) induces at best only mild protection.

Discussion. The possible immunogenicity of lipids extractable from tubercle bacilli has been investigated(5,9,10 and reviews 7,8), but the evidence is apparently contradictory and no thread of unity hitherto has been found in it. If a single antigen in tubercle bacilli accounts for induction of tuberculoimmunity,† this immunogen probably will be found in bacillary extracts of several different kinds, lipids as well as nonlipids. We have identified such a substance in a water-soluble extract of trypsin-digested tubercle bacilli (1); the present experiments provide data for selecting water-insoluble immunogenic lipoidal extracts for analyses to determine whether their activity is due to the same substance.

Since Pmko first was reported immunogenic in 1947(4), there appears to have been only one publication(10) regarding the protectiveness of this substance.‡ The data presented here show that Pmko can be immunogenic for mice, but that occasional batches may be relatively inactive.

Smith and coworkers(11) explained an anomaly which they observed, that some lipid fractions were more protective than the cruder lipids from which they were obtained, by postulating that the protective fraction coexisted with an antagonistic fraction which nullified its effectiveness. This theory seems to be the most likely to account for Wax B immunogenicity and the inactivity of crude chloroform extract from which this wax is obtained. Since whole bacilli are immunogenic, such antagonism probably is an artifact of the particular extraction procedure employed: it could account for the variability so often found in immunity experiments with tubercle bacillary lipids and, for example, for the inactivity reported(7) of crude oil extracts from which Pmko is derived.

Subfractions of tubercle bacillary methanol extracts reported protective for mice by Williams and Dubos(5) failed, in our experiments, to show any such activity, regardless of whether they were injected in aqueous milieu or given the advantage of water-in-oil emulsion adjuvant.

Little is known about Wax B, but it probably contains polysaccharide(12). Pmko itself is principally lipopolysaccharide(7). Although the crudeness of these 2 lipids forestalls conclusions that they may owe their immunogenicity to certain specific polysaccharides not associated with the other inactive bacillary lipids, this can be postulated in further investigation to find an immunogen common to various immunizing bacillary extracts.

† As distinguished from nonspecific resistance; *cf.* (8).

‡ Experiments with crude oil extract from which Pmko is obtained cannot be considered to bear directly on this point for reasons explained in *Discussion.*

Summary. Lipids extracted from tubercle bacilli and purified by Anderson's classic procedure, Choucroun's Pmko, and methanol extracts of the bacilli were tested for immunogenicity in mice. Pmko and Anderson's Wax B were the only fractions found capable of eliciting statistically significant immunity against challenge tuberculosis.

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Activity of Succinic Dehydrogenase and Cytochrome Oxydase of Liver Mitochondria in Rats Treated with Cortisone and with an Anabolic Androgen.* (27394)

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Activity of enzyme systems may be modified by hormones, and hormonal action on cellular metabolism may be mediated by enzymes. Enzyme-hormone interaction probably plays an important role in the processes of anabolism and catabolism. An attempt was made previously to elucidate the mechanism of known metabolic antagonism between anabolic steroids and cortisone. Tissue respiration, a function dependant upon enzyme activity, was studied in animals treated with some C^{19} and C^{21} steroids(1). Expected antagonism between these 2 groups of hormones was not found as far as their effect on Qo_2 of various tissues is concerned because both anabolic androgens and cortisone significantly depressed tissue Qo_2 in rats(2,3). It became evident that further studies were indicated to localize the site of enzymatic impairment of respiration, and that the activity of some respiratory enzymes should be determined in mitochondria. Mitochondria are known to metabolize steroid hormones(4). Several individual enzymes of the Krebs cycle are associated with mitochondria but succinic dehydrogenase and cytochrome oxydase, constituting an integral system of hydrogen transferring enzymes, were found almost exclusively in these granules(5,6).

In the present study rats were treated with cortisone and/or an anabolic androgen. Succinic dehydrogenase and cytochrome oxydase activity in liver mitochondria of these animals was determined.

Methods. Male Sprague-Dawley rats, weighing 250-275 g, kept in individual cages and fed special grain diet(7), were used for this study. Cortisone Acetate (Merck) was injected intramuscularly. Methandrostenolone (Ciba), an anabolic androgen(8), was given *per os,* in the form of crushed tablet mixed with food(9). Six groups of rats, 20

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