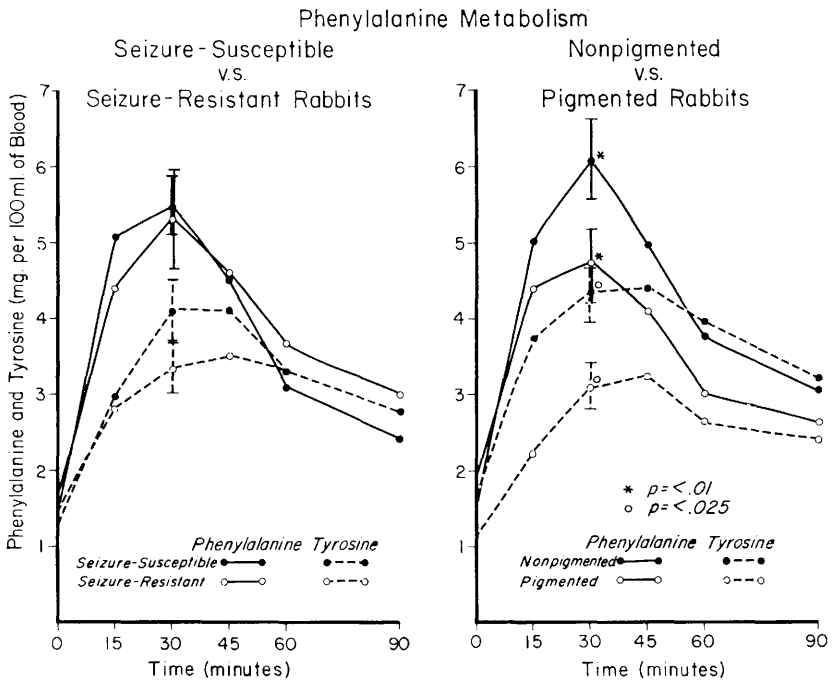

FIG. 1. Phenylalanine loading test in 12 seizure-susceptible and 12 seizure-resistant rabbits, 6 pigmented and 6 nonpigmented animals in each group. Animals were injected intraperitoneally at zero time with 100 mg L-phenylalanine/1 kg body weight. Values represent the averages in each group of 12 rabbits.

ance of phenylalanine from the bloodstream could be related to the degree of coat pigmentation since phenylalanine is a precursor of melanin. However, the metabolism of phenylalanine in these rabbits appeared to be unrelated to their susceptibility to audiogenic seizures.

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Lowered Delayed Skin Reactivity to Fractions of *Mycobacterium bovis* (BCG) in Guinea Pigs Sensitized with Large Doses of BCG. (30504)

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Guinea pigs sensitized with varying doses of heat-killed *Mycobacterium bovis* (BCG) were studied for delayed skin reactivity, complement fixing, and precipitating antibody activity towards 2 fractions of culture filtrates of BCG. A different amount of BCG was needed to induce optimum sensitization toward each fraction. Lesser reactivity to both fractions was observed when the sensitizing dose was greater than optimum. The circu-

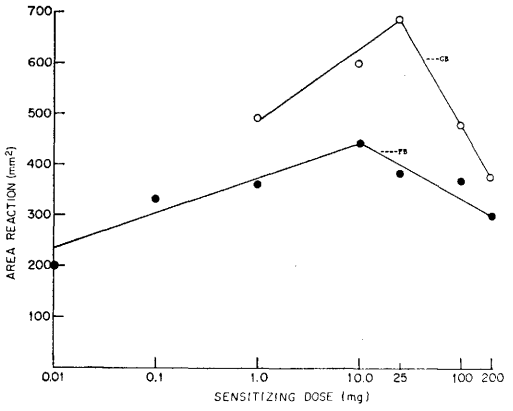

FIG. 1. Areas of reaction induced by intradermal injection of 0.1 ml saline containing 10 μg of FB or GB in guinea pigs sensitized with varying doses of BCG.

lating antibody levels did not decrease when the sensitizing doses were greater than the optimum required to induce maximum skin reactivity.

Materials and methods. One-month-old cultures of BCG, grown in Long's synthetic medium, were heated at 100°C for 30 minutes and dried in shallow pans at 35°C. Guinea pigs were injected with organisms that had been suspended in Arlacel-Drakeol (35:65) and emulsified with an equal volume of physiological saline. The amount per animal varied from 0.01 to 100 mg BCG in 0.6 ml distributed in 3 sites of the nuchal area. A 200 mg dose was administered in 1.2 ml. Six weeks after sensitization the animals were skin tested with 10 μg of either one or both of the 2 BCG fractions described earlier(1): FB is insoluble at pH 4.2 and consists of 95% protein and 5% carbohydrate; and GB is soluble at pH 4.2 and consists of 50% protein and 50% carbohydrate. Results of skin tests, which were read at 24 hours, were recorded as the product of 2 diameters of reaction. Serum taken one week prior to and one week post-skin testing was examined for gel precipitating antibodies by the method of Crowle(2) and for CF antibodies by the method of Wasserman and Levine(3).

Results. In 2 experiments in which a total of 12 animals were used for testing fraction GB and 19 animals for testing fraction FB at each sensitizing dose similar results were obtained. The degree of delayed skin reactivity towards fractions FB and GB increased with an increase in the sensitizing dose of BCG until an optimum dose was reached (Fig. 1). The optimum doses were 10 mg for FB and 25 mg for GB. Increasing the sensitizing dose beyond the optimum resulted in lower skin reactivity of the animals towards the fractions. FB reactions increased from 200 mm^2 in animals sensitized with 0.01 mg to 441 mm^2 at the optimum sensitizing dose of 10 mg and then decreased to 295 mm^2 at the 200 mg sensitizing dose. GB was the more reactive of the 2 substances. Reactions increased from less than 500 mm^2 in animals sensitized with 1 mg to 700 mm^2 at the optimum dose and then decreased to less than 400 mm^2 at the 200 mg sensitizing dose.

Although GB was more skin reactive, circulating antibodies to this fraction could not be detected in the pre- or post-skin test sera by complement fixation and gel precipitation techniques. On the other hand, gel precipitating and CF antibodies were induced toward FB in animals sensitized with 1 mg to 200 mg of BCG (Tables I and II). The number and intensity of precipitin bands did not vary appreciably with the dose.

Some animals showed a large anamnestic CF response while others showed only a small anamnestic response (Table II). The ratios of CF titers of the post- to pre-skin testing sera for 7 of the 19 animals showed rises in titers that ranged from 9.2- to 14.3-fold (avg. 11.9); while for the remaining 12 there was either no increase or no more than a 4.4-fold increase (avg. 2.9). The significance of this observation is being investigated.

Discussion. The optimum dose of heat-

TABLE I. Precipitating Antibodies in Guinea Pigs Injected with Varying Doses of BCG.

BCG (mg)	Gel precipitins* FB	GB
1	13 (3)	0
10	13 (5)	0
25	11 (4)	0
100	14 (5)	0
200	15 (6)	0

* No. of animals out of 15 that produced precipitins. No. in parentheses refers to No. of animals producing multiple precipitins.

TABLE II. Titers of Complement Fixing Antibodies Against Fraction FB.

	Sensitizing dose of BCG								
	1 mg			10 mg			200 mg		
	Pre*	Post*	Ratio post/pre	Pre	Post	Ratio post/pre	Pre	Post	Ratio post/pre
	1100	15800	14.3	240	3300	13.7	1300	14500	11.1
	1100	15800	14.3	400	1400	3.5	950	10000	10.5
	340	3630	10.7	1900	4800	2.5	1100	4800	4.4
	1580	14500	9.2	6000	11000	1.8	500	2100	4.2
	4560	14500	3.2	980	1320	1.3	4500	17300	3.8
	1800	5250	2.9				4500	17300	3.8
	6660	6600	1.0				430	950	2.2
Mean	2448	10869		1904	4364		1897	9564	
Median	1580	14500		980	3300		1100	10000	

* Reciprocal 50% hemolytic titers for sera taken one week prior to skin testing (pre) or one week post skin testing (post).

Fraction FB was used at a concentration of 10 μg.

killed BCG for inducing in guinea pigs delayed skin reactivity to each of 2 fractions, FB and GB, differed. The number of skin reactive substances found in culture filtrates must, therefore, be greater than one. If the same substance was responsible for eliciting the skin reaction in both FB and GB we would expect the optimum sensitizing dose to be the same. We have shown previously that carbohydrates of *Mycobacterium bovis* as well as proteins can elicit delayed skin reactions(4). It is likely that skin reactivity of tuberculins of other species of mycobacteria are induced also by mixtures of antigens. In the standardization of such tuberculins, efforts to determine the most suitable conditions and doses for sensitization should result in more uniform and predictable test procedures.

A lack of correlation between circulating antibodies and degree of skin reactivity was very obvious. GB produced the largest skin reactions in sensitized guinea pigs but no circulating antibodies were detected regardless of the size of the sensitizing dose. In other unpublished experiments GB has been shown to be very effective in fixing complement and precipitating with sera containing specific antibodies induced by injecting the homologous antigen in complete Freund's adjuvant. Skin reactivity, therefore, could not be correlated with circulating antibodies. The possibility should be considered that antigens present in the mixture which do not precipitate with antibody may play a role in eliciting the delayed skin reaction.

Although CF and precipitating antibodies were produced to FB, the titer did not appear to be dependent on dose. The mean and medium pre-skin test titers per sensitizing dose did not vary significantly. There was, however, a dose dependency with regard to delayed skin reactivity in that doses lower and higher than the optimum sensitized guinea pigs to a lesser degree.

Summary. Different optimal doses of heat-killed *Mycobacterium bovis* (BCG) were required to sensitize guinea pigs maximally toward fractions FB (largely protein) and GB (50% protein and 50% carbohydrate) prepared from culture filtrates of BCG. Increasing the sensitizing dose beyond the optimum resulted in lesser skin sensitivity for each antigen. No circulating antibodies were detected towards GB, which was the more skin reactive fraction. Complement-fixing and precipitating antibodies were produced toward FB but could not be correlated with the dose of injected organisms.

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