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Assessment of the Potency of Tuberculin in Humans and Guinea-pigs

OTTO GALTUNG HANSEN,[1] KARE LINDQVIST [2] & HANS WAALER [3]

The biological effects of tuberculin in an organism infected with tubercle bacilli are due to an unknown number of different substances, which are present in varying concentrations in different tuberculin preparations. Consequently, the response to tuberculin tests with various products is likely to vary with the biological material, the test technique, the tuberculin dose, etc., and potency estimates of tuberculin products will depend to a considerable extent on how the assessment is made. The present paper describes an experiment in which this dependence was demonstrated in the case of two purified protein derivative tuberculin preparations (RT 23 and PPD-Weybridge). The potency ratio of these products was found to vary widely according to whether it was determined in humans by the Pirquet test or in guinea-pigs by the Mantoux test, and the authors therefore conclude that tuberculin products must be assessed under the conditions in which they will subsequently be used.

It is generally admitted today that there is no chemical method available that can, with satisfactory accuracy, be used for the assessment of the biological potency of a tuberculin product. The potency of a product or the relative potencies of several products must therefore be assayed by means of biological material.

In most laboratories this biological assessment is performed in guinea-pigs, which are sensitized either with virulent tubercle bacilli, with BCG or with heat-killed tubercle bacilli suspended in mineral oil. The potency values thus obtained are in general considered to be valid also for humans and for any technique applied.

It is today regarded as a fact that the characteristic biological effects of tuberculin in the infected organism are due not to a single substance, but, rather, to an unknown number of different substances, which are present in varying concentrations in different preparations. The use of a single strain of human tubercle bacillus does not give sufficient guarantee that a qualitatively homogeneous tuberculin is produced. This fact makes it *a priori* unreasonable to expect the response to tuberculin tests with various products to be unaffected by the biological material, the method of application, the level of concentration of the tuberculin product and the various substances added for sterilizing, stabilizing and other purposes. Evidence that this is the case has been given in various reports.

Long, Miles & Perry (1954), in a comparative study of the effects of varying both the sensitizing antigens and the test allergens in guinea-pigs, found sufficient heterogeneity among the different types of tuberculins to render invalid their comparison in terms of a single standard. They considered their results to throw some doubt on the safety of assuming that information on the strength of tuberculin products from guinea-pig studies adequately reflects the potency when applied in human subjects sensitized by natural infection or by BCG.

In the paper by Guld et al. (1958) on the standardization of the purified product RT 23, clear evidence is given that a tuberculin comparison will not always give the same result in humans and in guinea-pigs.

Edsall et al. (1962) report considerable differences in the potency of diluted tuberculin preparations when tested in guinea-pigs and in humans.

In *Årsberetning for 1951* (Statens Skjermbildefotografering, 1954), the results are reported of an experiment where a Danish and a Norwegian tuberculin product were compared. The potency ratio of the two products was observed to differ according

[1] Senior Medical Officer in charge of Tuberculosis, Directorate of Health, Oslo, Norway.

[2] Scientific Consultant, Veterinary Institute, Oslo.

[3] Consultant Statistician, State Mass Radiography Service, Oslo.

to whether the assessment was made in BCG-vaccinated or in naturally infected humans.

On the basis of the *a priori* suspicion and the published evidence, in Norway the new batches of tuberculin produced at the Veterinary Institute, Oslo, have, since 1951, been assayed in two groups of humans—previously BCG-vaccinated and naturally infected persons (i.e., the populations for which the tuberculin test is intended) before the products are released for general use. This has been considered necessary in order to ensure that the tuberculin is (*a*) sufficiently potent to be used in case-finding surveys with a single tuberculin test, and (*b*) of sufficiently constant potency to be used for measuring the post-vaccination allergy with such a degree of accuracy that the BCG vaccine could be standardized in terms of post-vaccination allergy.

During a round-table discussion on tuberculin at the 7th International Congress for Microbiological Standardization, held in London in 1961 (*Proceedings* . . . , 1962), the need for comparative studies of tuberculin standardization at the same time in animals and in humans was stressed. In this paper we shall present some evidence from an experiment especially designed to throw light on this question.

MATERIALS AND METHODS

Biological material

Humans. 4516 persons covered by the mass-radiography survey of Oslo in 1962, of whom 1373 were not and 3143 were BCG-vaccinated. Of the non-vaccinated persons, 557 were assumed, on the basis of a tuberculin test, to be infected with virulent tubercle bacilli. Of the BCG-vaccinated persons, 2209 had " large " reactions to a post-vaccination tuberculin test. The BCG status of a person was assessed partly by the Oslo Health Board's index, partly by the person's vaccination certificate, and partly on the reading of the local reaction. Such an assessment is considered to be fairly accurate.

Guinea-pigs. 31 guinea-pigs were sensitized with three different allergens, as follows:

(*a*) 9 guinea-pigs with killed *Mycobacterium tuberculosis* in oil suspension. (This method is the usual way of sensitizing guinea-pigs and has great practical advantages. The animals survive and can be repeatedly used, and the tuberculin reactions are relatively large, very marked and legible.)

(*b*) 14 guinea-pigs with virulent *Myco. tuberculosis* (strain E 9655).

(*c*) 8 guinea-pigs with BCG.

Tuberculin products

The following tuberculin products were tested:

(*a*) RT 23 (a purified protein derivative (PPD) tuberculin prepared by the Statens Seruminstitut, Copenhagen) in phosphate buffer with 10% glycerol and 0.5% phenol.

(*b*) PPD-W, batch 2/62 (a PPD tuberculin prepared by the Central Veterinary Laboratory of the Ministry of Agriculture, Fisheries and Food, Weybridge, England) in the same diluent as RT 23.

(*c*) SMT 6, 75% with adrenalin (a synthetic medium tuberculin prepared by the Veterinary Institute, Oslo). This tuberculin was employed in the present experiment as a reference test in humans.

Tuberculin doses and concentrations and test technique

In humans. 120 000 TU/ml and 30 000 TU/ml RT 23; 100 000 TU/ml and 25 000 TU/ml PPD-W. The TU values given here refer to the tuberculin units as specified by the manufacturers of the various products.

The testing technique applied in humans was a modified Pirquet test. A drop of the concentrated tuberculin was placed on a site on the volar aspect of the underarm. A scarification, 5 mm long, was then produced in the superficial layer of the skin by means of a specially constructed pen-nib, and the site was left to dry for 5 minutes.

The test reactions were read after 2-3 days. The present analysis is based on the width of the scar, measured in millimetres.

In guinea-pigs. Concentrations of 1000, 250 and 62.5 TU per 0.1 ml of diluent were used for each tuberculin product and the testing technique employed was the Mantoux test (intradermal injection of 0.1 ml). The reactions (diameter of the induration in mm) presented in this paper were read after 24 hours. The tuberculin reactions were read by trained readers who had no knowledge either of the allergen or of the tuberculin product and dose.

Statistical design

Humans. The Pirquet standard test consists of two parallel scarifications on the volar side of the left underarm. In the present study one scarification was made for one of the 4 test concentrations, and the

TABLE 1

MEAN SIZE OF REACTIONS (mm) TO THE VARIOUS TUBERCULIN CONCENTRATIONS: HUMANS

Group	Absolute reactions				Difference from reference test with 75 % SMT 6			
	RT 23 (1 000 TU/ml)		PPD-W (1 000 TU/ml)		RT 23 (1 000 TU/ml)		PPD-W (1 000 TU/ml)	
	30	120	25	100	30	120	25	100
Non-vaccinated (" small " reactions excluded)	7.0	7.7	4.6	6.5	−0.9	+0.5	−3.0	−1.3
BCG-vaccinated (all reactions)	3.4	4.7	1.6	2.7	−1.1	+0.3	−2.7	−1.5
BCG-vaccinated (" small " reactions excluded)	4.5	6.1	2.3	3.9	−1.4	+0.5	−3.7	−2.1

other for the reference concentration. The two concentrations were alternately assigned to the upper and lower scarification. The analysis presented here has been based on the difference between the two reactions. For the non-vaccinated, reactions smaller than 3 mm to both concentrations have been excluded from the analysis as such reactions are supposed to be non-specific. For the BCG-vaccinated, the analysis has been performed on the basis of the total distribution as well as on that of the distribution with the small reactions (less than 3 mm) excluded.

FIG. 1

DOSE-RESPONSE CURVES EXPRESSED IN TERMS OF MEAN DIFFERENCE IN SIZE BETWEEN REACTIONS TO TEST CONCENTRATIONS AND TO REFERENCE CONCENTRATION: PIRQUET TEST IN HUMANS

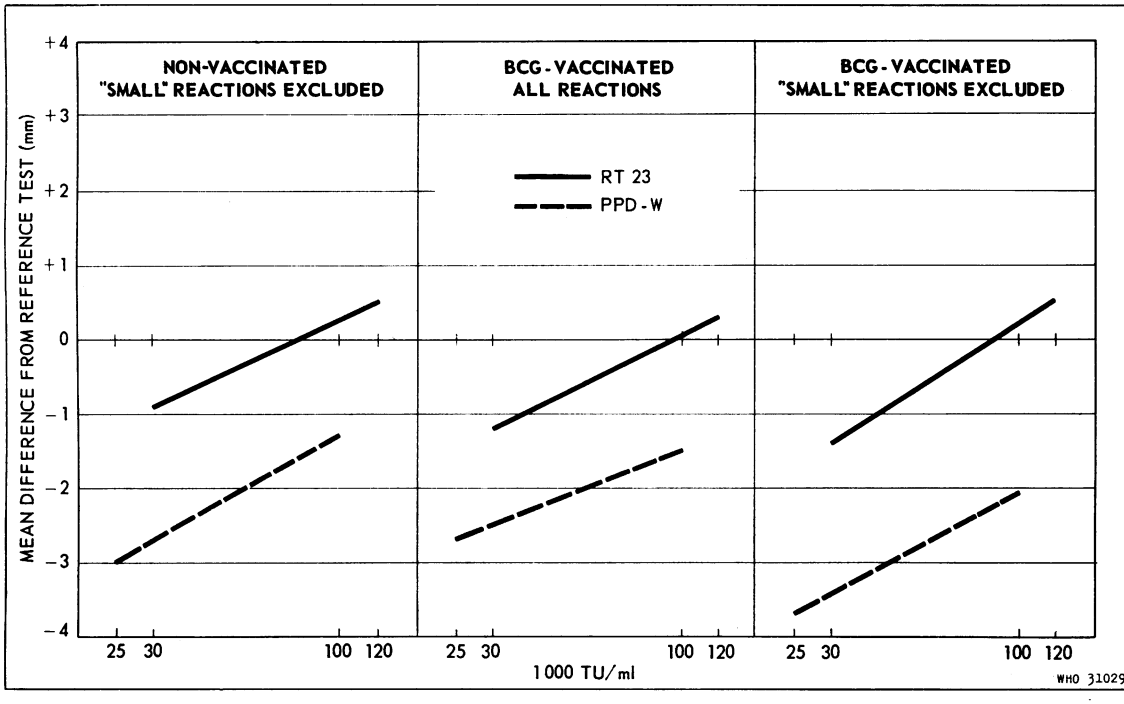

TABLE 2
MEAN SIZE OF REACTIONS (mm) TO THE VARIOUS TUBERCULIN DOSES: GUINEA-PIGS

Allergen	RT 23 (TU/0.1 ml)			PPD-W (TU/0.1 ml)		
	62.5	250	1 000	62.5	250	1 000
Virulent *Myco. tuberculosis*	12.0	14.7	17.5	11.6	15.0	17.1
Killed *Myco. tuberculosis* in oil suspension	11.4	14.3	16.6	12.8	15.7	17.9
BCG	11.5	14.5	15.9	11.1	13.1	15.8

Guinea-pigs. The experiment was planned for each of the 3 allergens as 6×6 Latin-square designs with 1 or 2 replicates, giving the 6 combinations of 2 products and 3 doses to each animal. In addition some reserve animals were included. Owing to a few deaths among the guinea-pigs, the material has been analysed as complete block designs.

FINDINGS

The distributions of reactions, in terms of size in millimetres, for each of the biological groups are given in Appendix Tables 1-10. The mean sizes of reactions are presented in Tables 1 and 2, and the corresponding dose-response curves in Fig. 1 and 2.

FIG. 2
DOSE-RESPONSE CURVES EXPRESSED IN TERMS OF MEAN SIZE OF REACTIONS: MANTOUX TEST IN GUINEA-PIGS

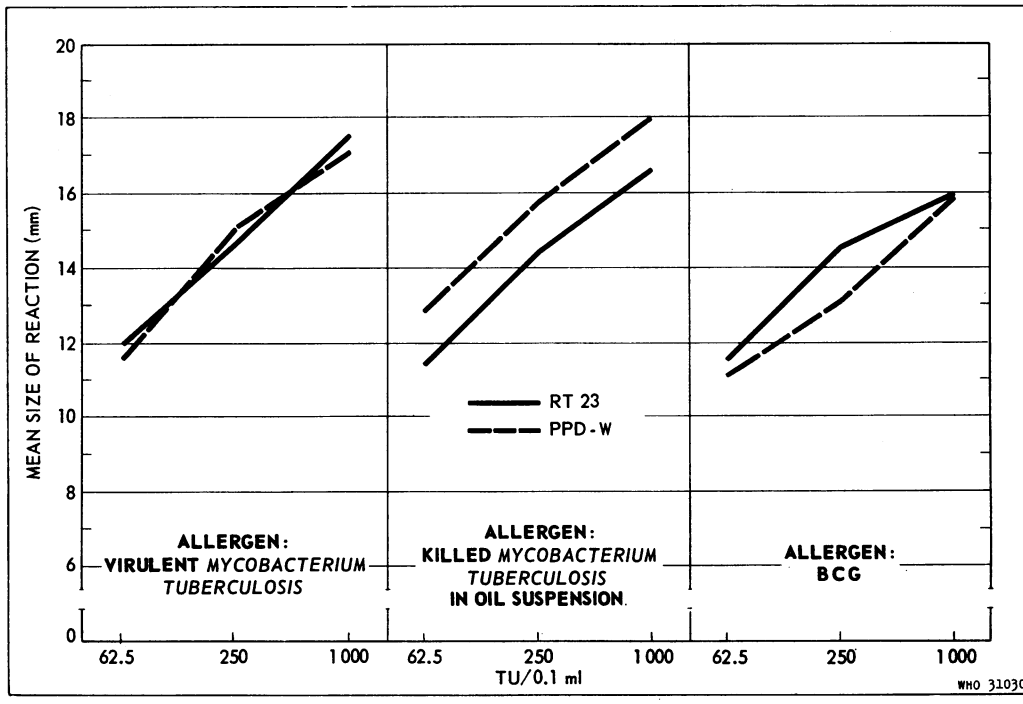

TABLE 3
POTENCY RATIOS OF THE TWO TUBERCULIN PRODUCTS PPD-W AND RT 23

Biological material; method	Allergen	Potency ratio (PPD-W: RT 23)	95 % fiducial limits
Humans; Pirquet test	Non-vaccinated (" small " reactions excluded)	0.21	0.09-0.38
	BCG-vaccinated (" small " reactions excluded)	0.17	0.13-0.22
Guinea-pigs; Mantoux test	Virulent *Myco. tuberculosis*	1.1	0.91-1.34
	Killed *Myco. tuberculosis* in oil suspension	2.2	1.37-3.74
	BCG	0.71	0.49-1.0

The potency ratio of the two test products, together with 95% fiducial limits, is shown in Table 3 for each of the five biological groups. The analyses of variance for the two groups (humans and guinea-pigs) are given in Tables 4 and 5, respectively.

In BCG-vaccinated humans the PPD-W was found to have a potency of hardly one-fifth that of the RT 23; and a very similar potency ratio was observed in naturally infected humans. In guinea-pigs, the potency ratios (PPD-W: RT 23) were very different, varying from 0.7 : 1 in BCG-vaccinated animals to 2.2 : 1 in animals sensitized with killed *Myco. tuberculosis* in oil suspension.[1]

[1] The estimates of potency ratios are subject to rather large experimental errors, owing to the substantial difference in the potency of the two preparations when applied in humans. The findings have, however, been confirmed in a later experiment, in which the strength of the doses of PPD-W was adjusted to be equivalent to that of the doses of RT 23.

TABLE 4
ANALYSIS OF VARIANCE: HUMAN POPULATION

Source	Non-vaccinated (" small " reactions excluded)			BCG-vaccinated (all reactions)			BCG-vaccinated (" small " reactions excluded)		
	d.f.	s.s.	m.s.	d.f.	s.s.	m.s.	d.f.	s.s.	m.s.
Product	1	3.686		1	2.907		1	5.881	
Regression	1	2.341		1	1.756		1	3.010	
Parallelism	1	0.036		1	0.018		1	0.021	
Error	553		0.064	3 138		0.006	2 205		0.01

TABLE 5
ANALYSIS OF VARIANCE: GUINEA-PIGS

Source		Virulent *Myco. tuberculosis*			Killed *Myco. tuberculosis* in oil suspension			BCG		
		d.f.	s.s.	m.s.	d.f.	s.s.	m.s.	d.f.	s.s.	m.s.
L_p	Product	1	0.76		1	28.17		1	4.08	
L_1	Regression	1	429.02		1	235.11		1	175.78	
L_1'	Parallelism	1	0.02		1	0		1	0.03	
L_2	Quadratic curvature	1	1.34		1	0.33		1	0.84	
L_2'	Difference of quadratics	1	1.72		1	0.33		1	3.76	
Σ	Tuberculin/dose	5	432.86	86.57	5	263.94	52.79	5	184.50	36.90
	Animal	13	49.36	3.80	8	53.33	6.67	7	34.25	4.89
	Residual	65	51.07	0.786	40	37.56	0.939	35	34.50	0.986
	Total	83	533.29		53	354.83		47	253.25	

DISCUSSION

In the present paper clear evidence is given that the observed potency ratio of two tuberculin products differs with the biological material used and possibly with the technique applied. This is demonstrated here with the Mantoux test in guinea-pigs and the Pirquet test in man, but there is no reason to suppose that similar findings would not be obtained with other tuberculin test techniques. The point to be emphasized is that the assessment of a tuberculin product must be carried out under the conditions in which the product will subsequently be employed (Guld et al., 1958, p. 881). It should be pointed out that our findings demonstrate that the method generally used in laboratories today—namely, comparison of the products in guinea-pigs sensitized with killed *Myco. tuberculosis* in oil suspension—is particularly unsuitable for forecasting how the products will behave when applied to human material. For instance, if we had used the PPD-W product in Norway and based the required concentrations on findings in English laboratories from experiments in guinea-pigs and with the international product RT 23 as standard, we would have observed a potency of just one-tenth of the expected value.

RÉSUMÉ

Les effets biologiques de la tuberculine sur un organisme infecté par le bacille tuberculeux dépendent d'un nombre indéterminé de substances qui existent en concentrations variables dans les différentes tuberculines. Ainsi, la réponse aux tests effectués avec diverses préparations tuberculiniques variera selon les organismes auxquels on les applique, selon la technique du test et la dose de tuberculine; d'autre part, l'évaluation de l'activité de la tuberculine dépendra dans une large mesure des critères d'évaluation.

Les auteurs décrivent, à l'appui de cette thèse, une expérience comparant deux tuberculines, la RT 23 et la PPD de Weybridge. Ils montrent que, pour être valables, les évaluations de l'activité des tuberculines doivent être effectuées dans les conditions mêmes où le produit sera utilisé dans la pratique. Leurs expériences indiquent que la méthode généralement en usage dans les laboratoires — la comparaison des tuberculines sur le cobaye sensibilisé par une suspension huileuse de bacilles tuberculeux tués — ne permet nullement de prévoir la réaction que ces tuberculines provoqueront dans l'organisme humain. C'est ainsi que, si l'on avait utilisé en Norvège la PPD de Weybridge, d'après les résultats des tests effectués avec cette tuberculine sur le cobaye, dans les laboratoires anglais, en utilisant RT 23 comme étalon, on aurait obtenu une activité ne représentant que le dixième de la valeur escomptée.

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APPENDIX TABLE 1

DISTRIBUTION OF DIFFERENCES BETWEEN REACTIONS TO THE TEST CONCENTRATIONS AND REACTIONS TO THE REFERENCE CONCENTRATION (75 % SMT 6) IN HUMANS, ACCORDING TO TYPE OF INFECTION

Group	Test product	Test concentration (1 000 TU/ml)	Difference between reactions to the test concentration and to the reference concentration (mm)																											N	$\bar{X}$	S^2
			−14	−13	−12	−11	−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	1	2	3	4	5	6	7	8	9	10	11	12			
Non-vaccinated (" small " reactions excluded)	RT 23	30	.	.	.	.	.	1	.	1	2	7	10	13	15	18	11	17	10	8	2	1	1	1	.	.	.	.	.	118	− 0.87	7.80
	„	120	.	.	.	.	.	.	2	.	2	2	7	11	14	18	19	20	15	13	8	10	2	2	1	1	.	.	.	147	+ 0.47	9.54
	PPD-W	25	.	1	.	.	2	3	2	4	11	10	18	22	23	12	11	2	3	1	1	2	.	1	.	.	.	.	.	129	− 2.98	9.12
	„	100	.	.	.	.	1	.	1	1	5	8	19	18	31	26	16	14	7	7	3	2	.	1	1	1	.	.	1	163	− 1.26	9.20
BCG-vaccinated (all reactions)	RT 23	30	1	.	.	.	.	1	1	8	15	24	47	78	99	177	181	74	30	21	1	4	3	.	.	.	.	.	.	765	− 1.12	4.65
	„	120	.	.	.	.	.	.	.	4	2	11	17	37	63	97	187	144	81	63	23	18	8	4	.	.	.	.	.	759	+ 0.34	4.94
	PPD-W	25	.	.	.	.	1	3	14	38	49	73	89	118	122	109	130	24	3	.	1	1	.	.	.	1	.	.	.	776	− 2.69	5.56
	„	100	.	.	.	.	.	1	4	6	26	34	75	88	139	183	188	56	27	9	5	1	1	.	.	.	.	.	.	843	− 1.50	4.31
BCG-vaccinated (" small " reactions excluded)	RT 23	30	1	.	.	.	.	1	1	8	15	24	47	78	81	113	73	59	28	21	1	4	3	.	.	.	.	.	.	558	− 1.39	5.88
	„	120	.	.	.	.	.	.	.	4	2	11	17	37	49	55	88	111	74	63	23	18	8	4	.	.	.	.	.	564	+ 0.49	6.28
	PPD-W	25	.	.	.	.	1	3	14	38	49	73	89	118	73	37	15	10	3	.	1	1	.	.	.	1	.	.	.	526	− 3.67	4.85
	„	100	.	.	.	.	.	1	4	6	26	34	75	88	110	102	50	28	21	9	5	1	1	.	.	.	.	.	.	561	− 2.08	5.10

APPENDIX TABLE 2

CORRELATION OF SIZE OF REACTIONS (mm) TO 75 % SMT 6 AND RT 23, 120 000 TU, FROM PIRQUET TESTS IN HUMANS: NON-VACCINATED GROUP

RT 23, 120000 TU	75 % SMT 6 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
17	.	.	.	.	.	.	.	.	.	1	.	1	.	.	.	.	.	2
16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	.	.	.	.	.	.	.	.	1	.	.	1	1	1	.	.	1	5
14	.	.	.	.	.	.	.	.	1	.	.	.	1	.	1	.	.	3
13	.	.	.	.	.	.	.	.	.	.	.	2	1	1	.	.	.	4
12	.	.	.	1	.	.	.	.	2	1	1	2	.	1	.	1	.	9
11	.	.	.	.	1	.	.	1	.	1	3	2	.	.	.	1	.	9
10	.	.	.	.	.	2	2	2	.	4	.	3	1	3	.	.	.	17
9	.	.	.	.	1	.	1	.	3	3	2	2	.	.	.	.	.	12
8	.	.	.	1	2	2	2	3	2	2	3	1	2	.	1	.	.	21
7	.	.	1	.	1	1	1	1	2	3	1	.	.	.	.	.	.	11
6	.	2	.	1	1	.	2	2	1	.	2	.	.	.	.	.	.	11
5	3	.	.	2	2	3	.	.	.	.	.	.	.	.	.	.	.	10
4	.	.	3	.	1	1	2	1	.	.	.	.	.	.	.	.	.	8
3	4	.	1	3	3	1	1	.	.	.	.	.	.	.	.	.	.	13
2	5	4	6	1	.	1	.	1	1	.	.	.	.	.	.	.	.	19
1	13	22	8	1	.	.	.	.	.	.	.	.	.	.	.	.	.	44
0	130	34	3	2	2	1	.	.	2	.	.	.	.	.	.	.	.	174
Total	155	62	22	12	14	12	11	11	15	15	12	14	6	6	2	2	1	372

APPENDIX TABLE 3

CORRELATION OF SIZE OF REACTIONS (mm) TO 75 % SMT 6 AND RT 23, 30 000 TU, FROM PIRQUET TESTS IN HUMANS: NON-VACCINATED GROUP

RT 23, 30 000 TU	75 % SMT 6 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Total
16	.	.	.	.	.	.	.	.	.	1	.	.	.	1	.	.	.	.	.	.	2
15	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	1	2
14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
12	.	.	.	.	.	.	.	.	.	3	2	1	.	.	.	.	.	.	.	.	6
11	.	.	.	.	.	.	.	2	.	.	1	1	1	.	.	.	.	.	.	.	5
10	.	.	.	.	.	.	.	.	2	3	2	1	3	.	2	.	.	.	.	.	13
9	.	.	.	.	.	.	.	4	2	2	6	.	1	1	.	.	.	.	.	.	16
8	.	.	.	.	.	1	.	3	.	3	6	2	.	.	.	.	.	.	.	.	15
7	.	.	.	.	.	1	1	4	3	.	4	.	2	.	.	.	.	.	.	.	15
6	.	.	.	.	.	2	1	1	.	.	1	.	.	.	.	.	.	.	.	.	5
5	.	.	.	.	2	1	2	2	1	.	1	.	.	.	.	.	.	.	.	.	9
4	.	.	1	1	.	.	.	.	1	2	2	.	.	.	.	.	.	.	.	.	7
3	3	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4
2	6	3	4	1	3	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	19
1	12	16	9	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	39
0	88	28	16	5	2	1	.	1	.	1	.	.	.	.	.	.	.	.	.	.	142
Total	109	47	31	8	7	7	5	18	10	16	25	5	7	2	2	.	.	.	.	1	300

APPENDIX TABLE 4

CORRELATION OF SIZE OF REACTIONS (mm) TO 75 % SMT 6 AND PPD-W, 100 000 TU, FROM PIRQUET TESTS IN HUMANS: NON-VACCINATED GROUP

PPD-W, 100 000 TU	75 % SMT 6																Total
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
20	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1
15	.	.	.	.	.	.	.	.	.	.	.	1	2	.	.	.	3
14	.	.	.	.	.	.	.	.	.	.	.	2	1	.	.	.	3
13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1
12	1	.	.	.	.	.	.	.	.	1	.	.	2	.	.	.	4
11	.	.	.	.	.	.	.	1	.	.	2	2	1	.	.	1	7
10	.	.	.	.	.	.	1	.	1	.	2	6	4	.	2	.	16
9	.	.	.	.	1	.	.	4	2	2	3	1	.	.	.	.	13
8	1	.	.	1	.	.	.	2	3	6	4	1	1	.	1	.	20
7	1	.	.	.	1	.	3	1	2	9	3	2	1	.	.	.	23
6	.	.	.	1	.	3	1	2	1	2	3	.	1	.	.	.	14
5	.	.	.	.	.	2	2	2	1	4	1	1	.	.	.	.	13
4	.	.	.	2	.	1	1	1	.	.	.	.	.	.	.	.	5
3	.	1	.	1	1	2	2	1	.	.	.	.	.	.	.	.	8
2	1	3	5	2	2	1	4	1	.	.	.	.	.	.	.	.	19
1	13	13	11	4	1	.	1	1	1	.	.	.	.	.	.	.	45
0	107	37	17	6	1	4	1	.	1	.	1	.	.	.	.	.	175
Total	124	54	33	17	7	13	16	16	12	24	19	17	13	.	3	2	370

APPENDIX TABLE 5

CORRELATION OF SIZE OF REACTIONS (mm) TO 75 % SMT 6 AND PPD-W, 25 000 TU, FROM PIRQUET TESTS IN HUMANS: NON-VACCINATED GROUP

PPD-W, 25 000 TU	75 % SMT 6																					Total
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
15	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	1
14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
12	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	1
11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	.	.	.	.	.	.	1	.	1	.	1	.	2	.	.	.	.	.	.	.	1	6
9	.	.	.	.	.	.	.	.	1	2	1	2	.	.	.	.	.	.	.	.	.	6
8	.	.	.	.	.	.	.	.	1	1	1	2	1	1	2	.	.	.	.	.	.	9
7	1	.	.	.	.	.	.	4	.	4	1	4	.	1	1	.	.	1	.	.	.	17
6	.	.	.	.	.	.	1	1	1	.	1	1	1	.	2	.	.	.	.	.	.	8
5	1	.	.	.	.	1	4	3	5	2	1	1	1	.	.	.	.	.	.	.	.	19
4	.	.	1	.	.	1	4	3	2	1	.	1	.	.	.	.	.	.	.	.	.	13
3	1	1	1	1	2	.	2	3	1	.	.	.	.	.	.	.	.	.	.	.	.	12
2	1	2	5	1	3	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	16
1	12	15	13	3	1	1	1	2	.	.	1	.	.	.	.	.	.	.	.	.	.	49
0	104	32	18	7	3	3	3	1	.	2	.	.	.	1	.	.	.	.	.	.	.	174
Total	120	50	38	12	9	7	17	18	13	12	8	11	5	4	5	.	.	1	.	.	1	331

APPENDIX TABLE 6

CORRELATION OF SIZE OF REACTIONS (mm) TO 75 % SMT 6 AND RT 23, 120 000 TU FROM PIRQUET TESTS IN HUMANS: BCG-VACCINATED GROUP

RT 23, 120 000 TU	75 % SMT 6 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
18	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1
17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1
14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.	2
12	.	.	.	.	.	.	.	1	.	.	4	1	3	1	.	.	10
11	.	.	.	.	1	2	1	3	2	4	7	3	.	1	.	.	24
10	.	.	.	.	1	4	.	12	7	5	6	.	1	1	.	.	37
9	.	.	.	3	3	4	6	6	10	10	5	1	.	.	.	.	48
8	.	.	1	5	3	5	3	14	3	5	9	1	2	.	.	2	53
7	3	1	2	6	7	5	8	18	3	6	5	3	.	.	.	.	67
6	.	.	3	5	9	15	4	13	3	5	.	.	.	.	.	.	57
5	1	.	11	12	18	19	6	13	7	4	.	.	.	.	.	.	91
4	2	6	9	17	11	10	3	3	1	5	.	1	.	.	.	.	68
3	8	15	16	11	7	5	2	3	.	1	.	.	.	.	.	.	68
2	7	13	9	5	2	2	.	1	.	.	.	.	.	.	.	.	39
1	20	15	11	5	4	.	.	1	1	.	.	.	.	.	.	.	57
0	75	31	14	7	4	5	.	.	.	.	.	.	.	.	.	.	136
Total	116	81	76	76	70	76	33	88	37	46	37	11	6	4	.	2	759

APPENDIX TABLE 7

CORRELATION OF SIZE OF REACTIONS (mm) TO 75 % SMT 6 AND RT 23, 30 000 TU, FROM PIRQUET TESTS IN HUMANS: BCG-VACCINATED GROUP

RT 23, 30 000 TU	75 % SMT 6 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
13	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	1
12	.	.	.	.	.	.	.	1	.	1	.	.	.	1	.	1	.	4
11	.	.	.	.	.	.	.	.	.	.	2	1	.	.	.	.	.	3
10	.	.	.	.	.	1	.	2	3	5	3	1	.	.	1	.	.	16
9	.	.	.	.	.	1	1	7	2	5	5	3	1	.	2	.	.	27
8	.	.	.	.	.	3	.	5	2	7	2	3	2	.	.	.	.	24
7	.	.	2	.	4	2	8	11	9	7	9	1	2	.	.	.	.	55
6	2	.	.	2	2	6	11	10	8	2	2	.	.	.	.	.	.	45
5	.	.	2	7	14	18	15	17	3	5	5	.	.	.	.	.	.	86
4	.	2	3	9	12	26	12	14	5	6	5	.	1	.	.	.	.	95
3	4	4	8	10	19	11	8	3	1	1	.	.	.	.	.	.	.	69
2	2	5	13	20	14	9	5	2	2	2	.	.	.	.	.	.	.	74
1	10	22	11	7	5	5	1	1	1	.	.	.	.	.	.	.	.	63
0	73	53	18	23	18	5	6	5	.	1	.	.	.	.	1	.	.	203
Total	91	86	57	78	88	87	67	79	36	42	33	9	6	1	4	1	.	765

APPENDIX TABLE 8

CORRELATION OF SIZE OF REACTIONS (mm) TO 75% SMT 6 AND PPD-W, 100 000 TU, FROM PIRQUET TESTS IN HUMANS: BCG-VACCINATED GROUP

PPD-W, 100 000 TU	75% SMT 6 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
16	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1
15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
14	.	.	.	.	.	.	.	.	.	.	1	.	.	.	1
13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
12	.	.	.	.	.	.	.	.	1	.	1	.	.	.	2
11	.	.	.	.	.	.	.	.	.	1	1	.	1	.	3
10	.	.	.	.	1	.	1	2	1	.	1	2	1	.	9
9	.	.	.	.	.	.	.	2	.	1	6	.	1	.	10
8	.	.	.	.	.	1	.	3	5	4	5	.	.	1	19
7	.	.	.	.	3	6	5	6	9	9	9	.	1	.	48
6	.	.	1	.	2	1	7	10	12	7	10	1	.	.	51
5	.	.	.	3	3	11	17	16	10	5	2	3	.	.	70
4	1	1	3	4	5	11	13	17	12	7	6	2	.	.	82
3	2	2	11	14	17	25	5	10	3	2	.	1	.	.	92
2	6	6	18	25	15	10	3	6	1	2	1	.	.	.	93
1	22	22	20	14	10	10	5	5	.	2	.	.	.	.	110
0	98	61	29	19	25	8	9	2	.	1	.	.	.	.	252
Total	129	92	82	79	81	83	65	79	54	41	43	10	4	1	843

APPENDIX TABLE 9

CORRELATION OF SIZE OF REACTIONS (mm) TO 75% SMT 6 AND PPD-W, 25 000 TU, FROM PIRQUET TESTS IN HUMANS: BCG-VACCINATED GROUP

PPD-W, 25 000 TU	75% SMT 6 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
11	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1
10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	1	.	.	.	.	.	.	.	.	.	.	2	4	.	.	.	7
8	.	.	.	.	.	.	1	1	.	1	1	.	.	.	.	1	5
7	.	.	.	.	.	2	1	.	4	1	4	1	1	1	.	.	15
6	.	.	1	.	.	2	2	5	2	2	4	1	2	.	.	.	21
5	1	.	.	.	1	4	2	9	4	2	6	3	1	.	.	.	33
4	.	.	.	.	5	4	3	13	7	9	5	3	.	1	.	.	50
3	.	.	5	4	8	14	9	17	6	3	1	1	.	.	.	.	68
2	.	4	7	13	16	25	9	10	8	5	3	.	.	.	.	.	100
1	10	16	29	24	19	18	9	11	6	4	1	1	.	.	.	.	148
0	92	43	49	38	31	31	16	21	6	1	.	.	.	.	.	.	328
Total	104	63	91	79	80	100	52	87	43	28	25	12	8	3	.	1	776

APPENDIX TABLE 10

DISTRIBUTION OF SIZE OF REACTIONS IN GUINEA-PIGS SENSITIZED WITH THREE DIFFERENT ALLERGENS

Allergen	Tuber-culin	Dose (TU/ 0.1 ml)	Size of reactions (mm) 6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	N	$\bar{x}$
Virulent *Myco. tuberculosis*	RT 23	62.5	.	.	.	.	1	4	3	6	.	.	.	.	.	.	.	14	12.0
	,,	250	.	.	.	.	.	.	.	2	3	6	3	.	.	.	.	14	14.7
	,,	1 000	.	.	.	.	.	.	.	.	.	1	2	3	5	3	.	14	17.5
	PPD-W	62.5	.	.	.	.	2	4	6	2	.	.	.	.	.	.	.	14	11.6
	,,	250	.	.	.	.	.	.	.	1	3	6	3	1	.	.	.	14	15.0
		1 000	.	.	.	.	.	.	.	.	.	1	5	3	3	.	2	14	17.1
Killed *Myco. tuberculosis* in oil suspension	RT 23	62.5	.	.	1	.	1	2	2	3	.	.	.	.	.	.	.	9	11.4
	,,	250	.	.	.	.	.	.	.	4	1	2	1	1	.	.	.	9	14.3
	,,	1 000	.	.	.	.	.	.	.	.	.	1	4	3	.	1	.	9	16.6
	PPD-W	62.5	.	.	.	.	.	1	3	3	1	1	.	.	.	.	.	9	12.8
	,,	250	.	.	.	.	.	.	.	1	1	2	2	2	1	.	.	9	15.7
	,,	1 000	.	.	.	.	.	.	.	.	.	1	.	2	3	2	1	9	17.9
BCG	RT 23	62.5	.	.	.	.	3	.	3	2	.	.	.	.	.	.	.	8	11.5
	,,	250	.	.	.	.	.	.	.	2	1	4	1	.	.	.	.	8	14.5
	,,	1 000	.	.	.	.	.	.	.	.	.	4	2	1	1	.	.	8	15.9
	PPD-W	62.5	.	.	.	.	2	3	3	.	.	.	.	.	.	.	.	8	11.1
	,,	250	.	.	.	.	.	1	1	3	2	1	.	.	.	.	.	8	13.1
	,,	1 000	.	.	.	.	.	.	.	1	.	4	.	2	.	1	.	8	15.8