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THE ASSAY OF TUBERCULIN

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SYNOPSIS

All types of tuberculin, crude and pure alike, have in the past been assayed by "matching" the skin reactions they produce in sensitized animals with those produced by the International Standard for Old Tuberculin ; furthermore, a variety of sensitizing antigens have been used. Such "matching" assays are not easy to analyse statistically.

An assay is described, based on the linear relationship between the diameter of the skin reaction and the logarithm of the dose of tuberculin. This type of assay is shown to be precise. It eliminates the need for preliminary titrations and provides information about the slope of the dosage-response lines, and it yields fiducial limits of error from the internal evidence of the assay.

Using this method, a comparative study is made of the effects of varying both sensitizing antigens and test allergens. It is shown that there is sufficient heterogeneity among these different types of tuberculins to make invalid their comparison in terms of a single standard, namely, the International Standard for Old Tuberculin.

The assay of preparations of tuberculin and of purified protein derivative (PPD) has usually been carried out by matching visually for size and intensity the lesions produced by the intradermal injection of the tuberculin into guinea-pigs previously sensitized with living virulent tubercle bacilli. This "matching" by visual appreciation has been the basis of calibrating the unitage of a preparation in terms of the International Standard.

Difficulty in assessing minimal reactions in animals in which skin sensitivity was impaired by progressive tuberculous infection led to the use, as sensitizing agents, of partially or completely attenuated living strains,

or of a suspension in liquid paraffin of heat-killed fully virulent tubercle bacilli. These strains of *Mycobacterium tuberculosis* may vary in degree and specificity of sensitization, and, moreover, the test allergens made from any such strains range from crude tuberculin to PPD. Nevertheless, in all these circumstances, assays have been carried out in terms of the International Standard for Old Tuberculin (ISOT).

In the absence, in such " visual-match " assays, of any information about the slope of the dosage-response lines, the error of the estimated potency, and the effects of variability of the sensitizing agents and test allergens employed, it is not unlikely that the principles of bio-assay are often contravened. Thus, if two types of tuberculin vary in composition so greatly that their dosage-response lines are not parallel, any measure of relative potency is invalid ; and, even if dosage-response lines are parallel, the potency ratios obtained may differ significantly when different sensitizing antigens are used (see, for example, Green [2]).

In spite of the fact that visual matching has been used for many years without its limitations becoming obvious, there is little justification, in the light of its potential invalidity, for its continued use if a valid method of assay can be devised without undue elaboration of the test.

The smaller lesions (5-8 mm), arbitrarily chosen by the individual worker as the minimal reacting dose (MRD), tend to be less defined than 10-15-mm lesions, and the error of their measurement is proportionally larger. Using these larger lesions, therefore, we have devised an assay method for tuberculin capable of statistical analysis and yielding an estimate of potency with fiducial limits of error based on the internal evidence of the assay. Our method has been based on the observations of Wadley,[7, 8] who showed that the diameter of the intradermal tuberculin-reaction in certain areas of the skin of sensitized cows and guinea-pigs was directly proportional to the logarithm of the dose of tuberculin injected in a constant volume of liquid ; and on those of Long & Miles,[4] who confirmed this relationship in guinea-pigs sensitized with BCG. Moreover, this method of assay has provided a means of investigating the effects on slope, potency, and error of varying either the sensitizing antigens or the test allergens being compared.

This paper describes the assay method and examines its precision and accuracy ; it gives an account of the variation observed with changes in either the sensitizing antigens or the type of tuberculin tested, and makes certain recommendations about the principles to be followed in assaying tuberculins. Preliminary accounts of this work were presented to the Royal Society of Medicine [6] and to the fifth session of the WHO Expert Committee on Biological Standardization (1951).[5]

Methods

Albino guinea-pigs of the Hampstead strain, maintained at the National Institute for Medical Research, London, and weighing from 350 g to 550 g, were used throughout the experiments. Animals were sensitized to tuberculin by injection into the adductor muscles of the right leg of one of the following :

(1) Living *M. tuberculosis* : 0.25 mg wet weight of strain CN 844 of the Wellcome Research Laboratory collection. This is a moderately virulent human strain.

(2) Dead *M. tuberculosis* : 0.5 mg dry weight of dead, human tubercle bacilli in liquid paraffin, as used by Dr. H. H. Green at the Ministry of Agriculture and Fisheries Veterinary Laboratory, Weybridge.

(3) BCG : 2 mg wet weight of BCG provided by the Statens Serum-institut, Copenhagen.

The sensitivity of the guinea-pigs was assessed by the intradermal injection of the test allergens three weeks after sensitization. All doses of test allergens were given in 0.2-ml volumes. Three different test allergens were used, namely :

(1) Old Tuberculin : the International Standard for Old Tuberculin, containing 100,000 International Units (IU) in 1.0 ml.

(2) PPD : the International Standard for the PPD of Tuberculin (Mammalian). The International Unit of PPD (Mammalian) is contained in 0.000028 mg and is approximately equivalent in activity to the International Unit for Old Tuberculin. The relationship is necessarily approximate, since it is doubtful whether the two preparations can legitimately be compared by direct assay.

(3) IP 48 : a highly purified preparation of tuberculin prepared by Dr. Bretey at the Institut Pasteur, Paris.

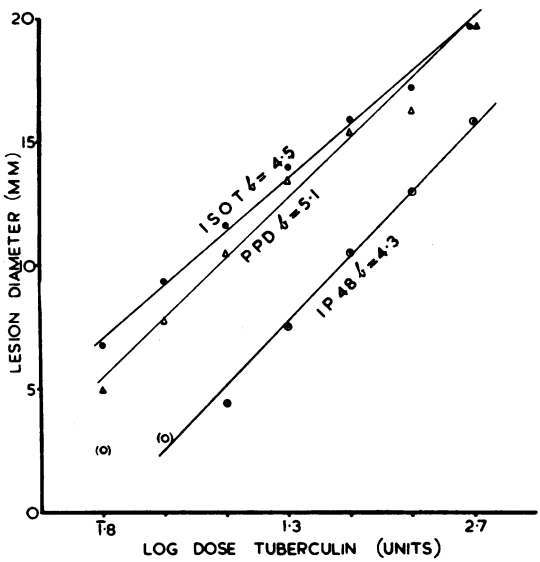
The details of the method of assay which we have developed are given in full in Annex 1 (see page 997). A similar technique was used in all other experiments ; any modifications in design are indicated in the text.

Results

1. *Linearity of dosage-response lines for tuberculin*

Both Wadley [8] and Long & Miles [4] showed that the diameter of the intradermal tuberculin-reaction in sensitized guinea-pigs is directly proportional to the logarithm of the dose of tuberculin injected in a constant volume of liquid. Both used guinea-pigs sensitized with BCG, followed by intradermal Old Tuberculin as the test stimulus. We have confirmed these findings in guinea-pigs sensitized with BCG over a wide range of doses of Old Tuberculin, PPD, and IP 48 respectively. The results of these experiments are shown in fig. 1. Eight doses, increasing in twofold steps, of each of

FIG. 1. COMPARISON OF DOSAGE-RESPONSE LINES FOR ISOT, PPD, AND IP 48



the three tuberculins were arranged in a Latin square of which the eight sites of injection on each animal and the eight animals in the experiment were the rows and columns respectively. The analysis of variance for the experiment with ISOT is shown in table I. It will be seen that the site variation was just significant at the $P = 0.05$ level, but that it was considerably smaller than the between-animals variation, which was highly significant. There were no significant deviations from linearity of the dosage-response lines for a range of doses producing lesions between 6 mm and 20 mm in diameter (i.e., a 64-fold dose range). Similar results were obtained with PPD and IP 48, and in these experiments the site variation was not significant. Although the slopes of the dosage-response lines for each of these substances are similar, they cannot be validly compared since the experiments illustrated were carried out on different days.

2. *Operator variation*

Experiments were carried out by two operators simultaneously and the lesion diameters in the two sets of tests were read by both operators. Analysis of the results showed that, although the size of the lesions might be read on a different basis by different operators, the bias was consistent, the estimates of lesion diameter of one operator all being larger than those of the other, so that slopes and relative potencies were unaffected.

TABLE I. ANALYSIS OF VARIANCE IN EXPERIMENT DESIGNED TO DETERMINE VARIABILITY OF REACTION AT DIFFERENT SITES

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio (F)	P
Between animals	83.23	7	11.89	8.96	< 0.001
Between sites	25.17	7	3.60	2.71	0.01 - 0.05
Between doses	549.42	7	78.49	59.14	< 0.001
Linear regression	534.42	1	534.42	402.66	< 0.001
Deviations from regression	15.00	6	2.50	1.88	> 0.05
Residual	55.74	42	1.33		
Total	713.56	63			

3. *Site variation*

The experiments determining the dosage-response lines, exemplified in table I, indicate that there was little variation in the sensitivity of the guinea-pig's skin from one injection site to another. All these sites were on the trunk—posterior to the shoulder-blade and anterior to the knee-joint in the sitting animal, with the exception of the thin skin on the ventral aspect of the belly. Further experiments on site variations indicated that there was a significant difference in sensitivity between the lateral and the paravertebral area of each flank, but that there was little or no difference anteroposteriorly and that the left and right sides of the guinea-pig behaved similarly. For these reasons, we have, in later experiments, randomized four doses on the four sites on each side of each animal.

4. *Routine method of assay*

As previously mentioned, a detailed description of the method of assay is given in Annex 1. The design is such that the large animal-variation is eliminated from the error of the estimated potency. The potency and slope are both independent of animal and site variation and the error is calculated from the variance of the replicate doses given to each guinea-pig. This type of assay (2+2) has been used for our further investigations of the effects of varying the test allergen and the sensitizing antigen.

5. *Reliability of the assay method*

A number of trial assays were done by the routine method, using guinea-pigs sensitized with BCG or with dead, human tubercle bacilli (TB) and using ISOT as the "Standard" tuberculin. The "unknown" tuberculin was also ISOT, in a concentration unknown to the operators. Three operators carried out assays ; the results are summarized in table II.

TABLE II. TRIAL ASSAYS OF ISOT

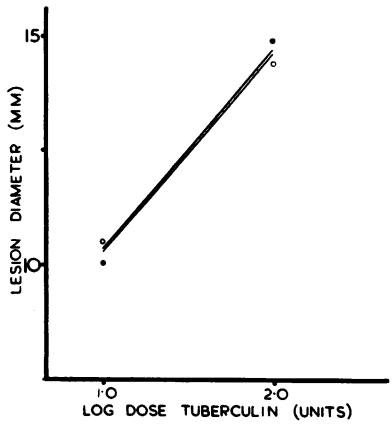
Assay	Operator	Sensitizing antigen	Actual potency of "unknown"	Estimated potency of "unknown"	Fiducial limits of error (P = 0.05)
1	A	BCG	0.99	1.15	0.870 - 1.53
2	B	BCG	0.99	0.792	0.631 - 0.99
3	C	BCG	0.99	1.12	0.835 - 1.51
4	A	BCG	0.936	0.921	0.706 - 1.20
5	B	BCG	0.936	1.09	0.885 - 1.35
6	A	dead TB	0.936	0.936	0.725 - 1.21
7	B	dead TB	0.936	0.72	0.576 - 0.900

Of five assays on animals sensitized with BCG, the fiducial limits of error of the estimated potency included the true value in all cases, although in one case the true value fell at one extreme of the limits of error. In the two assays carried out with animals sensitized by dead, human tubercle bacilli, one estimate of potency was exactly equal to the actual potency, and the other was only 77% of the actual value, which was not included within the limits of error.

6. *Effect of varying the test allergen or the sensitizing antigen*

Since, in the analysis of assays of the type we have described, differences in the slopes of the dosage-response lines can be detected, it is possible to determine whether or not the different tuberculins can, in practice, be considered similar ; i.e., whether it is statistically valid to measure the potency of one kind of preparation in terms of another. Differences in slopes of this kind represent one of the ways in which heterogeneity of the standard and test substances can be detected; another way is the recognition of differences in the potency ratio of the two preparations when the biological system in which they are compared is changed, for example, by changing the sensitizing agent used. We have carried out experiments to investigate both these factors simultaneously. The results of one such experiment are

FIG. 2. PARALLELISM OF DOSAGE-RESPONSE LINES FOR ISOT AND PPD



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shown in table III, and a typical group of another of these experiments is shown in fig. 2.

TABLE III. AN ASSAY OF PPD AND IP 48 AGAINST ISOT IN GUINEA-PIGS SENSITIZED BY: (A) DEAD TUBERCLE BACILLI (TB/D); (B) LIVING TUBERCLE BACILLI (TB/L); (C) LIVING BCG (BCG/L)

Sensitizing agent	Substance assayed	Slope of common regression line	Parallelism (P)	Potency (IU per mg)	Percentage limits of error (P = 0.05)
TB/D	PPD	4.35		48,750	71.4 - 140.1
	IP 48	4.94	< 0.01	55,366	78.2 - 127.9
TB/L	PPD	5.45		81,800	79.9 - 125.2
	IP 48	4.19	< 0.01	57,438	73.8 - 135.5
BCG/L	PPD	3.73		35,000	71.5 - 139.9
	IP 48	3.61		35,934	72.0 - 139.0

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These results exemplify the substantial heterogeneity that may be observed. Although all comparisons carried out by assays of the type we have described yielded estimates of potency having approximately the same fiducial limits of error—namely, from 75% to 135%—the same consistency was not observed in the estimates of potency. Thus, with changes in the sensitizing agent, the potency of PPD in International Units per mg of Old Tuberculin varied from 35,000 to 81,800, and that of IP 48 from 35,934 to 57,438. In general, the smallest potency was obtained in assays where the sensitizing agent was BCG and the largest in assays where the sensitizing agent was living virulent human tubercle bacilli. These differences reflect the fact that the different tuberculo-proteins are heterogeneous and that they cannot, therefore, be estimated in terms of the same standard preparation.

Before the PPD used in these experiments was established as the International Standard, its relative potency was estimated in order that, as nearly as possible, the International Unit for Old Tuberculin should be of the same magnitude as the International Standard for PPD Tuberculin. On this basis, the International Unit for PPD Tuberculin was fixed at 0.000028 mg. The expected potency of PPD would thus be 36,000 IU of Old Tuberculin per mg. The nearest approximation to this figure was obtained in our experiments when BCG was used as the sensitizing agent.

The slopes of the common regression lines, fitted in each assay, also varied with the sensitizing agent, and were significantly flatter when BCG was used as the sensitizing agent. However, when BCG was used, the slopes were fairly consistent and there was no evidence of non-parallelism between the different tuberculins. When IP 48 was compared with ISOT, using the other sensitizing agents, however, there were significant departures

from parallelism, and this is additional evidence of the heterogeneity of the two tuberculins. Furthermore, the slope of the common regression lines varied rather more from assay to assay than was the case with BCG-sensitized animals.

Discussion

These results show that, with a single experiment on eight guinea-pigs, it is possible to assay tuberculin by a method susceptible to statistical analysis and yielding, from the internal evidence of the assay, fiducial limits of error to the estimated potency. We feel that this has advantages over the commonly used methods of assay, which depend on " matching " concentrations of the Standard and test tuberculins and so determining an " end-point ", i.e., a concentration which just fails to produce a response. The method which we describe is less laborious than older methods, which may entail preliminary experiments to determine approximately equipotent doses of the two tuberculins, and no more animals are required than for a " matching test ".[9] On the other hand, a full statistical analysis may take upwards of one hour, a drawback which applies to most methods that provide figures for potency and limits of error and a check on the validity of the assay, rather than merely an opinion as to the probable strength of the unknown. It may, however, be possible to amend the design of the assay in such a way as to enable " short-cut " computing methods—for example, the use of a nomogram (see Leech & Grundy [3])—to be applied.

We recommend the use of guinea-pigs sensitized by BCG, first, because the risk to laboratory workers and the time-consuming precautions required for the maintenance and depilation of animals infected with human pathogens are eliminated ; secondly, because BCG-sensitized guinea-pigs maintain perfect condition with good skin reactivity for many months, so that, not surprisingly, the results of assay in such animals are more consistent and the estimated potencies more in line with expectation ; and, finally, because trial assays on animals sensitized with BCG gave more consistently satisfactory results than those on animals sensitized with either living or dead human tubercle bacilli.

The results of varying the sensitizing antigens and the test allergens suggest very strongly that there is a considerable heterogeneity among different preparations of tuberculoprotein ; and we cannot recommend the assay of these materials in terms of a single standard. This heterogeneity has been clearly noted by Green,[2] and the WHO Expert Committee on Biological Standardization also recognized it in establishing (at its fifth session, held in 1951) an independent International Standard for the PPD of Tuberculin (Mammalian).[10] The heterogeneity which we observed is compatible with the speculation that BCG does not induce sensitivity to *all* tuberculous allergens, and that, compared with IP 48, ISOT contains a relatively low proportion of the allergens to which the BCG-sensitized

guinea-pig is insensitive. Whatever the explanation, it is clear that current assays of one kind of tuberculin in terms of another are not necessarily valid, and that the results are not independent of the type of sensitizing agent, even among the available bovine and human types of antigen.

Moreover, the influence of the sensitizing agent in the guinea-pig throws some doubt on the safety of assuming that potency in the guinea-pig necessarily reflects the diagnostic potency of a tuberculin in the human subject, who may be sensitized by natural infection or by BCG and allied immunizing agents. Among the diagnostic tuberculoproteins used in man, however, the range of estimated potencies obtained by these various methods of assay may be immaterial, since these reagents are commonly used in coarsely graded doses (for example, the tenfold differences in Mantoux test doses).

Whatever the relevance of these discrepancies to the clinical problem, we contend that the assay we propose serves better than the usual routine assays, both for the estimation of potency ratios, and for discovering when potency estimations are not to be trusted.

Annex 1

DETAILS OF ASSAY METHOD

Eight albino guinea-pigs of at least 350 g in weight are sensitized by intramuscular injection of 2 mg wet weight of BCG. It is better to use female animals since this diminishes skin damage by fighting. Both flanks are depilated after three weeks to provide space for eight reactions.

Two doses of the standard preparation and two of the test preparation are used, the dose levels being chosen, on the basis of previous experience, so that the low doses are expected to give lesion diameters of at least 10 mm. The high doses should be ten times the low doses. Since the average slope in assays of this kind is about 4.0, the difference between the mean response to high and low doses is about 4 mm. The four doses—namely, Standard preparation high dose (S_H), Standard preparation low dose (S_L), unknown test preparation high dose (T_H), and unknown test preparation low dose (T_L)—are randomized in duplicate on each animal with the restriction that at each site (numbered 1-8, fig. 3) each dose must appear twice and twice only. An example of this type of randomization is shown in table IV.

Doses are injected intradermally in a volume of 0.2 ml, and the lesions are read 24 hours later. Lesions between 7 mm and 25 mm in diameter fall on the linear part of the line relating log dose and lesion diameter. Smaller lesions (5-7 mm) may be confused with non-specific inflammation, and the erythematous flare accompanying large lesions makes measurement difficult. Only the diameter is noted for the purpose of the assay. (The diameters of round lesions are read to the nearest 0.5 mm. The long and short

FIG. 3. GUINEA-PIG INJECTION SITES

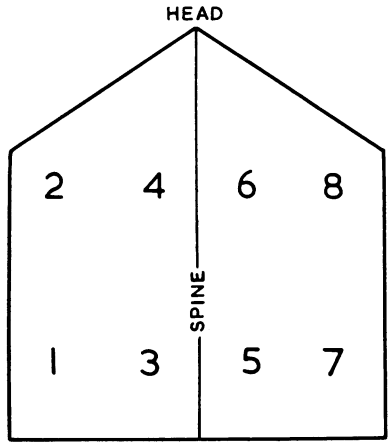

TABLE IV. RANDOMIZATION OF DOSES IN LATIN SQUARE

Guinea-pigs	Sites							
	1	2	3	4	5	6	7	8
1	S_H	S_H	T_L	T_H	T_L	S_L	T_H	S_L
2	T_H	T_L	T_L	S_L	S_H	S_H	S_L	T_H
3	S_L	S_H	T_H	T_L	S_L	T_L	T_H	S_H
4	T_L	T_H	S_L	S_H	S_L	T_H	S_H	T_L
5	S_H	T_L	S_L	T_L	T_H	S_H	S_L	T_H
6	T_L	S_L	S_H	S_H	T_H	T_H	T_L	S_L
7	T_H	T_H	S_H	S_L	S_H	S_L	T_L	T_L
8	S_L	S_L	T_H	T_H	T_L	T_L	S_H	S_H

S = Standard preparation ; H = high dose ; T = unknown test preparation ; L = low dose

diameters of elliptical lesions are measured and the diameter is recorded as the square root of their product.) The intensity of the induration and the presence of central necrosis are irrelevant. The results of an assay and the analysis thereof are shown in tables V-VII.

TABLE V. RESULTS OF AN ASSAY

Guinea-pig	Flank	Lesion diameters (mm), with doses			
		S_H	S_L	T_H	T_L
1	L	19.5	16	21	14.5
	R	19.5	13.5	20.5	15
2	L	16.5	12	17.5	15.5
	R	17	16.5	22	13.5
3	L	18	14.6	18.5	15.5
	R	19.5	13	19.5	14.5
4	L	18	14	19.5	13.5
	R	17.5	13.5	19	13.2
5	L	20	14.5	22.5	13.5
	R	17.5	13.5	19.2	13.7
6	L	19.5	14.7	18.5	15
	R	18	15	19.5	14.5
7	L	15.5	12	15.7	11.7
	R	16	10.7	16.2	11.6
8	L	22	16	20.5	14.5
	R	22.5	15.5	18.5	13.5

S_H = 1/x dilution of ISOT ; S_L = 1/10x dilution of ISOT ; T_H = 1/x dilution of test tuberculin ; T_L = 1/10x dilution of test tuberculin

TABLE VI. ANALYSIS OF VARIANCE OF ASSAY RESULTS GIVEN IN TABLE V

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio (F)	P
Between doses (D)	386.51	3	128.84		
between substances	1.50	1	1.50	1.08	> 0.2
common linear regression .	382.20	1	382.20	276.02	< 0.001
departure from parallelism .	2.81	1	2.81	2.03	> 0.1
Between animals (A)	89.71	7	12.82	9.26	< 0.001
A/D interaction	35.35	21	1.68	1.22	> 0.2
Within animals within doses .	44.31	32	1.38		
Total	555.88	63			

TABLE VII. CALCULATION OF POTENCY

Orthoganol coefficients (see Emmens [1])

	S_H	S_L	T_H	T_L	L		
Substance	−1	−1	+1	+1	64	9.8	1.501 = D^2
Linearity	−1	+1	−1	+1	64	156.4	382.20 = B^2
Departure from parallelism .	+1	−1	−1	+1	64	13.4	2.81
							386.51

k = 1

l = log 10 = 1

$M = \frac{klD}{B} = 0.06266$

Potency = antilog M = 1.155

i.e., the test tuberculin contains 115.5 International Units per ml

s^2 = error variance = 1.38

$s = \sqrt{1.38} = 1.177$

$s_M = skl\sqrt{B^2 + D^2} = 0.06031$

At P = 0.05 with 32 degrees of freedom t = 2.038

Log limits of error (P = 0.05) = M ± tsM

= 0.06266 ± (0.06031 × 2.038)

= T. 9398 — 0.1856

i.e., limits of error = 0.8705 - 1.533

i.e., 75.3 % - 132.7 %

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RÉSUMÉ

L'essai d'activité des tuberculines et des PPD a été effectué jusqu'à maintenant par la mesure des dimensions et de l'intensité de la réaction cutanée produite par l'injection intradermique de ces préparations au cobaye, préalablement sensibilisé par des bacilles tuberculeux, et la comparaison visuelle de cette réaction avec celle que provoque la Préparation Internationale de Vieille Tuberculine. Cette méthode, bien que couramment employée, comporte des causes d'erreur. L'absence de tout renseignement sur la courbe dose-réponse, l'erreur d'estimation de l'activité, l'emploi de divers agents sen-

sibilisants, le dosage de tuberculines de composition différente par rapport à un seul et même étalon sont autant d'infractions potentielles aux principes mêmes de tout essai biologique.

Les auteurs ont mis au point une méthode d'essai plus précise, susceptible d'analyse statistique et donnant une estimation de l'activité avec des marges d'erreur établies d'après les données mêmes de l'essai. Ils ont fondé cet essai sur la constatation de Wadley, confirmée par Long & Miles, selon laquelle le diamètre de la réaction à la tuberculine, dans certaines zones de la peau, chez la vache et le cobaye sensibilisés par le BCG, est proportionnel au logarithme de la dose de tuberculine injectée, sous un volume constant de liquide. Les auteurs discutent la précision de la méthode, exposent les variations des résultats, si l'on fait varier soit le type de tuberculine, soit l'agent sensibilisant. En annexe, ils décrivent les détails de la méthode. Ils ont utilisé, comme agent sensibilisant, une souche modérément virulente de bacille tuberculeux de type humain, des bacilles tués en suspension dans l'huile de paraffine, et le BCG; comme allergène ils ont employé les étalons internationaux de Vieille Tuberculine (VT), et de PPD ainsi qu'une tuberculine hautement purifiée, de l'Institut Pasteur (IP 48).

Les courbes dose-réponse ne se sont pas éloignées de façon significative de la ligne droite, pour huit concentrations de tuberculine provoquant des réactions de 6 mm-20 mm, qu'il s'agisse de VT, de PPD ou de IP 48. L'expérience a montré que les injections peuvent être faites en des points quelconques du thorax de l'animal.

La variation de l'allergène et celle de l'agent sensibilisant ont donné des résultats montrant l'hétérogénéité des tuberculines et l'influence considérable de l'agent sensibilisant sur l'activité estimée des tuberculines. C'est ainsi que l'activité du PPD (en unités internationales de VT) a varié selon l'agent sensibilisant, de 35.000 (avec le BCG) à 81.000 (avec des bacilles virulents) et celle de IP 48, de 35.934 à 57.438 (dans les mêmes conditions respectives). D'une façon générale, l'activité la plus faible a été constatée avec le BCG comme agent sensibilisant et la plus forte avec les bacilles vivants virulents. L'inclinaison des courbes de régression a varié selon l'agent sensibilisant et a été nettement plus faible, lorsqu'on utilisait le BCG. Ces observations montrent que les tuberculoprotéines sont hétérogènes et qu'elles ne peuvent par conséquent pas être évaluées de façon précise par rapport à la même préparation étalon. Cette hétérogénéité a déjà été reconnue puisque le Comité d'experts de l'OMS pour la Standardisation biologique a établi un Etalon International pour le PPD de mammifères. L'Unité Internationale de PPD (correspondant à l'activité de 0,000028 mg) a été établie de façon qu'elle soit du même ordre de grandeur que celle de la VT. L'activité du PPD correspond à 36.000 unités internationales de VT par milligramme. C'est lorsqu'on utilise le BCG comme sensibilisant que les résultats des essais se rapprochent le plus de ce chiffre. L'activité de IP 48, selon les mêmes calculs, devait être de 50.000 unités internationales de VT/mg. Ce chiffre est beaucoup plus élevé que celui qu'ont donné les essais, après sensibilisation par le BCG. L'hétérogénéité observée vient à l'appui de l'idée selon laquelle le BCG ne sensibilise pas l'organisme à tous les allergènes tuberculeux, et que, comparée à la tuberculine IP 48, la Vieille Tuberculine ne contient que peu des allergènes auxquels le cobaye est insensible. Quoi qu'il en soit, les essais d'un type de tuberculine par rapport à un autre ne sont pas nécessairement valables, et les résultats des essais ne sont pas indépendants de l'agent sensibilisant utilisé, même s'il s'agit d'antigènes appartenant au groupe des bacilles de type humain ou bovin.

On peut se demander en outre, en constatant le rôle joué par l'agent sensibilisant, s'il est légitime d'appliquer à l'homme les résultats observés sur le cobaye, l'homme pouvant être sensibilisé par une infection naturelle, par le BCG ou un agent similaire. Toutefois, pour diverses raisons, ces différences peuvent n'avoir pas d'importance pratique chez l'homme.

En terminant, les auteurs soulignent l'avantage que présente leur méthode sur la méthode courante, lorsqu'il s'agit d'évaluer des rapports d'activité et de déceler les cas où l'évaluation de l'activité est sujette à caution.

REFERENCES

1. Emmens, C. W. (1948) *Principles of biological assay*, London
2. Green, H. H. (1951) *Memorandum on mammalian (human) purified protein derivative tuberculin (P.P.D.)* (Working document WHO/BS/127). Quoted in *Wld Hlth Org. techn. Rep. Ser.* 1952, **56**, 6
3. Leech, F. B. & Grundy, P. M. (1953) *Brit. J. Pharmacol.* **8**, 281
4. Long, D. A. & Miles, A. A. (1950) *Lancet*, **1**, 492
5. Long, D. A., Miles, A. A. & Perry, W. L. M. (1951) *Assay of tuberculin* (Working document WHO/BS/120). Quoted in *World Hlth Org. techn. Rep. Ser.* 1952, **56**, 7
6. Miles, A. A. (1951) *Proc. roy. Soc. Med.* **44**, 1050
7. Wadley, F. M. (1948) *Biometrics*, **4**, 100
8. Wadley, F. M. (1949) *Amer. Rev. Tuberc.* **60**, 131
9. World Health Organization (1951) *Pharmacopoea Internationalis*, Geneva, vol. 1, p. 354
10. World Health Organization, Expert Committee on Biological Standardization (1952) *Wld Hlth Org. techn. Rep. Ser.* **56**, 6