

A BIOLOGICAL ASSAY OF TUBERCULINS

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A FEW YEARS AGO it was decided to carry out under the authority of the Agricultural Research Council a specific test, using bovine subjects, of the relative potency of two tuberculin preparations, which may be designated as Standard and Weybridge respectively. Such a test constitutes essentially a biological assay of the tuberculins, and a report of its results may be of interest, since little seems to be known of the statistical problems involved in the use of the tuberculin reaction for such a purpose.

For the test ten herds in different parts of England were used, and from each, twelve cows were chosen and assigned to four treatment groups, each group thus receiving three cows of each of ten herds. The groups differed only in the sites at which the tuberculin was applied. The treatments applied at each site were

<i>A</i>	Standard	0.1 mgm.
<i>B</i>	Standard	0.05 mgm.
<i>C</i>	Weybridge	0.05 mgm.
<i>D</i>	Weybridge	0.025 mgm.

The sites of application, four on each side of the neck, were numbered from one to eight in such a way that numbers five to eight on the left side corresponded with numbers one to four on the right. At each site, the measurement made was a thickening of the skin observable in a set number of hours after intradermal injection of the tuberculin. The treatments of the four classes of cow are set out in the following table:

Sites	Treatment Class			
	1	3	2	4
3 and 6	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
4 and 5	<i>B</i>	<i>A</i>	<i>D</i>	<i>C</i>
1 and 8	<i>C</i>	<i>D</i>	<i>A</i>	<i>B</i>
2 and 7	<i>D</i>	<i>C</i>	<i>B</i>	<i>A</i>

The following sections will incorporate parts of the report made by the author to the Agricultural Research Council in March 1944. The interest of the matter to biometricians lies in the fact that tuberculin readings present in an acute form the need to develop *ad hoc* an appropriate theory of errors. This need is often present and sometimes unrecognised in other types of biological response. The preliminary investigations, by which a theory of errors appropriate to these readings was built up, may therefore be of assistance to workers with other material.

It will be seen that the method judged by these tests, and verified *a posteriori* to be appropriate to the material, is essentially that of χ^2 analysis as ordinarily used with observations of *frequency*. This was adopted not only because it works well, but because it lies ready made to the hand of the statistician. I do not think it is the only mode of analysis which could have been usefully applied. Indeed the Eulerian distribution

$$\frac{1}{p!} x^p e^{-x} dx$$

having, for variable p , variances proportional to the means and giving exhaustive simultaneous estimation based only on the arithmetic and geometric means of each sample, would seem to supply an equally effective mode of approach and one which it would be of considerable mathematical interest to develop. I cannot, however, imagine that it should give a different answer to the practical question at issue.

I should add, what was not known to me when I wrote the report, that careful comparative tests with guinea pigs, well designed and of high precision, gave in fact a ratio of 0.9 instead of 2.2 for the two materials. They must, therefore, in reality be qualitatively different, although there is no indication of this within the scope of the bovine test.

The analysis of the experiment designed to assay the potency of Weybridge P.P.D. H₇ Tuberculin encountered two difficulties:

(a) That arising from the very great variation in the reaction of different cows. This of course had been foreseen as inevitable in unselected material, and it had been proposed that the series of trials first carried out should be regarded in one aspect as a means of selecting animals of uniformly high reactivity, a panel of which could be used for a more accurate assay.

As this had been found impracticable, it was necessary to utilize data involving the full variation in reactivity of unselected material.

(b) It was anticipated that equivalent reactions would be obtained from like sites on opposite sides of the same neck. The data available from the repetition of the test on 120 cows in all show that no such similarity is to be relied on, but that significant differences between Right and Left occur, and that these differences are strikingly different at the four chosen sites. In fact the data have to be examined as if each of the eight test-points on each animal had its own characteristic sensitivity.

In consequence of these two drawbacks the methods of reduction which we had hoped to use appeared on examination to be quite inadequate for the purpose of combining the information available from the different parallel sets of animals. In forming a judgement as to the manner in which such factors as tuberculin-potency interact with the differences in sensitivity of different animals and of different sites, the most valuable information is provided by the fact that on each animal certain pairs of sites, namely 1 and 8, 2 and 7, 3 and 6, 4 and 5, are invariably treated alike, although the actual treatments used on these pairs of sites are varied for animals of the four different classes.

Preliminary analysis seemed to indicate that the difference in response at two sites on different cows was proportional to the sensitivity of the cows, and that the difference in response to different treatments was proportional to the general average response which such treatments provoke. This, so far as it may prove to be true, is a most valuable generalisation. Together with a second observation, namely that the variance to be ascribed to any observation, whether owing to the individuality of the animal or to errors of measurement, is approximately proportional to the magnitude of the measurement to be expected, it does allow of a rational and comprehensive form of analysis.

To demonstrate the approximate truth of these views, the animals in each class were divided, using the total reaction at 48 hours, into 4 groups of reaction-intensity: thus, of the 30 animals in class 1, four, giving reaction at 48 hours of 0-19mm. in all at the 8 sites, are of the lowest class of reactors (α), nine give reactions of 20-49mm., i.e. on the average $2\frac{1}{2}$ to 6mm., (class β), eleven give total reactions of 50-79mm. i.e. 6-10mm. on the average, (γ), and six give total reactions of 80 or more, (δ).

Taking, for example, sites 1 and 8, which with these 30 cows both receive treatment *C* (Weybridge 0.05mgm), if a and b are the measurements observed at any stage, e.g. 72 hours, we can calculate $[(a - b)^2]/(a + b)$ for each cow, and for any group of cows $[(A - B)^2]/(A + B)$ where A and B are the sums of a and b . Then for variation in the ratio

of measurement at site 1 to that at site 8 among the 4 cows of the lowest sensitivity-class, one has three degrees of freedom, yielding

$$S\left\{\frac{(a - b)^2}{a + b}\right\} = 6.7777$$

less $\frac{(A - B)^2}{A + B} = \underline{2.8824}$

leaving 3.8953

as the contribution of these three degrees of freedom. Since there are three other pairs of sites equally comparable on each cow, also with nearly equal total reaction, one can in this way make up 12 degrees of freedom, obtaining the total of 6.9948, measuring variation of the same sort within homogeneous material. The three other classes of cow, in which these same sites receive treatments, *A*, *D*, and *B* respectively, bring up the total degrees of freedom to 108, with a total sum of squares of 40.8580, and a mean-square measured in this way for the least responsive class of cows (α) of 0.38mm.

The point of this procedure is the comparison it allows between cows of very different absolute sensitivity. For the four classes of cows chosen one has the results shown below:

Reactivity-class	Degrees of Freedom	Sum of Squares	Mean-Square
α , 31 cows	108	40.8580	0.3783
β , 43 cows	156	70.0597	0.4491
γ , 35 cows	124	50.8053	0.4097
δ , 11 cows	28	14.3365	0.5120
120 cows	416	176.0595	0.4232

Measured in this way, therefore, the gross heterogeneity between cows of different sensitivity-classes has practically disappeared, and the contributions of unequal numbers of cows in these classes to the evidence may be satisfactorily weighted. Further it appears that the ratio of reaction-measurement at two comparable sites is nearly the same whatever treatment these sites receive. For each sensitivity-class of cow, twelve degrees of freedom have been excluded from the analysis above, representing possible differences of this kind. For the four sensitivity-classes, these are:

Class	Degrees of Freedom	Sum of Squares	Mean-Square
α	12	6.5598	0.5466 mm.
β	12	5.9111	0.4522
γ	12	1.7141	0.3862
δ	12	12.3481	1.0290
	48	26.5331	0.5528 mm.

Apart from the slight suggestion that in the most sensitive class of cows some heterogeneity in the site-ratio has been introduced by varying the tuberculin used, these figures show that there is little danger of being misled if the data are treated as though the ratio of the response at different sites, both in different cows and to different tuberculins, were a constant property of those sites. This is important, since of the four pairs of sites treated alike, three (namely 1 and 8, 3 and 6, 4 and 5) all show significantly unequal response in the aggregate examined. Finally not only is the variation homogeneous within groups of cows showing very varying sensitivity to tuberculin, but the ratio of response in the four classes of cows chosen for their different sensitivity is also the same. For this we have three degrees of freedom for each pair of sites, or twelve in all:

VARIATION AMONG DIFFERENT SENSITIVITY-CLASSES $\alpha, \beta, \gamma, \delta$

Degrees of Freedom	Sum of Squares	Mean-Square
12	5.0866	0.4239

On the basis of this preliminary investigation, which has been set out in detail above for readings at 72 hours, the problem of estimating the proportionate increase in swelling measured produced (a) by doubling the quantity of tuberculin, and (b) by replacing a given amount of Standard tuberculin by half the quantity of Weybridge 10, becomes tolerably straightforward.

The method used in the original report, although substantially accurate in the results it gave, was not well suited as a methodological model, and may be replaced for our present purposes by one of equivalent accuracy and perhaps greater clarity.

Taking, for example, the data for readings at 48 hours, and adding

together readings at the two sites treated alike and on the 30 cows treated alike, the aggregate results of the test may be expressed by the following 4×4 table, to which is appended on the right a key to the treatments used in the form of a non-cyclic Latin Square.

Sites	Cow Class								
	I	III	II	IV					
3 + 6	454	249	349	249	1301	A	B	C	D
4 + 5	408	322	312	347	1389	B	A	D	C
1 + 8	523	268	411	285	1487	C	D	A	B
2 + 7	364	283	266	290	1203	D	C	B	A
	1749	1122	1338	1171	5380				

Treating these aggregate measurements as quasi-frequencies, the data now have a form closely similar to that which arises with a three-point linkage test in genetics, in which also we have 16 observable frequencies, classifiable in three orthogonal categories, assigned arbitrarily to the rows, columns and letters. In such a case, for example, we have typically four different triple heterozygotes used as parents and assigned to the four rows, four modes of gamete formation (crossover classes) assigned to the four columns, and four pairs of complementary genotypes distinguishable associated with the four letters of the square.

If, as sometimes happens, these pairs of complementary genotypes are not equal in viability, the frequencies to be expected in the sixteen entries will be affected not only by factors representing modes of gamete formation and abundance of material from the four possible sources, but by a third unknown set of factors representing relative viabilities.

The statistical problem will then consist in assigning sixteen expectations to the sixteen cells of the table, each expectation being the product of three appropriate factors, all of them unknown.

An examination of this statistical problem shows that the solution of maximum likelihood is such that the sums, by rows, by columns, and by letters, of the expectations are equal to the corresponding sums of the observed frequencies. This is a statistical solution of the utmost simplicity, although the algebraic problem of constructing expectations fulfilling these marginal conditions, and the condition of being triple products, seems to be one of some intricacy. I have elsewhere discussed certain approximate methods of approach.¹

The tuberculin data are in one respect slightly simpler than the

¹R. A. Fisher (1949) Note on the test of significance for differential viability in frequency data from a complete three point test. *Heredity* 3, 2, 215-219.

corresponding genetical problem, for in this case it is to be presumed, unless the data indicate otherwise, that the effect of doubling the dose is the same whichever of the two tuberculins is used, i.e. that the factors corresponding with the letters *A*, *B*, *C* and *D* shall be in proportion. This circumstance opens the way to an effective approximate estimate of these factors.

It will be noticed in the symbolic square that the four quarters are constituted by 2×2 Latin Squares such as

$$\begin{array}{cc} A & B \\ B & A, \end{array}$$

so that the ratio $A : B$, representing the ratio of the readings for double and single injections, can be consistently estimated from the product ratio of the four observed total measurements, i.e. from

$$\sqrt{454 \times 322/408 \times 249}$$

In practice it is most convenient to work with natural logarithms, so that we have

Treatment	Total Measurement	Natural Logarithm
<i>A</i>	454	1.51293
<i>B</i>	408	-1.40610
<i>A</i>	322	1.16938
<i>B</i>	249	-.91228
		.36393
	<i>A : B</i>	.18196

The weight of this logarithmic estimate is ("The Design of Experiments", Section 70) the harmonic mean of the four frequencies, namely 339.69.

Taking in turn the three other similar included 2×2 squares, and remembering that the ratio $C : D$ is to be presumed equal to that of $A : B$, we have, in the four cases

	Log Ratio	Weight
<i>A : B</i>	.18196	339.69
<i>A : B</i>	.22624	304.19
<i>C : D</i>	.20844	335.45
<i>C : D</i>	.22197	308.44

from which we have the estimate of the weighted mean .20890, for the effect expressed as the natural logarithm of the measurement of doubling the tuberculin dosage.

It may also be seen that four more 2×2 Latin Squares, in this case overlapping, are available to estimate the ratio $A : C$ or $B : D$ for which, using again natural logarithms, we have the estimates

	Log Ratio	Weight
$A : C$.01102	424.94
$A : C$	-.02517	308.42
$B : D$	-.02269	328.87
$B : D$.03075	261.91

the weighted mean being in this case $-.00188$. It will be noticed at this stage that the estimates are showing a remarkable consistency.

The two estimations carried out above answer the practical question of the enquiry by assigning relative performance to the single and double doses of the two tuberculins used and show, in fact, that the Weybridge material was effectively a little more than twice as potent as the Standard. Questions of precision can, however, only be answered by constructing the expectations corresponding to the measurements observed. An approximate method of doing this, appropriate to cases like the present, in which all cells of the square are well occupied, is shown below.

We have the measurements of logarithmic relative potency

B	Standard single	0.0000
A	Standard double	0.2089
D	Weybridge half	0.0019
C	Weybridge single	0.2108

The antilogarithms of these give factors appropriate to the four treatments. Dividing the observed frequencies by these factors we have the adjusted frequencies

368.412	249.	282.673	248.532	1148.617
408.	261.297	311.413	281.053	1261.763
423.604	267.496	333.518	285.	1309.618
363.316	229.216	266.	235.329	1093.861
1563.332	1007.009	1193.604	1049.914	4813.859

From the margins we can reconstruct the table so that the rows

and columns are in strict proportion

373.0208	240.2787	284.8014	250.5161
409.7657	263.9476	312.8561	275.1936
425.3070	273.9584	324.7219	285.6307
355.2385	228.8243	271.2246	238.5736

Each value may now be multiplied by the appropriate treatment factor, so as to give an approximate expectation.

459.6817	240.2787	351.6274	250.9876	1302.5754
409.7657	325.2684	312.4430	339.7652	1387.2423
525.1013	274.4740	400.1619	285.6307	1485.3679
355.9071	282.5158	271.2246	293.9995	1203.6470
1750.4558	1122.5369	1335.4569	1170.3830	5378.8326

Since these do not give exactly the original total, they may be reduced to the correct total, as in the following table.

459.781	240.331	351.704	251.042	1302.858	-1.858
409.855	325.339	312.511	339.839	1387.544	+1.456
525.215	274.534	400.249	285.693	1485.691	+1.309
355.984	282.577	271.283	294.063	1203.907	-0.907
1750.835	1122.781	1335.747	1170.637	5380.000	
1749	1122	1338	1171		
-1.835	-0.781	+2.253	+0.363		

The marginal totals of this table of expectations, although not exactly equal to those of the observations on which they are based, are good approximations to these. Thus the column totals each of about 1300mm. have discrepancies -1.8 , -0.7 , $+2.3$, $+0.4$ mm. only. With the rows the largest discrepancy is only -1.9 mm., and with the letters (treatments) we have

	Expected	Observed	
<i>A</i>	1479.432	1477	-2.432
<i>B</i>	1207.162	1208	+0.838
<i>C</i>	1499.335	1503	+3.665
<i>D</i>	1194.071	1193	-1.071

Thus our method, though only tentative and approximate, can be seen after the event to have given a very satisfactory approximation to the ideal fitting required. Owing to the importance of this type of problem in genetics, and the probability of further analogous cases in biological assay, the problem of making a sufficiently rapid and sufficiently accurate fitting of this kind seems to deserve further study.

Given sufficiently good expectations, we can calculate the ingredients $(a - m)^2/m$, the sum of which supplies the analogue to χ^2 for the residual seven degrees of freedom.

$(a - m)^2/m$			
.0093	.1555	.2888	.0017
.1805	.0006	.1029	.0561
.0727	.3127	.0208	.0166
.0084	.0343	.0008	.1509
.2709	.5031	.4133	.2253
1.4126 mm.			

d.f.	s.s.	m.s.
7	1.4126	.2018 mm.
12	2.92564	.2438 mm.

In millimetres this comes to 1.4126, with a mean square .2018mm. only. This value is in good agreement with that obtained by contrasts between the aggregate readings on sites treated alike, which for twelve degrees of freedom gives 2.92564, or .2438mm. as the average value.

These values are rather surprisingly less than those obtained directly in the preliminary test set out above. The indications of precision available from individual readings were, therefore, recalculated more exactly, treating each set of three cows of the same herd and treatment as a 3×8 frequency table, giving 21 degrees of freedom within the herd, and each set of trios, one from each of ten herds for a given treatment, thus supplying 63 degrees of freedom between herds. Owing to a few cows giving completely zero readings, we have not quite the full number of degrees of freedom available, but using the same readings, i.e. at 48 hours, as those used in the illustration above, we have

	Degrees of Freedom	Sum of Squares	Mean Square
Within herds	539	242.7791 mm.	.4504 mm.
Between herds	252	144.1697 mm.	.5712 mm.

It is a puzzling feature, and one that I do not understand, that the comparisons used in the final aggregates should agree so appreciably more closely than do the individual readings on which they are based.

The ratio of potency of equal weights of the two tuberculins were

estimated, for the readings at the three periods used, to be as follows:

Period	48 hours	72 hours	96 hours
Estimated ratio	2.009	2.141	2.172

TABLE 1
PERCENTAGE INCREASE IN MEASUREMENT (MEASURED LOGARITHMICALLY)
ESTIMATED INDEPENDENTLY FOR EACH HERD

	48 hrs.	72 hrs.	96 hrs.
DOUBLE v. SINGLE DOSE			
Kent	30.7	33.8	26.2
Cheshire A	30.0	28.2	30.4
Berkshire	25.1	32.7	25.0
Lancashire B	27.6	20.5	18.4
Cheshire B	17.8	21.8	20.7
Durham	18.4	20.0	18.3
Cambridge A	18.9	17.3	15.7
Essex	15.7	18.3	16.4
Cambridge B	17.5	15.3	- 2.0
Lancashire A	15.2	6.9	- 2.7
Weighted mean	21.1	21.5	16.4
WEYBRIDGE v. STANDARD TUBERCULIN.			
Kent	3.2	3.3	5.4
Cheshire A	- 2.1	- 1.7	- 0.1
Berkshire	6.8	7.5	11.2
Lancashire B	11.3	7.5	8.4
Cheshire B	-10.8	- 7.6	0.7
Durham	11.3	3.7	- 0.9
Cambridge A	- 4.5	6.3	7.1
Essex	- 2.4	4.1	- 9.3
Cambridge B	2.6	4.2	6.8
Lancashire A	- 3.0	5.9	-10.0
Weighted mean	0.48	3.19	3.03
<i>Relative potency of equal weight of Tuberculin.</i>			
Weybridge v. Standard, with fiducial limits	2.032	2.217	2.274
	2.341	2.505	2.727
	1.764	1.961	1.897
Estimate from aggregated data	2.009	2.141	2.172

To examine the consistency of the differential responses on which these estimates are based, and to obtain an appropriate standard error and fiducial limits for the estimates, a parallel process was applied to the ten constituent herds individually. (The original report then discusses individual herds in detail at the different periods at which the swellings were read.) The herd values are shown in Table 1. It is upon these that the fiducial limits have been based.

Table 2 gives the relative performance at the eight sites. Of these the most forward (1, 5) are the most sensitive, and the hindermost (3,7) are least so. It is obvious that there is no consistency in the differences between Right and Left.

TABLE 2
PROPORTIONATE RESPONSE AT EACH SITE

	48 hours	72 hours	96 hours
Site			
1	1.141	1.131	1.143
2	.924	.931	.925
3	.931	.894	.892
4	.993	.990	1.017
5	1.100	1.115	1.107
6	.975	.982	.959
7	.895	.928	.930
8	1.040	1.029	1.027
	7.999	8.000	8.000

The complete data from the experiment are shown in Table 3.

SUMMARY

The above details and the result of the experiment reported have been published at the present time: partly in illustration of the fact that each type of reading which arises in biological assay deserves and may require the development for it of an appropriate theory of errors; secondly because previous work with tuberculin readings seems to have given no idea as to how they can be quantitatively interpreted; and thirdly because the precision of such readings regarded as a biological assay seems to have been much underrated.

TABLE 3
 TABLE OF INDIVIDUAL RESPONSES AT 48, 72, 96 HOURS
 (Data relate to 10 farms, 4 treatment classes at each, 3 cows per class).

Treatment class	Site	Tuber-culin	Kent												Cheshire A											
			Cow 1			Cow 2			Cow 3			Cow 1			Cow 2			Cow 3								
			48	72	96	48	72	96	48	72	96	48	72	96	48	72	96	48	72	96						
1	A	16	18	11	6	4	4	21	28	21	10	12	14	10	10	10	8	6	6							
	B	12	14	10	2	2	2	14	14	12	5	6	7	6	7	5	6	7	5							
	C	4	6	5	7	6	4	23	24	17	6	8	9	6	8	7	6	7	4							
	D	14	15	12	4	3	3	11	12	10	6	8	9	7	7	6	4	4	4							
	D	6	9	9	4	2	1	14	16	12	8	10	11	9	10	8	7	7	5							
	C	8	12	10	9	8	6	19	21	15	10	12	13	9	11	10	6	6	4							
	B	3	8	8	2	2	2	13	16	12	7	10	10	7	9	8	5	6	3							
	A	10	15	13	4	5	5	20	24	18	9	12	13	10	10	9	8	8	5							
2	C	8	9	4	3	8	9	18	22	14	9	10	12	14	13	11	12	11	11							
	D	4	5	4	2	3	7	7	9	4	5	8	7	9	8	8	8	7	7							
	A	8	8	4	2	2	5	10	13	9	6	10	8	11	13	9	10	10	9							
	B	5	6	3	1	2	3	9	12	8	5	8	7	12	12	10	8	8	7							
	B	5	8	4	2	3	5	7	7	3	6	9	8	13	13	11	11	11	9							
	A	5	6	4	3	3	5	9	12	7	8	11	9	12	12	9	9	10	10							
	D	3	4	4	3	3	5	10	12	7	7	10	8	7	7	6	10	12	9							
	C	7	8	6	1	1	1	9	17	11	11	7	10	9	12	10	11	16	13							
3	B	1	1	1	3	3	5	2	4	4	5	6	5	1	1	2.5	0	0	1							
	A	1	1	1	1	2	3	2	3	3	6	7	5	1	1	1	0	0	0							
	D	1	0	1	1	3	6	2	2	2	4	4	3	0	0	0	0	0	0							
	C	4	2	3	1	3	6	5	5	5	8	8	6	1	1	1	0	0	0							
	C	0	1	1	4	6	6	4	5	6	8	8	7	0	0	1	0	0	1							
	D	0	0	0	2	4	6	3	4	3	5	4	4	0	0	1	0	0	0							
	A	1	1	1	3	4	5	4	5	4	4	4	3	1	1	2.5	0	0	1							
	B	1	0	1	4	4	5	3	2	2	2	5	5	3	1	0	0	0	0							
4	D	1	4	2	7	8	7	3	4	5	5	7	6	2	2	3	4	7	6							
	C	2	4	3	8	11	7	3	4	5	4	5	5	1	0	2	5	8	7							
	B	2	6	3	7	7	7	2	4	8	4	4	4	0	0	2	0	0	0							
	A	3	6	4	8	11	6	3	4	6	6	6	8	0	0	3	5	8	8							
	A	3	7	7	11	12	7	4	7	6	6	7	6	1	1	4	5	9	8							
	B	3	5	2	7	10	6	3	3	4	4	4	5	0	1	3	3	5	5							
	C	3	4	5	8	10	6.5	2	5	5	4	5	5	0	0	3	4	7	6							
	D	1	3	2	7	9	6	2	4	4	4	3	4	0	0	3	4	2	4							

TABLE 3—Continued
 TABLE OF INDIVIDUAL RESPONSES AT 48, 72, 96 HOURS
 (Data relate to 10 farms, 4 treatment classes at each, 3 cows per class).

Treatment class	Site	Tuber- culin	Berkshire						Lancashire B											
			Cow 1		Cow 2		Cow 3		Cow 1		Cow 2		Cow 3							
			48	72	96	48	72	96	48	72	96	48	72	96						
1	1	A	0	6	6	1	4	4	10	8	4	4	9	9	2	2	8	4	10	11
	2	B	0	4	4	2	3	2	1	1	1	4	8	7	1	4	4	3	5	7
	3	C	1	6	7	2	6	6	7	8	8	4	8	7	1	4	6	4	9	11
	4	D	1	6	5	2	4	4	3	7	7	4	6	6	1	4	5	3	8	12
	5	D	2	7	6	2	4	5	6	8	8	3	3	6	1	3	5	3	11	10
	6	C	1	8	7	2	4	4	2	8	8	4	4	7	8	3	6	6	7	12
	7	B	0	1	3	2	3	4	0	0	2	3	5	7	1	3	4	7	5	7
	8	A	2	5	4	2	5	4	3	8	9	3	3	6	8	2	4	2	2	5
2	1	C	16	13	12	17	23	23	6	8	7	4	8	11	5	7	7	10	11	15
	2	D	8	9	9	18	18	14	2	3	5	3	4	8	6	8	6	6	10	8
	3	A	9	12	12	16	18	14	3	3	5	7	9	13	7	7	7	9	13	10
	4	B	9	9	10	17	12	20	2	6	4	3	7	11	4	6	6	4	10	10
	5	B	12	13	12	14	16	16	3	7	5	4	7	15	2	4	3	4	12	10
	6	A	9	9	9	19	22	20	2	5	4	12	12	13	7	9	8	8	14	12
	7	D	7	7	9	13	18	15	3	3	3	4	6	10	7	8	7	6	8	9
	8	C	14	16	15	16	17	18	6	6	4	5	7	11	6	6	6	8	11	10
3	1	B	0	1	5	9	9	9	2	9	8	3	9	9	10	11	12	6	10	10
	2	A	2	4	6	8	7	6	1	6	7	3	8	10	11	13	15	5	9	7
	3	D	2	2	7	10	9	7	2	8	8	2	8	10	10	12	11	4	7	7
	4	C	2	2	7	10	8	8	2	9	13	4	10	12	15	20	18	5	6	8
	5	C	3	4	5	17	16	18	2	10	4	12	16	16	18	18	18	5	8	8
	6	D	1	2	4	2	5	4	0	2	3	2	10	11	10	9	13	4	7	7
	7	A	2	3	6	10	10	8	3	7	8	1	11	11	8	12	12	8	7	6
	8	B	1	1	5	13	11	10	0	4	5	5	11	11	3	5	6	2	6	6
4	1	D	6	6	3	1	4	5	1	7	6	5	12	11	2	4	5	3	7	6
	2	C	6	6	4	1	4	4	3	9	6	7	12	11	3	7	9	3	9	7
	3	B	5	4	3	1	2	3	4	4	7	7	8	6	1	3	4	3	5	6
	4	A	7	7	4	1	2	2	4	15	13	5	10	8	2	5	6	3	8	6
	5	A	8	6	4	1	6	4	4	14	8	4	12	10	2	5	6	4	8	9
	6	B	5	5	4	1	3	3	8	4	4	6	11	8	2	4	5	4	7	6
	7	C	6	4	4	1	4	2	3	3	8	3	9	7	2	6	7	5	6	6
	8	D	9	6	4	1	1	1	2	1	7	7	14	9	2	5	6	3	8	7

TABLE 3—Continued
 TABLE OF INDIVIDUAL RESPONSES AT 48, 72, 96 HOURS
 (Data relate to 10 farms, 4 treatment classes at each, 3 cows per class).

Treatment class	Site	Tuber-culin	Cheshire B						Durham											
			Cow 1		Cow 2		Cow 3		Cow 1		Cow 2		Cow 3							
			48	72	96	48	72	96	48	72	96	48	72	96						
1	1	A	7	10	6	13	18	9	12	14	14	4	4	4	0	1	3	11	16	13
	2	B	5	8	5	7	9	9	7	8	8	2	2	4	1	0	2	9	9	11
	3	C	6	9	5	6	9	9	9	11	10	2	4	4	1	1	1	7	7	11
	4	D	6	9	6	7	9	9	8	9	10	2	2	4	0	1	1	10	11	11
	5	D	8	12	9	9	12	11	9	11	10	2	2	3	0	1	1	9	12	11
	6	C	6	11	8	7	10	10	8	10	9	2	3	0	2	2	2	6	8	9
	7	B	6	10	5	8	10	10	9	11	9	2	1	1	0	1	1	4	9	8
	8	A	9	13	6	12	18	15	9	11	10	2	2	4	1	2	2	11	14	13
2	1	C	13	14	14	14	13	10	9	9	8	2	0	1	11	10	11	1	1	2
	2	D	11	11	9	9	10	7	5	6	5	3	2	1	5	9	8	1	1	2
	3	A	14	14	12	11	11	7	6	8	6	2	0	2	2	2	1	0	0	0
	4	B	15	15	12	11	10	6	5	8	6	3	3	2	5	6	7	0	0	0
	5	B	22	23	17	16	16	10	7	8	6	2	2	4	9	3	5	0	1	1
	6	A	16	15	13	14	13	9	7	8	6	2	2	2	5	3	6	0	0	0
	7	D	9	12	9	12	11	9	5	8	6	2	1	1	6	3	4	0	0	0
	8	C	20	20	15	13	14	10	9	10	7	5	5	5	14	11	8	0	0	0
3	1	B	1	1	1	14	14	12	0	0	0	10	14	15	4	12	9	1	1	
	2	A	1	2	2	9	11	8	0	0	0	11	17	14	4	8	5	0	0	
	3	D	1	1	1	6	6	6	0	0	0	5	9	9	4	5	5	0	1	
	4	C	0	1	1	11	13	12	0	0	0	8	15	18	8	12	11	1	1	1
	5	C	2	3	3	11	14	12	0	0	0	13	16	17	7	12	8	1	1	1
	6	D	0	0	1	7	8	7	0	0	0	9	13	13	6	8	6	1	1	1
	7	A	1	1	2	5	6	6	0	0	0	13	21	26	6	14	8	1	1	0
	8	B	1	0	0	7	9	7	0	0	0	9	15	16	6	11	9	1	0	0
4	1	D	2	3	3	1	0	0	3	7	9	0	2	0	8	8	6	3	4	5
	2	C	2	4	4	1	1	0	6	8	9	2	4	3	9	9	5	6	6	7
	3	B	1	3	3	0	0	0	3	6	7	3	4	4	5	6	4	6	7	8
	4	A	4	4	7	5	0	1	6	8	11	2	5	3	4	9	5	3	3	5
	5	A	4	4	7	5	0	1	5	8	10	4	6	5	4	6	7	6	8	7
	6	B	2	6	4	1	0	0	6	7	9	3	4	4	5	5	3	4	6	6
	7	C	4	4	7	5	0	0	5	10	12	5	5	6	6	7	8	5	5	8
	8	D	2	3	2	1	1	1	4	7	8	2	6	6	5	8	8	4	5	6

TABLE 3—Continued
 TABLE OF INDIVIDUAL RESPONSES AT 48, 72, 96 HOURS
 (Data relate to 10 farms, 4 treatment classes at each, 3 cows per class).

Treatment class	Site	Tuber- culin	Cambridge A									Essex								
			Cow 1			Cow 2			Cow 3			Cow 1			Cow 2			Cow 3		
			48	72	96	48	72	96	48	72	96	48	72	96	48	72	96	48	72	96
1	1	A	4	7	3	11	11	7	3	2	2	4	5	3	4	12	7	9	12	7
	2	B	4	5	3	8	12	6	3	0	1	2	5	4	3	8	2	2	2	3
	3	C	5	6	4	10	12	7	3	2	2	4	5	4	4	10	6	4	6	4
	4	D	3	5	3	8	9	8	3	0	1	4	5	4	4	11	4	3	6	3
	5	D	5	6	4	12	14	10	3	3	2	4	5	4	4	3	8	7	4	4
	6	C	3	5	4	9	12	8	4	3	3	2	2	2	2	6	12	7	1	2
	7	B	4	4	4	7	9	6	2	1	2	3	2	2	2	5	8	5	5	7
	8	A	4	6	4	13	14	10	3	0	1	3	2	2	2	5	10	5	8	9
2	1	C	4	6	5	5	6	6	24	21	10	7	12	12	10	12	6	9	10	7
	2	D	4	6	5	1	3	3	16	13	9	5	8	8	7	8	4	6	7	
	3	A	5	4	4	3	4	4	22	18	11	5	5	6	10	9	8	8	7	
	4	B	2	5	3	3	6	4	18	11	7	6	10	10	10	7	4	5	5	
	5	B	5	5	4	3	3	4	24	15	11	5	8	9	10	10	6	8	9	
	6	A	5	6	5	3	3	4	27	23	13	5	8	8	10	9	4	7	7	
	7	D	5	5	4	2	2	3	19	14	9	5	8	7	7	6	5	8	5	
	8	C	4	5	4	3	4	4	29	21	13	6	12	12	11	11	6	10	10	
3	1	B	1	2	1	19	18	9	5	4	3	14	12	9	5	10	4	8	7	
	2	A	3	2	1	21	13	7	3	3	2	13	15	10	6	12	9	4	6	
	3	D	3	2	1	12	21	6	2	2	1	13	11	8	5	6	4	6	6	
	4	C	3	2	2	24	23	12	3	4	2	14	16	8	8	11	7	5	7	
	5	C	1	2	1	17	15	9	4	3	2	12	14	9	8	10	6	7	12	
	6	D	0	3	2	12	10	7	3	3	2	11	17	5	4	7	5	5	9	
	7	A	1	1	1	15	15	9	3	3	3	10	11	6	6	9	10	5	8	
	8	B	2	2	1	15	16	10	4	4	3	11	14	8	7	8	4	4	6	
4	1	D	10	8	6	7	7	4	10	9	8	3	4	2	13	5	7	8	6	
	2	C	8	7	5	4	8	5	8	9	7	3	3	3	8	12	4	7	12	
	3	B	9	7	3	5	5	3	7	6	4	1	2	1	7	8	4	11	9	
	4	A	12	10	6	6	6	4	9	10	8	5	6	6	9	10	3	10	13	
	5	A	12	10	5	7	9	4	11	11	8	2	3	1	14	14	9	11	9	
	6	B	9	5	3	5	7	4	7	9	7	3	4	3	7	9	2	7	9	
	7	C	8	9	5	5	7	7	8	11	9	3	4	3	11	13	4	7	10	
	8	D	10	8	4	4	8	5	5	7	10	3	5	5	8	10	4	7	10	

TABLE 3—Continued
 TABLE OF INDIVIDUAL RESPONSES AT 48, 72, 96 HOURS
 (Data relate to 10 farms, 4 treatment classes at each, 3 cows per class).

Treatment class	Site	Tuber-culin	Cambridge B									Lancashire A									
			Cow 1			Cow 2			Cow 3			Cow 1			Cow 2			Cow 3			
			48	72	96	48	72	96	48	72	96	48	72	96	48	72	96	48	72	96	
1	1	A	7	9	6	23	19	15	10	8	11	8	0	1	5	1	1	6	6	7	9
	2	B	3	4	4	12	15	16	8	7	11	7	0	1	1	1	1	7	5	6	7
	3	C	6	7	5	18	16	9	7	12	7	7	0	0	4	1	1	6	5	6	6
	4	D	2	3	3	16	17	7	11	6	0	4	1	1	4	1	1	5	5	5	6
	5	D	3	4	4	21	21	14	7	7	7	6	0	1	3	1	1	7	1	2	1
	6	C	6	6	5	31	28	8	7	10	8	0	1	4	1	1	5	4	6	7	7
	7	B	5	5	4	11	18	9	6	8	6	0	1	1	1	1	5	4	6	6	9
	8	A	4	5	6	22	11	6	10	11	7	7	0	1	3	1	1	6	5	0	8
2	1	C	8	18	9	11	14	10	8	9	9	9	1	2	3	1	1	3	7	8	8
	2	D	6	10	8	9	11	6	7	8	8	1	1	3	6	1	2	2	2	2	3
	3	A	9	10	9	12	7	11	10	9	1	2	6	1	1	1	1	1	5	7	9
	4	B	6	8	7	10	14	8	6	9	7	7	1	1	6	1	0	0	3	4	8
	5	B	3	5	4	9	16	9	6	9	9	7	1	2	6	2	1	2	4	5	8
	6	A	8	9	8	10	14	8	6	10	8	1	2	6	3	1	3	2	2	2	2
	7	D	6	8	10	9	13	7	6	7	6	1	2	5	0	0	1	5	6	7	7
	8	C	8	12	10	10	14	9	6	10	8	0	2	2	2	2	2	2	5	5	8
3	1	B	10	12	12	7	10	10	9	8	5	5	2	4	5	1	1	2	2	1	4
	2	A	11	12	12	6	8	7	9	10	5	4	4	6	5	1	1	2	2	1	3
	3	D	7	9	9	7	8	6	7	5	3	5	5	6	5	1	1	1	2	1	2
	4	C	8	7	8	5	10	6	8	8	3	3	5	8	3	1	1	2	1	2	2
	5	C	9	16	17	11	13	9	9	9	5	3	5	3	5	4	1	2	1	2	1
	6	D	16	17	18	7	8	8	10	8	7	4	6	6	5	1	1	2	1	1	4
	7	A	7	9	8	9	11	7	8	7	8	4	4	5	3	1	1	2	1	1	4
	8	B	7	13	13	7	10	7	9	7	7	6	4	5	3	1	1	2	1	1	1
4	1	D	19	15	17	2	6	5	12	12	10	10	1	4	4	2	2	2	1	2	3
	2	C	21	15	11	2	6	5	9	10	7	7	1	2	3	2	1	2	2	3	4
	3	B	18	9	7	2	6	4	8	7	6	6	0	2	4	2	1	2	1	2	2
	4	A	13	10	7	4	7	4	12	13	9	1	2	4	1	0	1	2	2	2	3
	5	A	23	22	13	2	6	5	8	12	6	2	2	3	4	2	2	2	2	3	4
	6	B	11	12	10	1	7	4	7	9	7	1	2	4	2	2	2	2	2	2	4
	7	C	9	11	7	1	8	4	8	12	8	1	2	4	2	2	2	2	2	2	4
	8	D	12	9	11	2	5	6	8	8	7	8	1	5	5	2	2	2	2	2	3