THE EFFECT OF SOME VARIABLES ON EXPERIMENTAL *KLEBSIELLA* INFECTIONS IN MICE

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(With 5 Figures in the Text)

INTRODUCTION

The primary object of these experiments was to investigate the range of variation in virulence among selected strains of *Klebsiellae*. As these organisms constitute respiratory tract pathogens in man, it was considered of interest to test by oral in addition to peritoneal routes, the former having been shown (Epstein, 1958; Epstein & Stratton, 1958) to be a simple and reliable technique for the initiation of pneumonic infections in anaesthetized mice. An opportunity was incidentally taken to study the effect of some factors considered likely to influence the outcome of these infections.

MATERIALS AND METHODS

Inbred albino male mice, 4–6 weeks old, weighing 19–24 g. were used. The animals were fed on M.R.C. diet, no. 41, water *ad lib.* and housed ten to a cage. Table 1 gives a description of the nine National Collection Type Culture (N.C.T.C.) strains of *Klebsiellae* tested. As can be seen, three of these were 'low type' pneumoniae

Table 1. *Description of nine N.C.T.C. strains of Klebsiellae under test*

Strain	Type	Source
9494	Pneumoniae type 1 (capsulate)	Sputum
5054	Pneumoniae type 1 (capsulate)	Pneumonia
9503	Pneumoniae type 2 (capsulate)	Urine
5048	'Rhinoscleroma' type 3 (capsulate)	Nose
5049	Acapsular form of 5048	Nose
5051	'Ozaenae' type 5 (capsulate)	Unknown
5053	Acapsular form of 5051	Unknown
9527	'Aerogenes' type 1 (capsulate)	Water
8167	'Aerogenes' type II (capsulate)	Water

strains. In addition strains of 'rhinoscleroma', 'ozaenae', their acapsular variants and two strains of 'aerogenes' were tested. These designations are used here without prejudice, their nomenclatural status having been recently considered in relation to serological, biochemical and enzymological criteria (Epstein, 1959*a*). Strains

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under test were grown overnight in digest broth for 16 hr. at 37° C. and then centrifuged at 3000 r.p.m. for 15 min. Centrifugates were washed once, suspended in saline and suspensions in the range of 10^3 to 10^9 organisms/ml. prepared, using Brown's opacity tubes. Inoculations were effected within 1 hr. of preparation of the suspensions. Under each set of experimental conditions ten animals were inoculated by oral and intraperitoneal routes respectively with 0.05 ml. of each suspension concentration.

It was decided on the basis of pilot experiments to limit the duration of observation to 21 days. Specific mortality generally occurred within the first 14 days. The precise time of death could obviously not be determined in most cases, so that a mouse dying on the 6th day has been considered for the purposes of statistical analysis to have a survival time of $5\frac{1}{2}$ days. Animals surviving the study period were sacrificed and examined; in no case was there any histological or bacteriological evidence of infection.

PREPARATION OF RESULTS FOR ANALYSIS

In most cases where organisms were administered orally, and in a few where they were administered intraperitoneally, the mice died from specific pneumonic lesions before the lapse of 21 days. In these it was therefore possible to assign a survival time to each animal within the limits defined above and so to examine the dose-survival relationship by quantitative methods, namely, 'least squares' regression analysis of survival on dose. In one group (strain 9494 injected intraperitoneally) three mice survived the period of observation and the simplifying assumption that they died on the 22nd day does not appear to have had any adverse effect on the analysis. Absence of virulence in a particular experiment was usually so obvious that all or nearly all the animals survived the period of follow-up thus making quantitative analysis inapplicable.

For convenience the dose variable has been expressed in terms of concentration and ranged from 10^3 to 10^9 organisms/ml. In fact, since the amount of inoculum in each case was 0.05 ml., the approximate number of organisms injected ranged from 50 to 5×10^7 . The experimental error is, of course, proportionately greater at the lower limits of this range where doses assume merely nominal value. Following common practice, the logarithm of the concentration has been used in the analysis. However, it was clear from graphical considerations that this transformation alone did not result in straight line regression. Linearity was, however, achieved by the use of a double logarithmic transform, i.e. by also using the logarithm of survival times.

In toxicological studies the ability to calculate the potency of one drug relative to another, i.e. *relative potency*, will depend on whether the regression lines can be considered parallel. Parallel regression lines may not, however, be observed unless the drugs under comparison possess similar active constituents. In such cases relative potency is given by the antilogarithm of the horizontal distance between the regression lines. In the same way it must not be expected that regression lines fitted to virulence data will necessarily be parallel if they relate to different strains of organisms or routes of administration. If, however, the lines can be considered

parallel, it is possible to estimate an analogous statistic which may be called *relative virulence*, for which a confidence interval can be calculated (Fieller, 1940). This concept can be used in two ways. The virulence of a given strain may be compared under different conditions or the virulence of different strains may be compared under similar conditions.

ANALYSIS OF RESULTS

The experiments can be considered in five groups:

(a) The effect of mouse weight.	} With reference to <i>Klebsiella pneumoniae</i> (type 2) strain 9503.
(b) The effect of prolonged bacterial incubation.	
(c) The effect of repeated subculture.	
(d) The effect of killed suspensions.	
(e) A comparison of variations in virulence between strains.	

(a) *The effect of mouse weight*

The effect on survival times of inoculation by oral and intraperitoneal routes of strain 9503 in concentrations of 10^5 and 10^7 organisms/ml. was tested on mature (19–24 g.) and young mice (9–11 g.), respectively (Table 2). The application of the

Table 2. *Distribution of times to death in an experiment to investigate the effect of mouse weight with reference to strain 9503*

(Dose in all cases = 0.05 ml. The table shows the numbers of deaths on each day out of ten mice tested.)

Route	Mouse weight (g.)	Log conc. (organisms/ml.)	Day of death							
			1	2	3	4	5	6	7	8
Oral	19–24	7	.	4	4	1	1	.	.	.
		5	.	.	.	5	2	.	2	1
	9–11	7	.	8	2	.	.	.	.	.
		5	.	7	1	2	.	.	.	.
Intraperitoneal	19–24	7	2	7	1	.	.	.	.	.
		5	.	7	2	1	.	.	.	.
	9–11	7	3	7	.	.	.	.	.	.
		5	10	.	.	.	.	.	.	.

analysis of variance to these data (Table 3) shows that the overall effects on survival times of different doses, routes and weights of animals are all highly significant ($P < 1/2000$). On the other hand, variation of route and weight does not significantly modify the slope of the regression lines relating survival time to dose. The effects of route and weight interact significantly ($P < 1/40$). Thus the data can be represented by four parallel regression lines, although the distances between pairs relating to similar routes are not equal.

As can be seen from Fig. 1, the strain under test is more virulent when inoculated intraperitoneally and in small mice. However, the effect of reduction in mouse weight is greater in those inoculated orally.

Table 3. *The analysis of variance of the results given in Table 2*

Component of variation	Sum of squares	D.F.	Mean square	F ratio	Significance
Dose (D)	0.532	1	0.532	21.0	$P < 1/2000$
Route (R)	1.070	1	1.070	42.2	$P < 1/2000$
Mouse weight (W)	0.546	1	0.546	21.6	$P < 1/2000$
D $\times$ R	0.004	1	0.004	0.17	$\frac{1}{2} < P$
D $\times$ W	0.087	1	0.087	3.42	$1/20 < P < 1/10$
R $\times$ W	0.161	1	0.161	6.35	$1/100 < P < 1/40$
D $\times$ R $\times$ W	0.072	1	0.072	2.68	$1/20 < P < 1/10$
Residual	1.824	72	0.025	—	—
Total	4.296	79			

In this and other analysis of variance tables the F ratio has been calculated by dividing the mean square for the particular component by the residual mean square. One more place of decimals was used in this calculation than is shown in the tables.

Fig. 1. Relationship between concentration, route of administration, mouse weight and survival time with reference to strain 9503.

(b) *The effect of prolonged bacterial incubation*

A comparison was here made between the effect on survival times of strain 9503 incubated for 16 and 72 hr., respectively. Suspensions of 10^5 , 10^6 and 10^7 organisms/ml. were prepared in each case. The results (Table 4) and their analysis of variance (Table 5) show the substantial dose effect and confirm the greater virulence of the intraperitoneal route (cf. (a) above). The effect of varying the period of incubation is not so clear cut since this depends on route. As can be seen from Fig. 2 there is no difference in virulence when the strain is inoculated intra-orally or intraperitoneally, however, the 'younger' is more virulent than the 'older' culture. As judged by these data (Table 9) it is about 100 times more virulent but the limits of error of this estimate are rather wide.

Table 4. *Distribution of times of death in an experiment to investigate the effect of prolonged incubation with reference to strain 9503*

(Dose in all cases = 0.05 ml. The table shows the numbers of deaths on each day out of ten mice tested.)

Route	Hours of incubation	Log conc. (organisms/ ml.)	Day of death											
			1	2	3	4	5	6	7	8	9	10	11	12
Oral	16	7	.	4	3	2	1	.	.	.	.	.	.	.
		6	.	2	3	2	1	.	.	.	1	.	.	1
		5	.	.	.	4	4	1	.	1	.	.	.	.
	72	7	.	4	3	1	1	.	.	.	1	.	.	.
		6	.	.	5	4	1	.	.	.	.	.	.	.
		5	.	.	2	1	1	4	1	1	.	.	.	.
Intraperitoneal	16	7	2	6	2	.	.	.	.	.	.	.	.	.
		6	.	7	3	.	.	.	.	.	.	.	.	.
		5	.	7	1	2	.	.	.	.	.	.	.	.
	72	7	.	5	2	3	.	.	.	.	.	.	.	.
		6	.	2	5	.	3	.	.	.	.	.	.	.
		5	.	.	4	1	4	.	1	.	.	.	.	.

Table 5. *The analysis of variance of the results given in Table 4*

Component of variation	Sum of squares	D.F.	Mean square	F ratio	Significance
Dose (D)	1.036	2	0.518	15.3	$P < 1/2000$
Route (R)	1.053	1	1.053	31.1	$P < 1/2000$
Period of incubation (P)	0.401	1	0.401	11.8	$1/2000 < P < 1/1000$
D × R	0.040	2	0.020	0.59	$\frac{1}{2} < P$
D × P	0.037	2	0.018	0.54	$\frac{1}{2} < P$
R × P	0.364	1	0.364	10.7	$1/1000 < P < 1/200$
D × R × P	0.101	2	0.005	0.15	—
Residual	3.658	108	0.034	—	—
Total	6.599	119

Fig. 2. Relationship between concentration, period of incubation of organisms, route of administration and survival time with reference to strain 9503.

There is no evidence that route or period of incubation significantly modifies the slopes of the regression lines. The data can thus be fitted by a set of four parallel regression lines as in Fig. 2.

(c) *The effect of repeated subculture*

Strain 9503 grown on nutrient agar, was subcultured on alternate days for a total of twenty times and tested orally at the dose levels in Table 6 before and after subculture. There was no significant difference between the responses observed under these conditions.

(d) *The effect of killed suspensions*

Groups of mice were inoculated by both routes with suspensions of strain 9503 killed by heating at 55° C. for 45 min. No deaths occurred with concentrations of 10^9 killed organisms/ml. in contrast to the high mortality obtained with living organisms.

(e) *A comparison of variations in virulence between strains*

In this, the principal experiment of the investigation, nine strains were examined for differences in response by both routes. As can be seen from Table 6, these were not all tested at the same doses.

Fig. 3. Histogram to show survival times of groups of ten mice injected with graded concentrations of three strains. ■, oral; □, peritoneal; S, sacrificed after 21 days.

It will also be evident from Table 6 that there were marked differences between the virulence of different strains and, further, that the effect of varying the route was not the same in all cases. Some strains, e.g. 9494 proved highly virulent orally, others, e.g. 5054 less so, while still others, e.g. 9527 appeared avirulent. Only two strains 9494 and 9503, were more virulent by the intraperitoneal route. All other strains showed either reduced virulence, e.g. 5054 or absence of virulence, e.g. 5048. Strain 8167 appeared avirulent by both routes. Three examples of different patterns of response are illustrated in Fig. 3.

In the first instance, consideration was given to the feasibility of applying the analysis of variance to these data as a whole and estimating the relative virulence

Table 6. *Distribution of times to death of all strains under test for both routes of administration*

(The table shows the numbers of deaths on each day out of ten mice tested. Dose in all cases = 0.05 ml.)

Strain	Log conc. (organisms/ ml.)	Oral															Peritoneal															
		day of death															day of death															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14-21	S	1	2	3	4	5	6	7	8	9	10	11	12	13	14-21	S	
9494	9	4	5	1	.	1	2	1	.	.	.	.	.	.	.	.	.	4	3	.	2	.	.	.	.	.	.	.	.	.	.	.
	8	.	5	1	.	1	2	1	2	.	.	.	.	.	.	.	.	4	3	.	1	3	.	.	.	.	.	1	.	.	.	2
	7	.	2	1	1	1	2	1	2	.	.	.	.	.	.	.	.	1	3	.	1	3	.	.	.	.	.	.	.	.	.	.
	6	.	.	2	1	.	1	2	2	2	.	.	.	.	.	.	.	.	3	3	1	2	.	.	.	.	.	.	.	.	.	7
5054	9	.	1	2	.	5	2	.	1	.	.	.	.	.	.	.	.	1	1	.	1	.	.	.	.	.	.	.	.	.	.	7
	8	.	.	1	4	3	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	7	.	.	1	1	3	1	2	2	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	9
	6	.	.	.	1	.	2	1	2	2	2	.	.	.	.	.	.	1	.	.	.	.	.	.	1	.	.	1	.	.	.	7
9503	9	.	9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	8	.	8	2	.	.	.	.	.	.	.	.	.	.	.	.	.	4	6	.	.	.	.	.	.	.	.	.	.	.	.	.
	7	.	4	2	1	3	.	1	.	.	.	.	.	.	.	.	.	2	7	1	.	.	.	.	.	.	.	.	.	.	.	.
	6	.	2	2	2	1	1	1	.	.	.	.	.	.	.	.	.	.	8	2	.	.	.	.	.	.	.	.	.	.	.	.
5048	5	.	.	.	5	5	.	.	.	.	.	.	.	.	.	.	.	.	7	2	1	.	.	.	.	.	.	.	.	.	.	.
	4	.	.	.	1	2	2	1	1	1	1	.	.	.	.	.	.	.	7	1	2	.	.	.	.	.	.	.	.	.	.	.
	3	.	.	.	.	.	.	.	.	.	.	.	2	1	.	7	.	.	6	1	2	1	.	.	.	.	.	.	.	.	.	
	9	.	.	.	1	3	.	1	1	.	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
5049	8	.	.	1	1	1	.	1	.	2	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	7	.	.	2	.	.	.	.	2	1	3	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	9
	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	9
	9	.	.	.	.	2	1	.	.	3	2	.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
5051	8	.	.	.	.	1	1	.	.	2	3	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	7	.	.	.	.	1	1	.	.	2	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	6	.	.	.	.	1	1	.	.	1	2	2	.	1	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	9	1	7	1	.	4	1	.	1	.	.	.	.	.	.	.	.	10	3	2	1	2	1	.	.	.	.	.	.	.	.	
5053	7	1	1	3	4	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	5	1	1	1	5	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	3	.	.	.	4	1	2	1	.	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	9	.	.	.	9	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9527	7	.	.	2	4	3	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	5	.	.	5	2	1	1	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	3	.	.	.	1	.	4	1	.	1	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	9	.	.	1	.	.	.	2	3	.	.	.	3	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
8167	8	.	.	1	.	.	.	1	2	.	.	.	.	.	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	7	.	.	2	.	.	.	1	.	1	1	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	6	.	.	.	.	.	.	.	.	.	.	1	.	.	7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	9	.	.	.	.	.	.	.	.	.	.	.	.	.	10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	7	.	.	.	.	.	.	.	.	.	.	.	.	.	10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	8	.	.	.	.	.	.	.	.	.	.	.	.	.	10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
	6	.	.	.	.	.	.	.	.	.	.	.	.	.	10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10

S, sacrificed after the 21st day.

S, sacrificed after the 21st day.

of each strain with reference to one of them regarded as a standard. This unified approach confirmed the value of the double logarithmic transformation in attaining linearity. It indicated clearly, however, that the regression lines could not be regarded as having a common slope. This precluded the calculation of any unique estimate of relative virulence and, as a result, the data were analyzed in two groups:

(1) Difference between three pneumoniae strains.

(2) Differences between capsulate and acapsulate variants of rhinoscleroma and ozaenae strains.

It is of course possible to compare those doses which yield the same arbitrary survival times for different strains or experimental conditions. The mean log survival time selected for this purpose should preferably be one which corresponds to doses within the experimental range for all the strains or experimental conditions considered. There does not appear to be any case in the present study where this approach might be useful or reliable.

Table 7. *The analysis of variance of the results relating to strains 9503 and 9494 given in Table 6*

Component of variance	Sum of squares	D.F.	Mean square	F ratio	Significance
Strain (S)	1.175	1	1.175	14.3	$P < 1/2000$
Route (R)	2.848	1	2.848	34.6	$P < 1/2000$
Dose (D)	6.777	3	2.259	27.4	$P < 1/2000$
Linear effect (D_l)	6.555	1	6.555	79.6	$P < 1/2000$
Quadratic effect (D_q)	0.194	1	0.194	2.35	$1/10 < P < 3/10$
Cubic effect (D_c)	0.028	1	0.028	0.34	$\frac{1}{2} < P$
S $\times$ R	0.344	1	0.344	4.17	$1/40 < P < 1/20$
S $\times$ D	0.690	3	0.230	2.79	$1/40 < P < 1/20$
S $\times$ D_l	0.434	1	0.434	5.27	$1/100 < P < 1/40$
S $\times$ D_q	0.246	1	0.246	2.99	$1/10 < P < 3/10$
S $\times$ D_c	0.010	1	0.010	0.12	$\frac{1}{2} < P$
R $\times$ D	0.149	3	0.050	0.60	$\frac{1}{2} < P$
S $\times$ R $\times$ D	0.338	3	0.113	1.37	$1/10 < P < 3/10$
Residual	11.857	144	0.082		
Total	24.178	159			

(1) *Comparison of responses between 3 strains of Klebsiella pneumoniae.* Three strains 9494, 9503 and 5054 were tested at four dose levels (10^6 , 10^7 , 10^8 , and 10^9 organisms/ml.) by both routes. The data for one strain, 5054, were not analyzed, since it was found to be avirulent peritoneally and the inclusion of oral figures alone would have complicated matters. The analysis of variance (Table 7) for the other two strains, 9503 and 9494 showed that the slopes of the regression lines were significantly modified by the strain ($P < 1/40$). The overall difference between individual strains was highly significant but depended to some extent on route. Fig. 4 shows that the greatest part of this difference arose from the very high peritoneal virulence of strain 9503. Table 9 gives the equations of the regression lines and estimates of virulence of peritoneal relative to oral routes, i.e. about six in the case of strain 9494 and nearly 500 in the case of strain 9503. It is not

possible to estimate relative virulence between these strains as the regression lines are not parallel. The regression line for strain 5054 orally has been added to Fig. 4 for comparison. It is clearly a much less virulent organism at least over the range of doses considered.

Fig. 4. Relationship between concentration, route of administration and survival time for three strains.

It will be seen from Table 6 that strain 9503 was studied over a wider dose range than other strains, viz. seven doses from 10^3 to 10^9 organisms/ml. inclusive.

(2) *Comparison of capsulate and acapsulate strains.* Strains of rhinoscleroma and ozaenae were tested by both routes at different levels as indicated in Table 6. It will be seen that all four strains (5048, 5049, 5051 and 5053) are almost completely avirulent peritoneally. The remaining data for the two strains orally were analyzed separately (Tables 8a, b) and in both cases the capsulate (5048, and 5051) were

Table 8a. *The analysis of variance of the results relating to strains 5048 and 5049 given in Table 6*

Component of variance	Sum of squares	D.F.	Mean square	F ratio	Significance
Capsule (C)	0.202	1	0.202	4.88	$1/40 < P < 1/20$
Dose (D)	0.285	3	0.095	2.30	$1/20 < P < 1/10$
Linear effect (D_1)	0.258	1	0.258	6.25	$1/100 < P < 1/40$
Quadratic effect (D_2)	0.011	1	0.011	0.27	$\frac{1}{2} < P$
Cubic effect (D_3)	0.016	1	0.016	0.37	$\frac{1}{2} < P$
C $\times$ D	0.026	3	0.009	0.21	$\frac{1}{2} < P$
Residual	2.976	72	0.041		
Total	3.489	79			

Table 8b. *The analysis of variance of the results relating to strains 5051 and 5053 given in Table 6*

Component of variance	Sum of squares	D.F.	Mean square	F ratio	Significance
Capsule (C)	0.742	1	0.742	20.8	$P < 1/2000$
Dose (D)	1.701	3	0.567	15.9	$P < 1/2000$
Linear effect (D_l)	1.660	1	1.660	46.4	$P < 1/2000$
Quadratic effect (D_q)	0.040	1	0.040	1.12	$1/10 < P < 3/10$
Cubic effect (D_c)	0.001	1	0.001	0.03	$\frac{1}{2} < P$
C $\times$ D	0.142	3	0.047	1.33	$1/10 < P < 3/10$
Residual	2.574	72	0.036		
Total	5.159	79

Fig. 5. Relationship between concentration, presence of capsules and survival time for strains of *K. rhinoscleroma* and *K. ozaenae*, orally.

found to be more virulent than the corresponding acapsulate forms (5049 and 5053). The former were more virulent by 1000 times in the 'ozaenae' and by 100 times in the 'rhinoscleroma' (Table 9 and Fig. 5). These estimates have, however, a low degree of precision. The rhinoscleroma strain is much less virulent than the ozaenae strain in the case of the acapsulate by a factor of about 5.5×10^6 and in the case of the capsulate by a factor of about 4×10^8 .

DISCUSSION

The necessity for accurate and consistent standardization of experimental procedures in virulence estimations need no emphasis. Dutton (1955) has recently stressed the dependence of measures of virulence on routes of infection, for a variety of organisms including *Klebsiellae*. He pointed out that the variation of

Table 9. *Summary of regression equations and relative virulences*

Experimental conditions	Comparison	Regression equations*	Estimated 'relative virulence'	90% Fiducial interval	
				Lower limit	Upper limit
9503 Oral	Period of incubation 16 relative to 72 hr.	16 hr. $Y_{16} = 1.193 - 0.114x$ 72 hr. $Y_{72} = 1.199 - 0.114x$	1.1	0.20	6.7
	16 relative to 72 hr.	16 hr. $Y_{16} = 0.896 - 0.114x$ 72 hr. $Y_{72} = 1.122 - 0.114x$			
9503 Intraperitoneal	Route intraperitoneal relative to oral	Intraperitoneal $Y_p = 1.973 - 0.228x$ Oral $Y_o = 2.147 - 0.228x$	97	14	5,000
	Route intraperitoneal relative to oral	Intraperitoneal $Y_p = 1.014 - 0.135x$ Oral $Y_o = 1.375 - 0.135x$			
9494	Capsulate relative to acapsulate	Caps. $Y_+ = 1.246 - 0.051x$ Acaps. $Y_- = 1.347 - 0.051x$	5.8	1.6	28
	Capsulate relative to acapsulate	Caps. $Y_+ = 0.851 - 0.065x$ Acaps. $Y_- = 1.045 - 0.065x$			
9503			470	85	6,000
5048			95	2.9	4,500,000
5051 Oral			1,000	65	37,000

* Y = estimate of the mean of the logarithms of survival times observed under the specified conditions.
 x = log. concentration of organisms/ml.

lethality with route is a manifestation of non-specific immunity. As has been indicated, the relative importance of any one variable may depend on the route of infection. The effect on survival time of reduction in mouse weight was for instance greater in orally infected animals, whereas animals inoculated by this route appear to respond equally to suspensions prepared from 'young' or 'old' cultures.

Since suspensions were standardized by an opacity technique, it is not possible to preclude the possibility that the observed interstrain variations in virulence may have been merely a reflexion of differences in viable counts of suspensions. This factor is obviously not material to a consideration of inter-route differences in virulence of the same strain. Moreover, it is difficult on this basis alone to account for the lower virulence of both acapsular variants studied. In these experiments, the problem of capsules has been considered on an 'all or none' basis, i.e. the pathogenicity of capsular strains has been compared with their acapsular variants. This is, however, a simplification of the problem. MacLeod & Krauss (1950) made quantitative studies on *Diplococcus pneumoniae*, indicating a relation between size of capsules and degree of virulence. Ehrenworth & Baer (1956) extended these studies and applied them to strains of *Klebsiella pneumoniae*, showing that, in addition to size, the rate of capsule production is an important determinant of virulence.

With the inocula of higher concentrations particularly, it is difficult to assess the relative roles of infection, toxicity or some interaction of these factors. Knoll (1953) demonstrated that *Klebsiella* capsular polysaccharide enhances the virulence of a given strain of *Klebsiella*, irrespective of whether the polysaccharide was derived from pathogenic or non-pathogenic strains. Further, Baer, Bringaze & McNamee (1954), showed that purified *Type 2* capsular polysaccharide is toxic in large concentrations—the approximate LD₅₀ for a 20 g. mouse being 500 µg. It is also recognized that many Gram-negative bacilli contain toxic somatic antigens of a lipopolysaccharide nature. Rowley (1956) has recently stated that the virulence of a given strain may be a reflexion of the amounts of somatic lipopolysaccharide produced, which in turn determines the host properdin response. Landy & Pillemer (1956) have further postulated that interstrain variations in virulence may depend on qualitative besides quantitative differences in these somatic components.

It has been claimed that the recognition of virulence in *Klebsiellae* is of taxonomic value. Kauffman (1951) maintained that *Types 1* and *2* which in man usually produce respiratory tract infections can be differentiated from *Types 8, 9* and *10*, which usually produce urinary tract infections, by their high and low mouse virulence, respectively. Such differences incidentally, are in part reflected in the histological patterns of pneumonic infections induced by these respective strains. The former produced an unusual type of pneumonia characterized by early and in some cases massive peri-arterial oedema (Epstein 1959*b*). The 'intraperitoneal mouse test' is a common laboratory procedure for the differentiation of *pneumoniae* from 'aerogenes' strains. In fact, strains of the former studied in the present series exhibited a wide range of virulence for both routes tested. It must be emphasized, however, that virulence is an ecological

concept and an expression of a host-parasite relationship, the individual components of which may vary independently. As such, it is obviously an unsatisfactory taxonomic discriminant. Even assuming a constancy of host factors the distribution of virulence among any strains of a type, or types of a species, is consequently better described on a statistical than 'all or none' basis.

SUMMARY

The virulence of nine strains of *Klebsiella* was tested in mice inoculated by peritoneal and oral routes, the latter being a convenient method of inducing pneumonic infections.

At similar doses, shorter survival was observed in young mice. Injected by the intraperitoneal route, organisms from 16 hr. old cultures proved more virulent than those from older cultures, but no such difference was obtained orally. Repeated subculture of a virulent strain failed to attenuate its oral virulence and killed suspensions of the same strain were without effect. With only two exceptions, all strains were more virulent when administered orally than intraperitoneally. Wherever possible the concept of *relative* virulence was employed in making statistical comparisons. It is, however, considered that virulence is an unsatisfactory taxonomic discriminant.

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