PRODUCTION OF NON-INFECTIVE PARTICLES AMONG INFLUENZA VIRUSES: DO CHANGES IN VIRULENCE ACCOMPANY THE VON MAGNUS PHENOMENON?

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(With 3 Figures in the Text)

Commenting on some recent studies by von Magnus, Fulton (1953) made the relevant suggestion that the phenomenon generally referred to as 'production of incomplete virus' might be by rights 'production of avirulent virus'. The oddity of the contradiction in terms should not mar a nice distinction which, indeed, touches upon points of fundamental theoretical interest, and has immediate bearing on our concepts of the intracellular development, genetic behaviour, infectivity and adaptation of influenza viruses. The notion of 'incomplete virus' implies that the developmental process has been interrupted at a particular stage, the product being indistinguishable from fully fledged virus as regards antigenic make-up, enzyme activity, adsorptive capacity, interfering ability, etc., but lacking infectivity. Accordingly, infectivity is regarded as the crowning step of the reproductive sequence, the latter being undisturbed up to this level, and completely blocked thereafter. The notion of 'avirulent virus' visualizes an end-product which is slightly imperfect, and does so without any unnecessary assumptions on the nature of the multiplication process and the stage (or stages) at which deviations from the normal occur. Thus 'avirulent virus' may differ from infective virus in several respects, both qualitatively and/or quantitatively, and cannot be regarded as its precursor. Essentially, 'incomplete virus' is yielded by a normal process of virus production, 'avirulent' by an abnormal one. Thus a preparation containing 'incomplete virus' would show low average infectivity because some of the particles do not infect at all; a preparation of 'avirulent virus' would show the same low infectivity because all particles, in general, are less infective.

Although Fulton has not defined what he meant by 'avirulent', it will be clear that the customary interpretations of virulence are meaningless in this context, since the response of the host organism to fully active and 'incomplete virus' is the same, as has been convincingly demonstrated by von Magnus (1951*b*) in the papers which actually provoked Fulton's criticism. Neither can it be maintained that 'avirulence' referred to a proportion of the yield only, nor that the change from 'virulent' to 'avirulent' meant that a smaller fraction only of host cells was infectible, as this would reduce the distinction between 'incomplete' and 'avirulent' to a verbal nicety. The latter follows from the fact, pointed out by Moran (1954*a*), that such variations alter the position of the dose-response curve, but not

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its shape. Thus, as set out formally in the Appendix, in terms of the definition given at the end of the previous paragraph, 'avirulent' is operationally meaningful only if it implies that the virus is less likely to overcome the natural defences of its host. The sum of these defences is what we called 'host resistance' in an earlier paper (Fazekas de St Groth & Cairns, 1952), and its presence and variability can be gauged from the shape of the infectivity curve. The implications of this are evident: the difference between 'incomplete' and 'avirulent' virus should show up in the slope of their dose-response curves. 'Avirulent' virus should give a relatively flatter titration curve than 'virulent' virus; a preparation containing 'incomplete' particles, on the other hand, should give the same slope as 'complete' virus but a lower infectivity end-point. Accordingly, the question can be decided by the critical comparison of infectivity tests on virus preparations showing the von Magnus phenomenon and preparations of standard virus.

Several hundred such titrations have been performed in our laboratory during the past 2 years, but the details were considered too trivial to be included in the final reports (Fazekas de St Groth & Graham, 1953, 1954). As the same holds for other workers in the field, with a consequent lack of published data, it is proposed to furnish the relevant information here and to examine whether it supports one or the other of the alternative hypotheses.

METHODS

Comparison of slopes would present no problem, were the shape of the dose-response curve known. This, however, is not the case with allantoic infectivity titrations of influenza viruses. It has been shown that the simplest assumption, viz. a Poissonian distribution of 'takes', does not hold as a certain degree of host resistance is operative against all strains and this property shows inter-egg variation (Fazekas de St Groth & Cairns, 1952). What is more disappointing, some of the most carefully obtained and extensive sets of data (e.g. von Magnus, 1951*a*) were fitted neither by the normal or logistic distributions, nor by the negative binomial (Moran, 1954*a*). Under the circumstances there are two possible ways of evaluation open. One might choose to remain uncommitted as regards the fundamental curve, and make use of one of the non-parametric methods. Or, one might use the discrepancy between the data and one of the theoretical dose-response curves as a measure of slope. Neither method is beyond reproach. The first, straightforward and comprehensible to the uninitiated, will be decried by the statistician as wasteful of information and altogether lacking 'intuitive meaning'. The second, indirect, is essentially a test for goodness of fit where good fit cannot be expected on existing knowledge.

(I) As the *non-parametric* method I have chosen the method of cumulative totals (Dragstedt & Lang, 1928; Behrens, 1929; Reed & Muench, 1938) for purposes of this study; partly because it is computationally the simplest, and partly because it has already been used for determination of infectivity end-points on the same experimental material. The interquartile range served as a measure of the slope, and thus the *cumulative quartile points*, called here ID_{25} and ID_{75}, had to be found

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first. The computation required is similar to that of the ID_{50}, where in a set of dilutions (x_1, x_2, ..., x_k) we seek the dose x_i satisfying the identity $S_i^+ = S_i^-$, that is, the point where the cumulative total of 'takes' $\left(S_i^+ = \sum_{x_k}^{x_i} r_x\right)$ and 'misses' $\left(S_i^- = \sum_{x_1}^{x_i} (n_x - r_x)\right)$ is equal. The cumulative upper quartile point is defined in this context by $S_i^+ = 3S_i^-$, while the cumulative lower quartile point by $3S_i^+ = S_i^-$. A sample calculation on the first entry of Table 1 from our earlier paper (Fazekas de St Groth & Graham, 1954) will make this clearer.

An allantoic fluid containing WSE virus was titrated for infectivity in a group of 30 eggs, subgroups of 5 eggs being inoculated with falling 3·16-fold (log 0·5) dilutions of the test material. Table 1 gives the results in full.

Table 1. *Allantoic infectivity test on WSE virus*

(Details of sample 1 from Table 1 of Fazekas de St Groth & Graham, 1954.)

Dilution of sample ($\log_{10}$ units/ml.)	Response: Eggs 1	2	3	4	5	'Takes' (r)	'Misses' ($n-r$)
5·0	+	+	+	+	+	5	0
5·5	+	+	+	+	+	5	0
6·0	+	+	+	−	+	4	1
6·5	−	D	+	+	+	3	1
7·0	+	−	−	−	+	2	3
7·5	−	−	−	−	−	0	5

+ = positive haemagglutination at 42 hr. ('take'); − = negative haemagglutination at 42 hr. ('miss'); D = embryo dead at 42 hr., egg discarded.

The infectivity end-point may be found now by cumulative summing of 'takes' and 'misses' for each dilution. For the determination of the cumulative quartile points these figures have to be multiplied by 3, giving two further half-columns, as the total 'takes' need be considered only below the 50%-end-point, and the 'misses' only above it (cf. Table 2). Linear interpolation may be done either graphically (Fig. 1) or arithmetically (Table 2). From the results of this particular test we derive thus (in $\log_{10}$ units/ml.) $ID_{25} = 7·05$, $ID_{50} = 6·75$, $ID_{75} = 6·43$, with a lower quartile of 0·30 ($Q_l = ID_{50} - ID_{25}$) and an upper quartile of 0·32 ($Q_u = ID_{75} - ID_{50}$), the interquartile range being, of course, 0·62 ($R = ID_{75} - ID_{25}$).

Since there is no justification for assuming *a priori* that the dose-response curve is symmetrical, the two quartiles might be different and should be considered separately. In the tables to follow the ID_{50}, the two quartiles and the interquartile range of every assay therefore appear, together with their geometric means and the standard error. This allows an analysis of variance for the comparison of yields from concentrated and dilute inocula, and differences between either of the quartiles or in the interquartile range should thus be revealed, i.e. any significant changes in the shape and/or the slope of the curves.

With a limited range of dilutions, as used in our studies, it is inevitable to have

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Table 2. *Evaluation of the infectivity test by Method I*

(First three columns taken from Table 1.)

Dilution of sample ($\log_{10}$/ml.)	Response		Cumulative totals			
	(r)	($n-r$)	$3S_i^+$	S_i^+	S_i^-	$3S_i^-$
5·0	5	0	.	19	0	0
5·5	5	0	.	14	0	0
6·0	4	1	.	9	1	3
				⑥ (S^+ to $3S^-$)		
				① (S^+ to $3S^-$)		
6·5	3	1	15	5	2	6
				③ (S^+ to S^-)		
				③ (S^+ to S^-)		
7·0	2	3	6	2	5	15
			① ($3S^+$ to S^-)			
			⑩ ($3S^+$ to S^-)			
7·5	0	5	0	0	10	.

Interpolation
$$X_i = X_a + \frac{|S_a^+ - S_a^-|\,d}{|S_a^+ - S_a^-| + |S_b^+ - S_b^-|},$$

where X_a and X_b are the dilutions bracketing the endpoint (X_i), and d is the dilution factor. The differences ($S^+ - S^-$), ($3S^+ - S^-$), and ($S^+ - 3S^-$), at particular levels are shown in circles.

$$\text{ID}_{50} = 6{\cdot}5 + \frac{3 \times 0{\cdot}5}{3+3} = 6{\cdot}75. \qquad \text{ID}_{75} = 6{\cdot}0 + \frac{6 \times 0{\cdot}5}{6+1} = 6{\cdot}43. \qquad \text{ID}_{25} = 7{\cdot}0 + \frac{1 \times 0{\cdot}5}{1+10} = 7{\cdot}05.$$

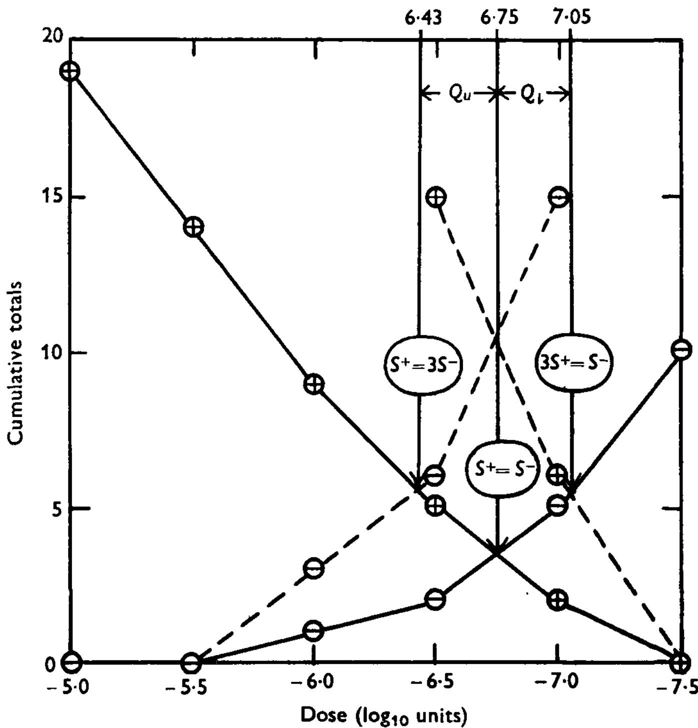

Fig. 1. Graphical evaluation of the infectivity test by Method I. (From data of Tables 1 and 2; the continuous lines are the cumulative totals, the broken lines the cumulative totals multiplied by three.)

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some assays where one of the quartile points was not bracketed. In these cases the point could have been determined only by extrapolation. To avoid such an adventurous procedure, truncated tests were omitted from the tables and substituted by others, from later experiments (Fazekas de St Groth & Graham, to be published); these are marked with an asterisk. Care was taken to select as closely similar substituents as possible, and the minor differences so introduced should not influence the validity of the conclusions, as controllable experimental conditions were kept constant throughout the two phases of our work.

The statistical evaluation was based on the null hypothesis that all samples (both the quartiles and their sums, the interquartile ranges) were drawn from a homogeneous population. An analysis of variance will answer whether discrepancies between the different groups in the tables are attributable to chance variation.

(II) When choosing the theoretical curve for *the second method*, no consideration need be given whether this curve will actually fit the data. In the present case, indeed, the choice could but be between bad and worse. The way is thus open to make the simplest assumption, namely, that uniformly infective particles are randomly distributed in an inoculum that reaches uniformly infectible cells. The corresponding dose-response curve, the Poissonian, is defined by a single parameter. The standard procedure here would be the maximum likelihood fitting of the negative exponential (the zero term of the Poisson distribution), with a χ^2 test for goodness of fit. Our infectivity tests, like the majority of virological data, hardly warrant an iterative method as laborious as this. Fortunately, we have now in the ingenious test of Moran (1954*b*) an alternative probably more powerful yet exceptionally simple to apply. First the statistic T has to be computed by multiplying the number of 'takes' and 'misses' at each dilution level, and summing the products ($T = \Sigma r_i\,(n-r_i)$). This involves mental arithmetic only. The deviation of T from the value expected on the Poisson distribution can be tested by the formula $(T-E(T))/\sigma_T = M$, the quantity M being a normal deviate. $E(T)$ and σ_T are uniquely defined by the dilution factor and the number of subjects at any dilution level. Moran (1954*b*) has published appropriate tables of these, and with their aid the M-values of an infectivity test may be computed in less than half a minute. A sample calculation on the data of Table 1 shall follow.

It will be noticed that only those groups are considered where the response is neither all 'takes' nor all 'misses', since in others the product $r(n-r)$ will be zero. The example is useful also in illustrating the corrections required in cases of natural mortality. At dilution $10^{6\cdot5}$ one of the embryos died during the incubation period. At that level we have thus 3 positives, 1 negative, and 1 missing. The missing entry is proportionately allotted to the other groups, making 3·75 positives and 1·25 negatives. Hence the value of T is $4+4\cdot69+6 = 14\cdot69$, as compared with 12·04, the expected value read from Moran's tables. To allow for the reduced precision in the estimate of M due to the missing entry, the error of T will have to be corrected too. With log 0·5 dilution steps σ_T equals 4·42 for 5 subjects per group, and 3·07 for 4 subjects per group. Since in our example there are three levels (dilutions 6·0, 6·5 and 7·0) giving finite products, two of which have 5 and one

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4 subjects, the theoretical error of T may be approximated by linear interpolation, making

$$\sigma_T = 4{\cdot}42 - \tfrac{1}{3}\,(4{\cdot}42 - 3{\cdot}07) = 3{\cdot}97. \text{ Hence } M = \frac{14{\cdot}69 - 12{\cdot}04}{3{\cdot}97} = 0{\cdot}67.$$

Were it used as a measure for goodness of fit, we would conclude that this value did not differ significantly from that expected and could occur by chance once in every four trials. Thus the hypothesis of a Poissonian dose-response relationship is

Table 3. *Evaluation of the infectivity test by Method II*

(First three columns taken from Table 1.)

Dilution of sample ($\log_{10}$/ml.)	Response		
	(r)	$(n-r)$	$r(n-r)$
5·0	5	0	.
5·5	5	0	.
6·0	4	1	4
6·5	3	1	4·69*
7·0	2	3	6
7·5	0	5	.

$$T = \Sigma r_i\,(n_i + r_i) = 14{\cdot}69$$

$$M = \frac{T - E(T)}{\sigma_T} = \frac{14{\cdot}69 - 12{\cdot}04}{3{\cdot}97^*} = 0{\cdot}67.$$

* Adjustment for missing entry; cf. text.

not contradicted by the outcome of that particular infectivity titration. This aspect of the results is, however, not directly relevant to the theme of the present study. The M-values, or more precisely their average, $\bar{M}$, will be used as the test criterion in the comparison of response curves obtained with preparations containing incomplete virus on the one hand, and fully infective virus on the other.

$\bar{M}$-values have been calculated for each of the assays evaluated by the non-parametric method. These, their arithmetic mean $\bar{M}$ and its standard error will appear in the tables. Since $\bar{M}$ is the mean of a number of independent variates its distribution will not be very far from normal, and the difference between two inocula of the same strain may be assessed by Student's t-test.

STATISTICAL

Comparison of infectivity curves by Method I

The results obtained with the non-parametric method are given in Table 4.

Although analyses of variance have been worked out for each of the ten strains, there is no need to give them here in detail, as in no case did any of the contrasts—variation between inocula, between quartiles, and their interaction—reach significance at the 5 % level. The standard errors of the means are shown in the table, and these should be sufficient to establish this point.

Since the cumulative quartiles are potential test criteria for comparing slopes of infectivity curves, it might be useful to examine the statistical properties of their

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distribution. To test for normality the deviations of the individual values about their means were plotted, giving the histogram of Fig. 2. The distribution shows positive skewness ((mean − median)/standard deviation = +0·15). Comparison of the standardized areas under this curve with the normal probability integral at $\frac{1}{3}\sigma$ intervals gives a χ^2 of 15·63 with 9 degrees of freedom. The corresponding probability, 0·08, although within the span usually allowed for chance variation, would suggest that the condition of normality is not strictly fulfilled. In the present case this is of little consequence, partly because it would only render comparisons

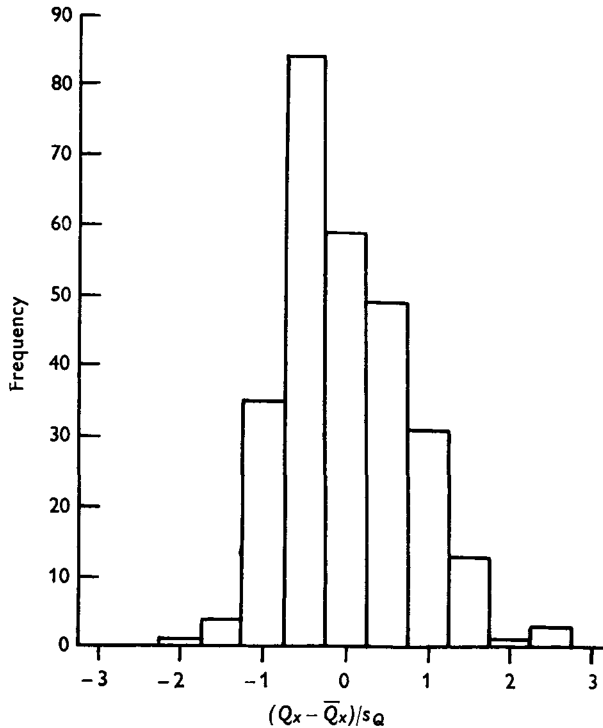

Fig. 2. Distribution of cumulative quartiles (Q-values) about their mean. (Plotted from data of Table 4.)

stricter by tending to underestimate the average error, and partly because intra-strain differences are uniformly so small that the need for exact tests of significance does not really arise.

Were a better estimate of error required, it would be advisable to use either a normalizing metameter of the quartiles, or some combined linear measure of slope, such as the interquartile range. The distribution of the latter would tend, on the central limit theorem, to be more nearly normal. As the less elaborate metametric transformations usually achieve normality at the expense of variance homogeneity, the second course seems sounder. We have actually examined the distribution of R-values and found that its skewness was minimal ($(M - Md)/s = +0{\cdot}09$), and that the test for goodness of fit gave $\chi^2 = 8{\cdot}72$ ($P \sim 0{\cdot}3$), i.e no serious discrepancy from the normal.

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The use of the range (R) instead of the quartiles may have also another point in its favour. Owing to the fact that the two quartiles have one common point (the ID_{50}), some negative correlation might exist between them. As estimates of variance would be vitiated if this were so, the independence of the observations had to be tested. However, no significant correlation was found within any of the 20 subgroups; neither was the overall correlation between the 140 pairs of quartiles

Table 4. *The infective behaviour of virus issuing from concentrated or dilute seed: I. Comparisons by a non-parametric method*

(Q_u = upper quartile = $ID_{75} - ID_{50}$; Q_l = lower quartile = $ID_{50} - ID_{25}$; R = interquartile range = $ID_{75} - ID_{25}$.)

	Inoculum: ~10^9 ID_{50}				Inoculum: ~10^4 ID_{50}			
Strain	ID_{50}/ml. of sample	Q_u	Q_l	R	ID_{50}/ml. of sample	Q_u	Q_l	R
WSE (A)	6·75	0·32	0·30	0·62	9·25	0·40	0·40	0·80
	7·42	0·17	0·22	0·39	10·20	0·67	0·32	0·99
	6·50*	0·23	0·43	0·66	10·40	0·55	0·23	0·78
	7·41	0·48	0·37	0·85	9·70	0·20	0·33	0·53
	7·75	0·61	0·53	1·14	10·20	0·23	0·67	0·90
	6·89	0·24	0·40	0·64				
	6·75	0·33	0·32	0·65	9·12	0·41	0·26	0·67
Mean ± s.e.		0·34 ± 0·06	0·37 ± 0·04	0·71 ± 0·09		0·41 ± 0·07	0·37 ± 0·07	0·78 ± 0·07
PR8 (A)	7·14	0·25	0·23	0·48	10·22	0·31	0·28	0·59
	7·72	0·58	0·30	0·88	10·34	0·29	0·19	0·48
	7·30	0·25	0·29	0·54	9·42	0·75	0·23	0·98
	7·55	0·20	0·18	0·38	10·30	0·37	0·36	0·73
	7·24	0·42	0·68	1·10	9·39	0·40	0·41	0·81
	7·70	0·51	0·52	1·03	10·19	0·44	0·58	1·02
Mean ± s.e.		0·37 ± 0·06	0·37 ± 0·08	0·74 ± 0·13		0·43 ± 0·07	0·34 ± 0·04	0·77 ± 0·09
MEL (A)	7·80	0·49	0·10	0·59	8·92*	0·37	0·36	0·73
	8·28	0·28	0·19	0·47	9·36	0·24	0·25	0·49
	7·50	0·33	0·45	0·78	8·75	0·31	0·42	0·73
	7·71*	0·48	0·47	0·95	10·00	0·32	0·20	0·52
	7·75	0·45	0·47	0·92	9·08*	0·68	0·37	1·05
	7·63*	0·38	0·30	0·68	8·79*	0·29	0·71	1·00
	7·25	0·58	0·63	1·21	9·40	0·54	0·54	1·08
Mean ± s.e.		0·43 ± 0·04	0·37 ± 0·07	0·80 ± 0·09		0·39 ± 0·06	0·41 ± 0·07	0·80 ± 0·09
BEL (A)	8·90	0·55	0·26	0·81	9·50*	0·50	0·23	0·73
	9·35	0·15	0·15	0·30	9·75	0·18	0·18	0·36
	8·33	0·33	0·34	0·67	9·80	0·38	0·55	0·93
	8·38	0·20	0·26	0·46	9·50*	0·44	0·44	0·88
	7·43	0·30	0·33	0·63	9·65	0·23	0·25	0·48
	8·63	0·46	0·15	0·61	9·70*	0·50	0·23	0·73
Mean ± s.e.		0·33 ± 0·06	0·25 ± 0·03	0·58 ± 0·07		0·37 ± 0·06	0·31 ± 0·06	0·69 ± 0·09
Cam (A′)	8·00*	0·92	0·40	1·32	9·17*	0·50	0·33	0·83
	9·78	0·23	0·22	0·45	9·72	0·53	0·40	0·93
	9·25	0·19	0·19	0·38	9·06	0·36	0·19	0·55
	8·42	0·29	0·50	0·79	9·63*	0·48	0·47	0·95
	9·30	0·15	0·30	0·45	9·25	0·17	0·17	0·34
	8·81*	0·33	0·22	0·55	9·15	0·17	0·16	0·33
	8·60	0·58	0·40	0·98	9·67*	0·45	0·55	1·00
Mean ± s.e.		0·38 ± 0·10	0·32 ± 0·04	0·70 ± 0·13		0·38 ± 0·06	0·32 ± 0·06	0·70 ± 0·10

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Table 4 (*cont.*)

	Inoculum: $\sim 10^9$ ID_{50}				Inoculum: $\sim 10^4$ ID_{50}			
Strain	ID_{50}/ml. of sample	Q_u	Q_l	R	ID_{50}/ml. of sample	Q_u	Q_l	R
FM1 (A′)	7·50	0·50	0·67	1·17	10·33*	0·76	0·46	1·22
	7·85	0·35	0·35	0·70	9·07	0·32	0·28	0·60
	8·35	0·48	0·33	0·81	8·20	1·01	0·59	1·60
	7·70	0·20	0·22	0·42	8·86	0·22	0·24	0·46
	8·88	0·42	0·30	0·72	7·90	0·40	0·35	0·75
	8·88	0·52	0·62	1·14	9·28	0·37	0·61	0·98
Mean ± s.e.		0·41 ± 0·05	0·42 ± 0·08	0·83 ± 0·12		0·51 ± 0·12	0·42 ± 0·06	0·94 ± 0·17
LEE (B)	9·81	0·20	0·24	0·44	9·45	0·40	0·35	0·75
	9·20	0·40	0·29	0·69	9·80	0·21	0·21	0·42
	9·62	0·20	0·33	0·53	9·40	0·97	0·35	1·32
	9·82	0·55	0·62	1·17	9·02	0·43	0·28	0·71
	9·63	0·21	0·18	0·39	9·80	0·37	0·22	0·59
	9·30	0·57	0·56	1·13	9·30	0·29	0·22	0·51
	10·05	0·67	0·33	1·00				
	9·80	0·53	0·25	0·78	7·55	0·29	0·21	0·50
	10·05	0·44	0·50	0·94				
	9·07*	0·42	0·15	0·57	9·17	0·61	0·55	1·16
	9·42	0·27	0·23	0·50	9·00	0·60	0·24	0·84
	8·63	0·33	0·33	0·66	7·86	0·32	0·47	0·79
	8·50	0·44	0·44	0·88	7·86	0·32	0·36	0·68
	9·38	0·25	0·45	0·70				
	8·76	0·22	0·23	0·45	9·67*	0·31	0·28	0·59
Mean ± s.e.		0·38 ± 0·04	0·34 ± 0·04	0·72 ± 0·07		0·43 ± 0·06	0·31 ± 0·03	0·74 ± 0·08
BON (B)	6·72	0·26	0·28	0·54	8·60	0·28	0·40	0·68
	6·35	0·46	0·29	0·75	8·06	0·42	0·21	0·63
	6·50	0·36	0·55	0·91	7·87	0·60	0·50	1·10
	5·81*	0·20	0·26	0·46	8·00	0·44	0·60	1·04
	5·65	0·44	0·22	0·66	7·92*	0·19	0·29	0·48
	6·28	0·46	0·49	0·95	8·57*	0·57	0·21	0·78
Mean ± s.e.		0·36 ± 0·05	0·35 ± 0·06	0·71 ± 0·08		0·42 ± 0·07	0·37 ± 0·07	0·79 ± 0·10
HUT (B)	7·42	0·37	0·45	0·82	8·93	0·49	0·28	0·77
	8·00	0·64	0·38	1·02	8·50	0·24	0·62	0·86
	7·22	0·36	0·80	1·16	7·85	0·33	0·32	0·65
	8·62	0·91	0·32	1·23	8·78	0·70	0·33	1·03
	7·42	0·32	0·47	0·79	8·36	0·43	0·62	1·05
	8·50	0·17	0·33	0·50	9·06	0·58	0·33	0·91
Mean ± s.e.		0·46 ± 0·11	0·46 ± 0·07	0·92 ± 0·11		0·46 ± 0·07	0·42 ± 0·06	0·88 ± 0·06
SW	7·43	0·43	0·52	0·95	9·20	0·78	0·59	1·37
	7·50	0·92	0·59	1·51	9·50	0·21	0·30	0·51
	7·58	0·58	0·47	1·05	8·81	0·27	0·23	0·50
	8·08*	0·63	0·21	0·84	9·28	0·56	0·49	1·05
	8·32	0·42	0·51	0·93	9·20*	0·87	0·66	1·53
	8·40	0·50	0·37	0·87	9·80*	0·72	0·58	1·30
Mean ± s.e.		0·58 ± 0·08	0·45 ± 0·06	1·03 ± 0·10		0·57 ± 0·11	0·47 ± 0·07	1·04 ± 0·19

The calculations are based on the data of Table 1 from Fazekas de St Groth & Graham (1954); those marked with * are substituents, drawn from later experiments (cf. text).

significant. Undoubtedly, this is due to the rather wide scatter of the quartiles about their mean, as such a state of affairs would mask any serial correlation assessed against the error variance. On the other hand, it also means that valid—even if not too precise—comparisons can be made using the same error term.

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To test for heteroscedasticity the variances of the subgroups were compared by Bartlett's test. The obtained χ^2 of 28·69 with 39 degrees of freedom corresponds to a probability of $>0{\cdot}5$. The variances are thus homogeneous, and the distribution of the quartiles homoscedastic.

The prerequisites of a valid analysis are met by the above results, and comparison of quartiles or of interquartile ranges may serve as a legitimate basis for investigating differences between strains. Accordingly an analysis of variance has been set up on the data given in Table 4.

Table 5. *Analysis of variance on the data of Table* 4

Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio	Probability
Strains	9	0·7580	0·0882	2·194	~0·02
Replicates within strains	62	2·4930	0·0402	.	.
Between experiments	71	3·2510			
Inocula	1	0·0252	0·0252	1·029	~0·5
Quartiles	1	0·1800	0·1800	7·347	~0·008
Interactions: $I \times Q$	1	0·0149	0·0149	0·608	>0·5
$I \times S$	1	0·0306	0·0306	1·249	~0·3
$Q \times S$	9	0·0622	0·0069	0·282	>0·5
$I \times Q \times S$	9	0·0527	0·0059	0·241	>0·5
Residual	178*	4·3643	0·0245	.	.
Within experiments	208	4·7299	.	.	.
Total	279	7·9809			

* $178 = (3 \times 62) - 8$ for missing readings.

The finding relevant to the theme of this study is contained in the third line of Table 5. The variance ratio for differences between inocula does not reach significance. This more stringent test thus confirms that the shape of infectivity curves remains the same by inoculating seed yielded by the von Magnus phenomenon instead of standard infective virus. Two further points of interest emerge from this table. First, the two quartiles differ significantly, that is, the infectivity curves of influenza viruses are not symmetrical about the ID_{50}. Secondly, there is a significant difference among the ten strains tested as regards their infective behaviour. The implications of these findings will be discussed at the end of the paper.

Comparison of infectivity curves by Method II

Moran's test for goodness of fit gives, with our data, the results shown in Table 6.

Again, as in the case of Method I, there is no difference exceeding that accountable by chance between the mean M-values obtained for any of the ten strains. The only instance where this is perhaps not obvious on inspection is the BEL strain. Here $\bar{M}$ is $-0{\cdot}21 \pm 0{\cdot}54$ and $1{\cdot}05 \pm 0{\cdot}61$ respectively for virus yielded by the von Magnus phenomenon and for standard virus. However, by Student's test the value of $t = 1{\cdot}32$ is obtained, with the probability $0{\cdot}3 > P > 0{\cdot}2$; clearly, the difference is far from significant.

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Table 6. *The infective behaviour of virus issuing from concentrated or dilute seed: II. Comparisons by Moran's test for goodness of fit*

(M = Moran's statistic = $T - E(T)/\sigma_T$)

Strain	Inoculum ~10^9: ID_{50}/ml. of sample	Inoculum ~10^9: M	Inoculum ~10^4: ID_{50}/ml. of sample	Inoculum ~10^4: M
WSE (A)	6·75	0·67	9·25	1·80
	7·42	−1·36	10·20	0·90
	6·50*	0·58	10·40	−0·08
	7·41	2·25	9·70	0·27
	7·75	3·07	10·20	0·90
	6·89	1·14		
	6·75	1·77	9·12	0·90
Mean ± S.E.		1·16 ± 0·54		0·78 ± 0·26
MEL (A)	7·80	1·57	8·92*	1·23
	8·28	−0·01	9·36	−0·46
	7·50	1·95	8·75	0·89
	7·71*	1·28	10·00	−0·40
	7·75	2·20	9·08*	3·77
	7·63*	0·13	8·79*	0·13
	7·25	3·50	9·40	2·53
Mean ± S.E.		1·52 ± 0·46		1·09 ± 0·61
CAM (A′)	8·00*	3·16	9·17*	2·53
	9·78	0·44	9·72	1·88
	9·25	−0·91	9·06	−0·86
	8·42	0·95	9·63*	2·00
	9·30	−0·01	9·25	−0·86
	8·81*	0·58	9·15	−1·38
	8·60	2·20	9·67*	1·88
Mean ± S.E.		0·92 ± 0·52		0·74 ± 0·64
LEE (B)	9·81	−0·27	9·45	2·28
	9·20	0·58	9·80	−0·40
	9·62	−0·27	9·40	1·88
	9·82	2·64	9·02	1·55
	9·63	−0·40	9·80	−0·40
	9·30	3·67	9·30	0·87
	10·05	2·53		
	9·80	0·87	7·55	−0·40
	10·05	3·29		
	9·07*	0·67	9·17	3·61
	9·42	−0·48	9·00	0·44
	8·63	1·63	7·86	1·59
	8·50	1·56	7·86	1·95
	9·38	0·44		
	8·76	1·28	9·67*	−0·65
Mean ± S.E.		1·18 ± 0·35		1·03 ± 0·25

Strain	Inoculum ~10^9: ID_{50}/ml. of sample	Inoculum ~10^9: M	Inoculum ~10^4: ID_{50}/ml. of sample	Inoculum ~10^4: M
PR8 (A)	7·14	−0·01	10·22	0·68
	7·72	0·90	10·34	−0·12
	7·30	−0·08	9·42	0·90
	7·55	−0·40	10·30	1·23
	7·24	2·28	9·39	2·28
	7·70	1·25	10·19	2·86
Mean ± S.E.		0·66 ± 0·41		1·31 ± 0·45
BEL (A)	8·90	1·14	9·50*	0·87
	9·35	−2·39	9·75	−0·91
	8·33	0·90	9·80	2·71
	8·38	−0·91	9·50*	2·86
	7·43	−0·46	9·65	−0·08
	8·63	0·44	9·70*	0·87
Mean ± S.E.		−0·21 ± 0·54		1·05 ± 0·61
FM1 (A′)	7·50	3·04	10·33*	1·55
	7·85	0·90	9·07	0·44
	8·35	2·27	8·20	4·35
	7·70	−0·46	8·86	−0·46
	8·88	0·06	7·90	1·06
	8·88	3·09	9·28	1·27
Mean ± S.E.		1·48 ± 0·63		1·37 ± 0·67
BON (B)	6·72	0·43	8·60	−0·01
	6·35	0·90	8·06	−0·01
	6·50	2·51	7·87	2·12
	5·81*	−0·01	8·00	1·28
	5·65	0·90	7·92*	−0·40
	6·28	−0·46	8·57*	1·88
Mean ± S.E.		0·71 ± 0·42		0·81 ± 0·44
HUT (B)	7·42	1·80	8·93	1·55
	8·00	1·88	8·50	−0·46
	7·22	4·76	7·85	1·55
	8·62	3·05	8·78	1·88
	7·42	1·88	8·36	1·62
	8·50	−0·27	9·06	2·37
Mean ± S.E.		2·18 ± 0·68		1·42 ± 0·40
SW	7·43	3·61	9·20	3·61
	7·50	2·47	9·50	−0·46
	7·58	3·16	8·81	−0·01
	8·08*	−1·05	9·28	1·44
	8·32	2·25	9·20*	3·29
	8·40	1·80	9·80*	3·51
Mean ± S.E.		2·04 ± 0·67		1·90 ± 0·75

The calculations are based on the data of Table 1 from Fazekas de St Groth and Graham (1954); those marked with * are substituents, drawn from later experiments (cf. text).

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The distribution of M-values about their mean, as shown in Fig. 3, is not skew ($M - Md/s = +0{\cdot}02$), and the test for goodness of fit gives a χ^2 of 5·32, with $P > 0{\cdot}5$. Normality of the distribution is thus not contradicted. Neither does the χ^2 in Bartlett's test reach significance: its value, 8·61, with 19 degrees of freedom indicates homoscedasticity since the corresponding probability ($P > 0{\cdot}5$) is in close agreement with what might be expected by chance.

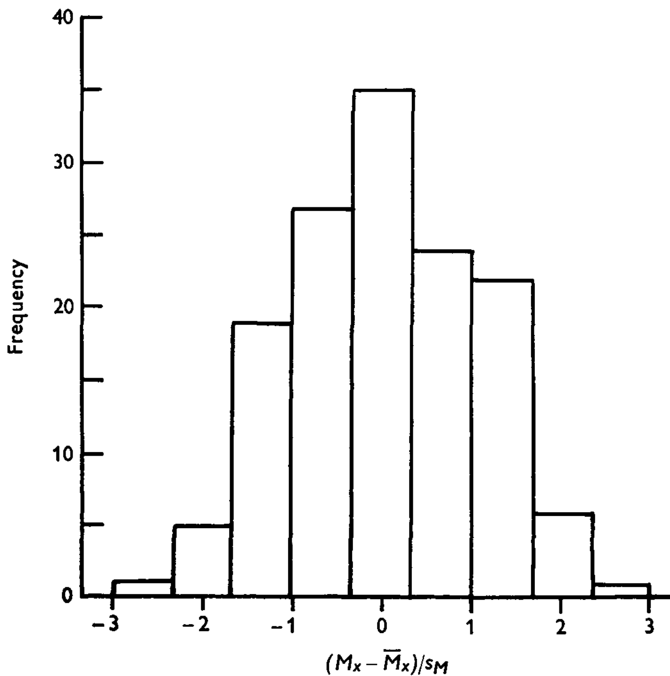

Fig. 3. Distribution of Moran's M-values about their mean. (Plotted from data of Table 6.)

The analysis of variance on the data furnished by Moran's test (Table 7) reveals, once more, no significant differences between the response to the two types of inocula. However, with this test the strains do not differ either—the probability

Table 7. *Analysis of variance on the data of Table* 6

Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio	Probability
Strains	9	24·7467	2·7496	1·176	~0·20
Replicates within strains	62	144·9498	2·3379	.	.
Between experiments	71	169·6965			
Inocula	1	0·0913	0·0913	0·064	>0·5
Interaction: $I \times S$	9	9·2474	1·0275	0·718	>0·5
Residual	58	82·9800	1·4307	.	.
Within experiments	68	92·3187	.	.	.
Total	139	262·0152			

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corresponding to their variance ratio is about 0·20. This lack of discrimination is due in great measure to the large error variance, and to this point we shall return later.

Interrelations of Methods I and II

In the foregoing sections the slope of infectivity curves has been compared by each of the two methods. Although these tests are independent in so far as their underlying principles differ, they were developed for a common practical purpose, namely the assessment of virulence. For this reason the results obtained with the two should be correlated, even though the means by which they approach their common aim are dissimilar. In this section the extent of their association will be determined, and also the way in which their results can be combined.

Table 8. *Analysis of covariance on R and M values from Tables* 4 *and* 6

Source of variation	Degrees of freedom	Sum of products	Mean product	Correlation coefficient
Inocula	1	−0·0482	−0·0482	.
Strains	9	5·7673	0·6408	+0·942
Interaction	9	−0·3915	−0·0435	−0·303
Error	120	33·6322	0·28027	+0·755
Total	139	38·9598		

First an analysis of covariance was set up for the two methods. To have comparable statistics, the R-values derived by Method I were matched with the corresponding M-values of Method II. By doing this some of the information was lost; however, as has been pointed out above, the choice is not without its compensating gain, since bias from correlation and non-normality could be excluded thereby. From the covariances of Table 8 and the variances of Table 5 and 7 the appropriate correlation coefficients were calculated; these, too, are shown in Table 8.

Strong overall correlation between the two methods is evident from the results, and the value obtained, 0·76, could have arisen by chance in less than once in a thousand cases.

As regards inocula, we have already seen that on its own neither of the two methods revealed significant differences between them. Now, as shown in the first row of Table 8, we find that the two are negatively correlated in this respect, although not too strongly. It may be hoped therefore that a combined function of the variables will give rise to a more efficient estimator of differences at this level. Although a discriminant function could be derived by regression analysis as there are only two inocula, we have used the more general method of canonical correlations for the purpose. The equation obtained, $D = R + 0{\cdot}110M$, has been applied to our data, with the results shown in Table 9.

Obviously, even this more powerful method could not discriminate between inocula, either in the overall test, or at the level of individual strains.

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Table 9. *Analysis of variance on the data obtained with the discriminant function D*

($D = R + 0{\cdot}11\,M$)

Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio	Probability
Inocula	1	0·0622	0·0622	1·93	~0·07
Strains	9	0·6129	0·0681	2·12	~0·03
Interaction	9	0·0585	0·0065	0·22	>0·5
Error	118	3·7920	0·0321	.	.
Total	137	4·5256			

DISCUSSION

No qualitative differences could be detected in the infective behaviour of elementary bodies issuing from large or small doses of influenza viruses in the allantois. The question put in the introduction can thus be answered in the negative.

Admittedly, the accuracy of the titrations is such that minor differences would pass unnoticed; but whatever their absolute magnitude, these differences are negligible in relation to the striking discrepancy between infective and haemagglutinating units characteristic of the von Magnus phenomenon. The main conclusion, then, is that the infective fraction of the yield after concentrated inocula is indistinguishable from standard passage virus, while the rest of the particles present do not contribute to the outcome of infectivity tests. This evidence runs counter to Fulton's suggestion of uniformly 'avirulent' virus, and such a presumption should not be invoked to account for the observations.

The discard of one hypothesis tends to lend support, by default, to the contending alternative. We should bear in mind though that information is restricted to the infective fraction, i.e. to the 1 in 100 minority, and has no bearing whatsoever on those particles which do not go on multiplying. Thus our ignorance of what the non-infective majority is has not been lessened, and for this reason the theoretical implications of Fulton's hypothesis remain as stimulating as before. Indeed, the idea of an imperfect cycle of multiplication is bound to have more general appeal, and will certainly not prejudice our eventual understanding of the phenomenon. The notion of an unfinished cycle, implicit in the term 'incomplete virus', is all too restrictive and sets artificial and unjustifiable limits to our thinking and experimentation. For the time being it is perhaps safest to call the minor portion of the yield 'infective' and the major one 'non-infective' virus. These names cover what has been actually observed; if found to be objectionably simple, they can be replaced by more appropriate terms at a later date, as our knowledge deepens.

Although the comparison of infectivity curves was undertaken with a special aim in view, several points of concomitant information bearing on the more general problem of infectivity have emerged. The first of these is the nature of virus-host interaction. In the simplest case, and some of the phage-bacterium relationships belong to this class, infection depends only on the probability of having a virus in the inoculum; the response curve here is the Poissonian, defined by a single

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parameter. At the next order of complexity not all the cells are susceptible and the success of infection is reduced in proportion; the probability distribution, however, is still Poissonian, although the parameter defining it is here a product of virus concentration and host susceptibility, and the two cannot be separated without independent further information. The behaviour of myxoma virus (Fenner & Woodroofe, 1953) is a good case in point. With influenza viruses the situation is even more involved. The Poisson distribution does not fit the infectivity data, as is clear from the results of Moran's test. On the other hand, both tests give evidence not only that some host cells are less susceptible than others, but that the degree of this insusceptibility varies from egg to egg. While the distribution of this 'host resistance' remains unknown, as it is at present, the dose-response curve cannot be defined in exact terms. All one can say is that it is asymmetric about its median (cf. Table 5: difference between upper and lower quartiles), and that its slope in the central portion shows considerable variation among strains.

The differences among strains are clearly demonstrable by the non-parametric method (cf. Table 5), but no more than suggestive when evaluated by Moran's test (cf. Table 7). The latter, a test for goodness of fit, is sensitive to discrepancies over the whole of the dose-response curve. With groups as small as in our infectivity titrations, the expectations at the flat ends of the curve will be small, and this is bound to increase variation between replicates. It is this inflated variance which tends to mask interstrain differences. The non-parametric method, restricted to the central portion of the curves, is free from this additional variation, and hence better suited to work on small samples. Indeed, a discriminant function derived for the strains showed only minimal improvement over the R-values on their own (variance ratio 2·41, as against 2·32), and was definitely inferior to comparisons based on the quartiles (cf. Table 5).

The strains can thus be ordered in a gradient according to the size of their cumulative quartiles, i.e. the central slope of their infectivity curves. With the appropriate tests of significance this value may serve as a statistical measure of how sensitive a particular strain is to variations in host resistance; or, conversely, as a measure of virulence, provided we accept overcoming of host resistance as one possible definition of virulence. In either case, and whether comparisons of different strains or different virus preparations are to be based on these criteria, it should be borne in mind that the methods have been proposed mainly for their simplicity, and that they are only approximate and essentially indirect. Their usefulness will cease as soon as the true nature of the infectivity curve of influenza viruses has been elucidated.

SUMMARY

Two new statistical methods—one parametric, the other non-parametric—are developed to compare the slopes of quantal infectivity curves.

Ten influenza strains were examined with the aid of these tests, and the results show that virus yielded by the von Magnus phenomenon (i.e. containing 'incomplete' particles) and standard infective virus do not differ in the slope of their infectivity curves.

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It is concluded that the von Magnus phenomenon yields a mixture of infective and non-infective particles; on this evidence Fulton's suggestion of uniformly changed virulence is untenable.

I am greatly indebted to Prof. P. A. P. Moran for helpful discussions of theory, and frequent corrections of statistical technique.

APPENDIX: A MATHEMATICAL MODEL OF VIRUS-CELL INTERACTION

BY S. FAZEKAS DE ST GROTH and P. A. P. MORAN

The interaction of a virus particle and a cell may or may not result in infection, that is, in the multiplication of the virus inside the cell. In what follows we shall enumerate and define the variables which enter into this process; construct a general theoretical model; and examine—in the particular case of influenza virus—what is the minimum of assumptions compatible with existing empirical knowledge.

Definitions

Whether a virus particle infects a cell or not is determined by the properties of both the particle and the cell. We suppose that in the simplest case these properties can be described by a single quantity for the virus and similarly for the cell.

Infectivity is the potency of a virus particle to multiply in cells. This potency is measured by the probability, p_i, that a given particle, i, will multiply when applied to fully susceptible cells. Infectivity may vary from particle to particle, and the probability density function of its distribution is $f(p_i)$, with a mean value over all particles, $\bar{p}$.

Susceptibility is the power of a cell to support viral multiplication. We measure the susceptibility of a cell j by the probability, s_j, of its being infected on coming into contact with a fully infective virus. Susceptibility may vary from cell to cell with a probability density function $g(s_j)$, and its mean value over the cells is $\bar{s}$.

If a particle, i, of infectivity p_i, comes into contact with a cell, j, of susceptibility s_j, we suppose that the probability of infection occurring is determined solely by p_i and s_j; we take this probability of infection to be a function

$$F(p_i,\, s_j)\ (0 \leqslant p_i \leqslant 1,\ 0 \leqslant s_j \leqslant 1),$$

the *interaction function*. Clearly:

$$F(p_i,\, 0) = F(0,\, s_j) = 0 \quad F(p_i,\, 1) = p_i \quad F(1,\, s_j) = s_j.$$

General hypothesis

Let c be the average concentration of virus per standard inoculum. The probability of exactly r particles in such an inoculum is

$$\frac{c_r}{r!}\, e^{-c}.$$

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These particles have infectivities $p_1, p_2, \ldots, p_r$. The probability that a particular particle does not infect is

$$P = 1 - \int_0^1 \int_0^1 F(p, s)\, f(p)\, g(s)\, dp\, ds, \quad \begin{matrix}(0 \leqslant p \leqslant 1)\\(0 \leqslant s \leqslant 1)\end{matrix} \tag{1}$$

and the probability that r particles do not infect is P^r. Thus the probability that there are r particles and they do not infect is

$$\frac{c^r P^r}{r!} e^{-c}. \tag{2}$$

Hence the probability that the host remains uninfected on receiving a standard inoculum with an average multiplicity of c virus particles in it will be

$$\begin{aligned} P_0 &= e^{-c} + \sum_{r=1}^{\infty} \frac{c^r P^r}{r!} e^{-c} \\ &= e^{-c} + e^{-c}\,(e^{cP} - 1) \\ &= e^{-c(1-P)} \\ &= \exp\left(-c\left\{\int_0^1 \int_0^1 F(p, s)\, f(p)\, g(s)\, dp\, ds\right\}\right). \end{aligned} \tag{3}$$

This is the zero term of a Poisson distribution; the bracketed part, corresponding to the effective proportion, will be referred to below as the *virulence integral* or *virulence*, for short.

Equation (3) describes the case when the distribution of cellular susceptibility does not vary from host to host. In the general case, however, inter-host variation may occur. Let therefore $g_k(s)$ be the probability distribution of cell susceptibility within the kth host (i.e. in the kth set of cells). Then the probability of no infection becomes the average over all k's of

$$\exp\left(-c \int_0^1 \int_0^1 F(p, s)\, f(p)\, g_k(s)\, dp\, ds\right). \tag{4}$$

The term
$$\int_0^1 \int_0^1 F(p, s)\, f(p)\, g_k(s)\, dp\, ds = V_k$$

is a number depending on k, and represents the virulence of the virus for the kth host. If the distribution of V amongst host systems is $h(V)$, the probability of no infection becomes

$$P_0 = \int_0^1 e^{-cV}\, h(V)\, dV. \tag{5}$$

Accordingly, the infectivity curve (c, P_0) will be a function of the distributions of viral infectivity and cellular susceptibility, and of the interaction function. This is the general case.

Special hypotheses

H. I and II. Under the boundary conditions where either the virus is 'incomplete' $(p = 0)$, or the cells 'insusceptible' $(s = 0)$,

$$P_0 = 1, \tag{6}$$

since $F(0, s) = F(p, 0) = 0$.

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H. III. In the simplest non-zero case, where 'complete' virus ($p=1$) enters a system of susceptible cells ($s=1$),

$$P_0 = e^{-c}, \tag{7}$$

and from such tests the number of particles could be determined directly. It has been shown, however, that the relation between influenza virus particles and infective units is not constant (reviews: von Magnus, 1954; Schlesinger, 1953), and can be varied experimentally over a thousand-fold range. Hence H. III cannot hold in general.

H. IV and V. If the virus particles are uniformly or variably less infective, than 'complete' virus $\left(0<\bar{p}=p_i<1, \text{ or } 0<\bar{p}=\int_0^1 f(p)\,p\,dp<1, \text{ respectively}\right)$, and the cells are fully susceptible ($s=1$), the response curve will be still Poissonian, and

$$P_0 = \exp\,(-cF(\bar{p},\,1)) = e^{-c\bar{p}}, \tag{8}$$

or

$$P_0 = \exp\left(-c\int_0^1 F(p,\,1)\,f(p)\,dp\right) = e^{-c\bar{p}}. \tag{9}$$

In this case the response curve will give no direct information about the number of particles inoculated.

H. VI and VII. If uniformly or variably less susceptible cells

$$\left(0<\bar{s}=s_j<1, \text{ or } 0<\bar{s}=\int_0^1 g(s)\,s\,ds<1, \text{ respectively}\right)$$

come into contact with 'complete' virus ($p=1$), the situation is analogous to H. IV and V, and

$$P_0 = \exp\,(-cF(1,\,\bar{s})) = e^{-c\bar{s}}, \tag{10}$$

or

$$P_0 = \exp\left(-c\int_0^1 F(1,\,s)\,g(s)\,ds\right) = e^{-c\bar{s}}. \tag{11}$$

H. VIII. If uniformly less infective virus meets uniformly less susceptible cells,

$$P_0 = \exp\,(-cF(\bar{p},\,\bar{s})). \tag{12}$$

H. IX and X. If either infectivity or susceptibility is distributed, and the other constant, the probability of no infection becomes

$$P_0 = \exp\left(-c\int_0^1 F(p,\,\bar{s})\,f(p)\,dp\right), \tag{13}$$

or

$$P_0 = \exp\left(-c\int_0^1 F(\bar{p},\,s)\,g(s)\,ds\right), \text{ respectively.} \tag{14}$$

H. XI. If variably less infective virus attacks variably less susceptible cells, Eq. (3) will obtain.

Hypotheses IV–XI postulate infectivity curves with a single parameter, c. These Poissonian curves differ from each other and from the curve of H. III (Eqn. 7) only by a constant, i.e. they have different means but the same shape on a ($\log c$, P_0) plot. As it has been shown that the most carefully obtained (c, P_0) curves are not fitted by the negative exponential (Moran, 1954) and that this holds for standard infectivity tests on most of the adapted strains of influenza virus (Fazekas de St Groth & Cairns, 1952), the assumptions underlying the above hypotheses are not sufficient.

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As a consequence a further postulate will have to be incorporated into the model, namely the variation from host to host of the distribution of cellular susceptibility. The general case was given by Eqn. (5); for discussion of special hypotheses the value of V, the virulence integral, need be considered only. Virulence, as here defined, is a complex function of the two independent distributions $f(p)$ ('infectivity) and $g(s)$ ('susceptibility'), and of the joint 'interaction function' $F(p, s)$.

H. XII. Virus particles are complete ($p=1$), cells are of uniformly intermediate susceptibility within any one host, but susceptibility varies from host to host. The situation within any host is as postulated by H. VI, and thus the virulence for the kth host will be

$$V_k = F(1, \bar{s}_k) = \bar{s}_k. \tag{15}$$

Analogously, by adding the postulate of inter-host variation to H. VII–XI, we can derive the following virulence integrals:

H. XIII. $$V_k = \int_0^1 F(1, s)\, g_k(s)\, ds = \bar{s}_k; \tag{16}$$

H. XIV. $$V_k = F(\bar{p}, \bar{s}_k); \tag{17}$$

H. XV. $$V_k = \int_0^1 F(p, \bar{s}_k)\, f(p)\, dp; \tag{18}$$

H. XVI. $$V_k = \int_0^1 F(\bar{p}, s)\, g_k(s)\, ds; \tag{19}$$

H. XVII. $$V_k = \int_0^1 \int_0^1 F(p, s)\, f(p)\, g_k(s)\, dp\, ds. \tag{20}$$

Hypotheses XII–XVII define (c, P_0) curves, all of which are flatter than the negative exponential. These curves differ from each other in shape, the latter being determined by the distribution amongst hosts of the virulence integral, which is different for each hypothesis. However, the shape of these response curves will vary with infectivity only if $F(p, s) \neq ps$.

H. XVIII. If we assume $F(p, s) = ps$, the general formula (Eqn. 20) becomes

$$\begin{aligned} V_k &= \int_0^1 \int_0^1 ps\, f(p)\, g_k(s)\, dp\, ds \\ &= \int_0^1 p\, f(p)\, dp \int_0^1 s\, g_k(s)\, ds \\ &= \bar{p}\,.\,\bar{s}_k, \end{aligned} \tag{21}$$

and the shape of the (c, P_0) curve will depend only on the variation from host to host of $\bar{s}_k$, the average cellular susceptibility. This would imply an infectivity curve flatter than e^{-c}, but independent of any variation in the virus and characteristic of and constant for a particular type of host. Since it is common knowledge (Andrewes, Laidlaw & Smith, 1934; Sherp, Flosdorf & Shaw, 1938; Burnet, 1941*a*, *b*, 1943; Burnet & Bull, 1943; Burnet, Beveridge & Bull, 1944; Knight, 1944; Knight & Stanley, 1944; Panthier, Cateigne & Hannoun, 1948; Fazekas de St Groth & Cairns, 1952) that non-adapted strains of influenza virus give flat infectivity curves

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('patchy titrations', 'bad end-points', in biological parlance), while later passages of the same virus give steeper curves ('clear-cut end-points') in the same host, H. XVIII can be dismissed as contrary to established facts.

Discrimination between the hypotheses of von Magnus and of Fulton

Virus yielded after very concentrated inocula may give lower median infectivity than could be expected from the number of particles present.

H. 'v. M.' von Magnus assumes that the yield is a mixture of 'complete' and 'incomplete' virus, i.e the particles are of two kinds, a fraction π with an infectivity of $p=1$ and the rest, $(1-\pi)$, with infectivity $p=0$. Formally expressed this yields a virulence integral

$$V_k=\int_0^1\{F(0, s)\ (1-\pi)+F(1, s)\ \pi\}\ g_k(s)\ ds$$

$$=\pi\int_0^1 F(1, s)\ g_k(s)\ ds, \quad \text{since } F(0, s)=0$$

$$=\pi\bar{s}_k. \tag{22}$$

The corresponding infectivity curves will have the same shape whatever the average infectivity of the particles; variations in π can alter the position only of the (c, P_0) curves.

H. 'F.' Fulton assumes that the yield is uniform and less virulent. Thus the value of V_k will be as postulated by H. XVII (Eqn. 20), and may be simplified further by restricting assumptions to give H. XIV, H. XV or H. XVI. The shape of these curves will vary, and will depend in every instance on the distributions of infectivity and susceptibility—that is, on a property of the virus and a property of the cell— as well as on their interaction, $F(p, s)$. Accordingly, a change in the average behaviour of the virus will alter both the shape and the position of the (c, P_0) curve.

Let the average infectivity in von Magnus's hypothesis equal the average infectivity in Fulton's hypothesis, i.e. $\pi=\bar{p}$, throughout. Then the discrimination between the two hypotheses under different modifying assumptions (cf. H. XIV–XVII) is based on the following inequalities:

(*a*) if both infectivity and susceptibility are constant

$$\bar{p}.\bar{s}_k\neq F(\bar{p}, \bar{s}_k); \tag{23}$$

(*b*) if infectivity is constant and susceptibility distributed

$$\bar{p}.\bar{s}_k\neq\int_0^1 F(\bar{p}, s)\ g_k(s)\ ds; \tag{24}$$

(*c*) if infectivity is distributed and susceptibility constant

$$\bar{p}.\bar{s}_k\neq\int_0^1 F(p, \bar{s}_k)\, f(p)\ dp; \tag{25}$$

(*d*) if both infectivity and susceptibility are distributed

$$\bar{p}.\bar{s}_k\neq\int_0^1\int_0^1 F(p, s)\, f(p)\ g_k(s)\ dp\ ds. \tag{26}$$

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It is also evident that under the boundary conditions where all particles are infective ($\overline{p}=1$), the difference between the two hypotheses disappears, since both reduce to either H. XII or H. XIII, and their virulence integrals become identical, $\bar{s}_k$.

REFERENCES

ANDREWES, C. H., LAIDLAW, P. P. & SMITH, W. (1934). *Lancet*, ii, 859.
BEHRENS, B. (1929). *Arch. exp. Path.* **140**, 237.
BURNET, F. M. (1941*a*). *Aust. J. exp. Biol. med. Sci.* **19**, 39.
BURNET, F. M. (1941*b*), *Aust. J. exp. Biol. med. Sci.* **19**, 101.
BURNET, F. M. (1943). *Aust. J. exp. Biol. med. Sci.* **21**, 231.
BURNET, F. M. & BULL, D. R. (1943). *Aust. J. exp. Biol. med. Sci.* **21**, 55.
BURNET, F. M., BEVERIDGE, W. I. B. & BULL, D. R. (1944). *Aust. J. exp. Biol. med. Sci.* **22**, 9.
DRAGSTEDT, C. A. & LANG, V. F. (1928). *J. Pharmacol.* **32**, 215.
FAZEKAS DE ST GROTH, S. & CAIRNS, H. J. F. (1952). *J. Immunol.* **69**, 173.
FAZEKAS DE ST GROTH, S. & GRAHAM, D. M. (1953). *Nature, Lond.*, **172**, 1193.
FAZEKAS DE ST GROTH, S. & GRAHAM, D. M. (1954). *Brit. J. exp. Path.* **35**, 60.
FENNER, F. & WOODROOFE, G. M. (1953). *Brit. J. exp. Path.* **34**, 400.
FULTON, F. (1953). *Bull. Hyg., Lond.*, **28**, 292.
KNIGHT, C. A. (1944). *J. exp. Med.* **79**, 487.
KNIGHT, C. A. & STANLEY, W. M. (1944). *J. exp. Med.* **79**, 291.
VON MAGNUS, P. (1951*a*). *Acta path. microbiol. scand.* **28**, 250.
VON MAGNUS, P. (1951*b*). *Acta path. microbiol. scand.* **28**, 278.
VON MAGNUS, P. (1954). *Dynamics of Virus and Rickettsial Infections*, p. 36. (Ed. Hartmann, Horsfall & Kidd.) New York: Blackiston Co.
MORAN, P. A. P. (1954*a*). *J. Hyg., Camb.*, **52**, 189.
MORAN, P. A. P. (1954*b*), *J. Hyg., Camb.*, **52**, 444.
PANTHIER, R., CATEIGNE, G. & HANNOUN, C. (1948). *C.R. Soc. Biol., Paris*, **142**, 1354.
REED, L. J. & MUENCH, H. (1938). *Amer. J. Hyg.* **27**, 493.
SCHLESINGER, R. W. (1953). *Ann. Rev. Microbiol.* **7**, 83.
SHERP, H. W., FLOSDORF, E. W. & SHAW, D. R. (1938). *J. Immunol.* **34**, 447.

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