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The first cycle of growth in the chick embryo of the agents of trachoma and inclusion blennorrhoea

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We have observed differences in the rate of growth of strains of trachoma and inclusion blennorrhoea agent in the chick embryo consistent with the assumption that increased virulence for the chick embryo is associated with a higher rate of multiplication (Taverne, Blyth & Reeve, 1964). Growth curves were plotted from the time of inoculation to the death of the embryo by daily measuring the amount of agent formed in terms of lethality for chick embryos, infectivity for HeLa cells and total particles; these curves represented the results of many cycles of multiplication. However, the differences in growth rate of strains of different virulence were small and the data were not amenable to tests of statistical significance; furthermore, the finding of such differences was at variance with earlier deductions from dose–response curves plotted from the results of egg titrations (Jawetz & Hanna, 1960; Reeve & Taverne, 1963).

We pointed out that differences in growth rate over a long period do not necessarily reflect differences of intracellular growth (Taverne *et al.* 1964), but could, for instance, result from variations in behaviour during the extracellular phase. To distinguish between these possibilities and to verify differences in growth rate, single-cycle growth curves were plotted for several strains of trachoma and inclusion blennorrhoea agents multiplying in the chick embryo yolk sac; we have compared their intracellular cycles of growth and related them to the growth rates previously observed.

MATERIALS AND METHODS

TRIC agents listed below are designated according to the system proposed by Gear, Gordon, Jones & Bell (1963). Abbreviated designations are used in the text (Collier, 1963).

For convenience, *f* is suffixed to the designation of strains that kill chick embryos relatively quickly ('fast-killing variants': Taverne *et al.* 1964) by contrast with the less virulent, slow-killing strains for which the suffix *s* is used where necessary for clarity.

Strains. TRIC/ /China/Peking-2/OT (T'ang, Chang, Huang & Wang, 1957) was obtained from freeze-dried material preserved by I.C.I. Laboratories, Cheshire.

TRIC/ /SAU/HAR-2/OT (Murray *et al.* 1960) (SA 2). The variant, HAR-2*f* was derived at the Lister Institute from material supplied by Dr S. Bell, School of Public Health, Harvard University.

TRIC/ /GB/MRC-4/ON (Jones, 1961) (LB 4) was isolated from material supplied by Professor Barrie Jones, Institute of Ophthalmology, London. The variant LB 4*f* was derived at the Lister Institute.

TRIC/ /WAG/MRC-1/OT (Collier & Sowa, 1958) (G 1) was isolated in the Gambia and maintained at the Lister Institute.

Diluent was phosphate-buffered saline containing streptomycin sulphate, 1000 μg./ml.

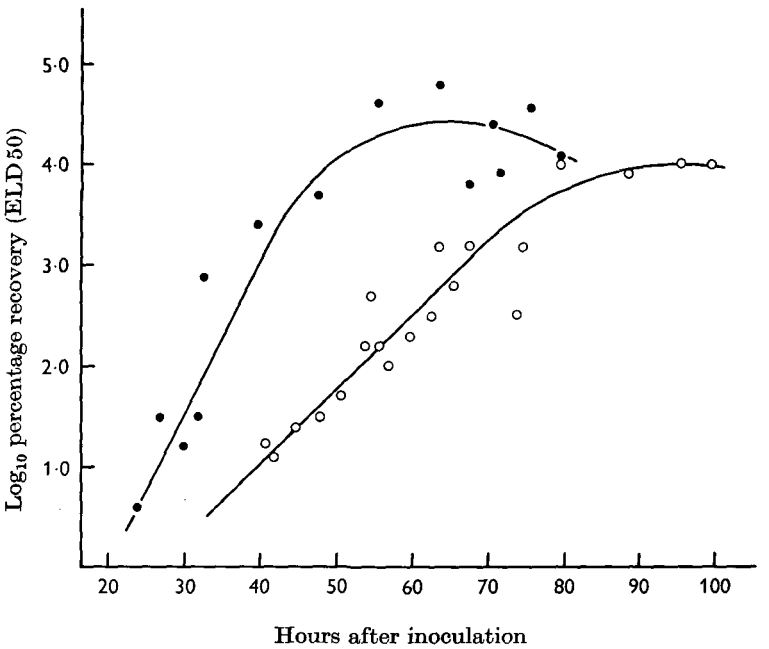

Fig. 1. The first cycle of growth in the chick embryo yolk sac of strains of trachoma and inclusion blennorrhoea of differing virulence. The points represent geometric means ($\log_{10}$ ELD50 per YS) expressed as a percentage of the dose inoculated. ●, Mean of strains HAR-2*f* and MRC-4*f*; ○, mean of strains MRC-1/OT, MRC-4 and PK-2.

Infectivity titrations were done in the yolk sacs of 7-day chick embryos kept at a continuously recorded temperature of 35° C. ± 0·5° C. in a humid atmosphere. Eggs were candled daily and tests were terminated 14 days after inoculation. Titres were calculated according to the method of Reed & Muench (1938) and expressed in terms of 50% lethal doses for eggs (ELD50). Specificity of death, when doubtful, was determined by examining yolk-sac smears for the presence of elementary bodies.

Growth curves. For each strain, the yolk sacs of fifty 7-day chick embryos were inoculated with 0·5 ml. of a yolk-sac suspension calculated to contain between 10 and 100 ELD50/ml. and titrated at the time of injection. At intervals of about 8 hr. the yolk sacs of 3 or 6 live embryos were harvested, shaken with glass beads in 10 ml. of diluent, and titrated in eggs using 3·2-fold serial dilutions.

Results were expressed as ELD 50 recovered per yolk sac, and in Fig. 1 as a percentage of the inoculum, i.e.

$$\frac{\text{ELD 50 inoculated per yolk sac}}{\text{ELD 50 recovered per yolk sac}} \times 100.$$

RESULTS

The amount of agent present in yolk sacs at different times during the first 3 days after inoculation of fast- and slow-killing strains was measured in ELD 50 per yolk sac (Table 1). Growth curves were plotted to show the increase in infectious organisms during this period by calculating the geometric means for the two *f* and three *s* strains respectively, and expressing them as a percentage of the mean dose inoculated for each group (Fig. 1).

With strains HAR-2*f* and MRC-4*f*, infectious organisms were detected 24 hr. after inoculation and thereafter their number increased exponentially to reach a peak about 60 hr. after inoculation. After this time the amount of agent decreased, indicating that one cycle of multiplication was complete. With strains MRC-4, MRC-1/OT and PK-2, organisms were first detected about 40 hr. after infection and their number increased exponentially until about 80 hr.

These results were analysed to determine any statistically significant differences in the behaviour of *f* and *s* strains in terms of (*a*) the times when exponential growth began and (*b*) the slopes of the exponential sections of their growth curves. Exponential growth was taken as starting when the amount of agent recovered per yolk sac, expressed as a percentage of the dose inoculated, was 1 ELD 50 by extrapolation; this time was on average 21 hr. after inoculation for the *f* strains and 38 hr. for the *s* strains. The difference between these times was significant at the 1% level ($t = 3{\cdot}19$ with 28 D.F.; $0{\cdot}01 < P < 0{\cdot}002$). If the exponential phase is taken as ending at 40 hr. with *f* strains and at 68 hr. with *s* strains, comparison of the slopes suggested a genuine difference which just fails to attain significance at the 5% level of probability ($t = 2{\cdot}03$, with 28 D.F.; for $t = 2{\cdot}05$, $P = 0{\cdot}05$). Estimates of the average slope of the growth curves gave growth rates of $10^{0{\cdot}14}$ ELD 50 per hr. for the *f* strains and $10^{0{\cdot}07}$ ELD 50 per hr. for the *s* strains.

DISCUSSION

The growth rate of an organism can be measured directly as the rate of division during the exponential phase of one cycle of growth, or it can be calculated from the yield in unit time. A rate derived in the first way is defined here as the 'actual growth rate', as it measures the true rate of division. The second method gives an 'apparent growth rate' since during the observation period there may be a lag phase in which the organism is not actively dividing, and some organisms may die before samples are titrated. Previously, we found that during several cycles of growth the more virulent strains had faster apparent rates of growth than the less virulent (Taverne *et al.* 1964). Measurement of the maximum yield obtained in unit time after one cycle of growth confirms the existence of these differences in apparent growth rate related to differences in virulence. Thus, calculated from

Table 1. *Yields of TRIC agents, in log_{10} ELD50 per yolk sac during the first cycle of growth*

Strain ...	HAR-2*f*			MRC-4*f*	MRC-1/OT		MRC-4					PK-2		
Suspension* ...	*a*	*a*	*b*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*b*	*c*
Dose inoculated ...	1·7	2·6	2·5	1·2	2·0	1·7	1·8	3·6	3·0	2·3	2·5	2·5	1·9	3·6
Hours after *inoculation*														
24	0·6	—	1·9	< 0·0	< 0·0	—	1·2	0·7	—	—	—	< 0·0	—	—
27	—	—	2·0	—	—	—	—	—	—	—	—	—	—	—
30	—	—	1·7	—	—	—	—	—	—	—	—	—	—	—
32	1·1	—	—	0·8	< 0·0	—	< 0·0	2·2	—	—	—	< 0·0	—	—
33	—	—	3·4	2·9	—	—	—	—	—	—	—	—	—	—
36	—	—	—	—	—	—	—	—	< 0·5	—	—	—	—	—
40	2·7	—	—	—	—	—	< 0·0	—	—	—	—	—	—	—
41	—	—	—	—	1·9	—	—	2·8	—	—	—	1·1	—	—
42	—	—	—	—	—	—	—	—	1·4	—	—	—	—	—
45	—	—	—	—	—	—	—	—	—	—	1·9	—	—	3·3
48	3·5	—	—	2·8	1·1	—	2·6	3·7	3·1	—	2·4	1·6	2·0	2·0
51	—	—	—	—	—	—	—	—	—	—	2·5	—	—	3·0
54	—	—	—	—	—	—	—	—	—	2·1	3·1	—	—	3·8
55	—	—	—	—	—	—	—	—	—	—	—	—	3·0	—
56	3·7	4·3	—	4·5	2·2	2·4	1·8	3·6	—	—	—	2·7	—	—
57	—	—	—	—	—	—	—	—	—	2·4	2·4	—	—	3·5
60	—	—	—	—	—	—	—	—	—	—	3·1	—	—	3·6
63	—	—	—	—	—	—	—	—	—	2·8	3·0	—	—	4·0
64	3·9	4·8	—	4·5	—	4·0	1·7	—	—	—	—	—	—	—
65	—	—	—	—	—	—	—	—	—	—	—	3·5	3·6	—
66	—	—	—	—	2·5	—	—	4·6	—	—	—	—	—	—
68	—	4·2	—	—	—	2·9	—	—	—	—	—	—	—	—
71	—	—	—	3·6	—	—	—	—	—	—	—	—	—	—
72	3·7	4·5	—	—	2·1	—	2·5	4·0	—	—	—	2·4	2·9	—
74	—	—	—	—	—	—	—	—	—	—	—	—	—	—
75	—	—	—	—	—	3·6	—	—	—	—	—	—	—	—
76	—	4·9	—	—	—	—	—	—	—	—	—	—	—	—
78	—	—	—	—	—	—	—	—	—	—	—	—	2·4	—
80	—	4·7	—	—	—	3·7	—	—	—	—	—	—	—	—
89	—	6·4	—	—	—	3·9	—	—	—	—	—	—	3·4	—
96	—	7·6	—	—	—	3·7	—	—	—	—	—	—	—	—
101	—	—	—	—	—	—	—	—	—	—	—	—	3·9	—
105	—	—	—	—	—	4·5	—	—	—	—	—	—	—	—

* Identity of pool used as inoculum.

Fig. 1, the apparent growth rate of *f* strains was $10^{1.9}$ ELD50 per day and of *s* strains was $10^{1.1}$ ELD50 per day.

Measurement of the slopes of the exponential phases of single-cycle growth curves strongly suggested concomitant differences in actual growth rate. The more virulent strains MRC-4*f* and HAR-2*f* multiplied at a rate of $10^{3.4}$ ELD50 per day, compared with a rate of $10^{1.7}$ ELD50 per day for the less virulent strains MRC-1/OT, MRC-4 and PK-2.

The significance of other differences observed in the growth cycle of the two sorts of strain is not clear. The observed difference in the length of their lag phase may not be real; the less virulent strains require 10–100 times more elementary bodies to form an ELD50 than do *f* strains (Reeve & Taverne, 1963; Taverne *et al.* 1964) so that there may be a period during which elementary bodies of *s* strains are increasing in number but are not sufficiently numerous to be detected by titration in eggs. The techniques available for counting elementary bodies are not sensitive enough to settle this question.

This study has confirmed statistically that the greater virulence of some TRIC strains for the chick embryo is associated with a faster growth rate. The fundamental difference between the strains lies in their particle/infectivity ratios and its basis will not be resolved until a method is devised for accurately enumerating those *s* strain elementary bodies that cannot be detected in eggs.

SUMMARY

Chick embryos were inoculated with measured doses of various strains of the agents of trachoma and inclusion blennorrhoea, and the number of infective organisms they contained was determined at intervals during the first 4 days after inoculation. From curves of the yield of agent at different times relative to the dose inoculated, it was evident that the lag phase before the exponential phase of growth began was shorter for fast-killing—and more virulent—variant strains than for slow-killing strains, and the difference was statistically significant; and that variant strains multiplied faster during the exponential phase of growth and produced their highest titres sooner.

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