J. Pharm. Pharmac., 1968, **20**, *Suppl.*, 8S–13S Received May 1, 1968

Cell size changes during the growth of *Escherichia coli* partially inhibited by some antibacterial agents

R. M. RYE AND DAVID WISEMAN

The effects of tetracycline, phenol, chloramphenicol and ampicillin on the rates of division and mass increase in growing cultures of *Escherichia coli* have been examined by comparing the number, carbon content and the size of the cells from partially inhibited cultures with those of control cultures. In cultures treated with low concentrations of tetracycline, phenol or chloramphenicol the increase in cell mass is inhibited more than cell division, thus causing a decrease in the cell size. At higher concentrations of these agents and at all concentrations of ampicillin, cell division is inhibited more than increase in cell mass so causing an increase in cell size.

THE growth of bacteria in the presence of low concentrations of antibacterial agents can result in cell size changes. Increases in cell size resulting from cellular division being inhibited to a greater extent than cellular growth have frequently been observed (Dean & Hinshelwood, 1966). This paper describes the results of studies of the relative inhibition of growth and division in *Escherichia coli,* produced by different concentrations of phenol, tetracycline, chloramphenicol and ampicillin; all results were obtained during the early stages of growth when complications due to breakdown of the antibacterial agents or adaptation by the cells were unlikely to have occurred.

Experimental

Escherichia coli (NCTC 1013) was cultivated in a mineral salts medium containing 2mg/ml of uniformly labelled [^{14}C]glucose (specific activity 0·01μc/mg). The media, conditions of culture and methods for measuring radioactivity and absorbance have been described previously (Rye & Wiseman, 1966).

Total cell counts and size (volume) distributions were determined using a model B Coulter electronic particle counter fitted with a 30μ orifice tube. The electrolyte solution and methods for obtaining total cell counts and size distributions have already been described (Rye & Wiseman, 1967).

Antibacterial agents. Freshly prepared solutions of the following compounds in a glucose-free medium were used; tetracycline B.P., phenol B.P., chloramphenicol B.P. and ampicillin B.P. Preliminary experiments were made to determine the concentrations of these agents required to partially inhibit the growth of *E. coli.*

Inhibition of cultures. The absorbance of exponentially growing cultures of *E. coli* was measured at intervals and when this reached a value of 0·200, 10ml volumes were mixed with equal volumes of solutions of the antibacterial agents at 37°. Incubation of these partially inhibited cultures, together with control cultures diluted with glucose-free medium alone, was continued with shaking; growth was followed by means of absorbance measurements. When the absorbance of each culture reached

From the School of Pharmacy, University of Bradford, Bradford 7, England.

0·200, the time taken was noted, duplicate samples were diluted with electrolyte solution for determining total cell counts and size distributions; cells from duplicate 2ml samples were collected on membrane filters for radioactivity measurements.

Results

Fig. 1a shows the results of measurements made during the growth of *E. coli* in the presence of concentrations of tetracycline between 0 and 1·5μg/ml. The abscissa represents the growth rate of the tetracycline-treated cultures relative to that of the uninhibited control; the values were calculated for each culture by dividing the time required for the absorbance to increase from 0·100 to 0·200 in the control by the time required for the same increase to occur in the treated cultures. The ordinate shows the ratios of (a) the total cell count and (b) the total cellular carbon ^{14}C-content of each of the treated cultures to those of the control.

The total cellular carbon ^{14}C-content is approximately the same in all the treated cultures and the control suggesting that an absorbance of 0·200 represents the same total cell mass in all cases. In the treated

TABLE 1. THE PERCENTAGE OF CELLS OF *E. coli* FALLING WITHIN DIFFERENT SIZE RANGES DURING UNINHIBITED AND PARTIALLY INHIBITED GROWTH IN THE PRESENCE OF TETRACYCLINE, PHENOL, CHLORAMPHENICOL AND AMPICILLIN

	Size range (μ^3)			
Antibacterial agent (μg/ml)	0–0·53	0·53–1·06	1·06–2·12	> 2·12
Tetracycline				
0·00	1·1	54·4	37·7	6·7
0·075	4·1	63·6	29·0	3·3
0·15	6·8	64·7	25·6	2·9
0·25	5·4	65·0	26·9	2·7
0·375	2·1	62·3	32·2	3·5
0·50	0·9	58·1	37·1	4·0
1·00	0·1	33·7	58·0	8·3
1·50	0·3	27·0	62·7	10·1
Phenol				
0·00	1·1	57·0	36·1	5·8
125	2·3	57·7	34·4	5·6
250	4·3	59·9	31·0	4·8
375	4·7	60·4	30·3	4·6
500	4·0	58·2	32·1	5·6
625	3·6	57·8	32·7	5·9
750	2·7	58·3	32·9	6·1
1,000	2·3	52·4	37·9	7·4
1,250	0·6	32·6	52·5	14·4
Chloramphenicol				
0·00	4·3	68·2	25·9	1·7
0·25	5·1	69·1	24·2	1·5
0·50	5·2	69·3	24·0	1·5
0·75	4·2	68·2	26·2	1·5
1·00	2·3	66·0	29·9	1·8
1·50	0·0	61·1	36·6	2·3
2·00	0·0	50·3	46·6	3·5
3·00	0·2	35·4	57·7	6·7
Ampicillin				
0·00	10·1	67·3	21·1	1·5
0·125	8·4	66·3	23·8	1·6
0·25	8·1	67·7	22·7	1·5
0·50	6·4	63·3	28·1	2·2
1·00	0·5	51·9	42·9	4·6
1·50	0·6	42·0	50·7	6·8
2·00	0·0	43·1	49·6	7·3
4·00	0·6	40·6	51·1	7·7
10·00	0·6	38·5	52·6	8·3

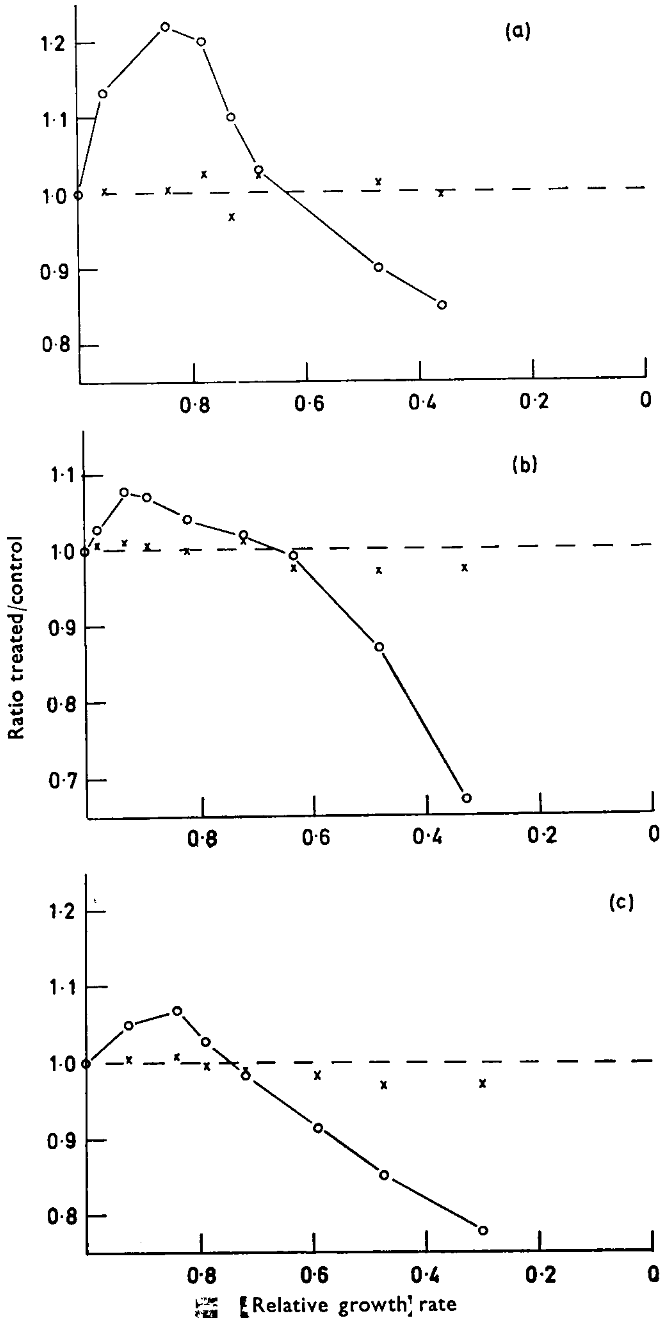

FIG. 1. The ratios of the total cellular ^{14}C-content and total cell counts of (a) tetracycline, (b) phenol and (c) chloramphenicol treated cultures to those of untreated controls. All measurements were made at an absorbance of 0·200. × Carbon content, ○ total cell counts.

cultures with relative growth rates between 0·65 and 1·0, however, the total cell count is greater than in the control thus indicating a decrease in the ^{14}C-content/cell. At slower growth rates (relative growth rate $<0{\cdot}65$) the total cell count in the treated suspensions is less than in the control and the ^{14}C-content/cell is consequently greater.

Table 1 shows the proportion of cells in different size ranges in these treated and untreated cultures and the actual concentrations of tetracycline used to partially inhibit growth. These results show that the variations in ^{14}C-content/cell are accompanied by corresponding changes in cell size. Thus in the presence of low concentrations of tetracycline the cells are smaller than in the control whilst in the higher concentrations they are larger.

Fig. 1b and c and Table 1 show that when cells are treated with phenol and chloramphenicol the results are qualitatively similar to those obtained with tetracycline but the decrease in cell size and ^{14}C-content/cell at the higher relative growth rates is not as marked.

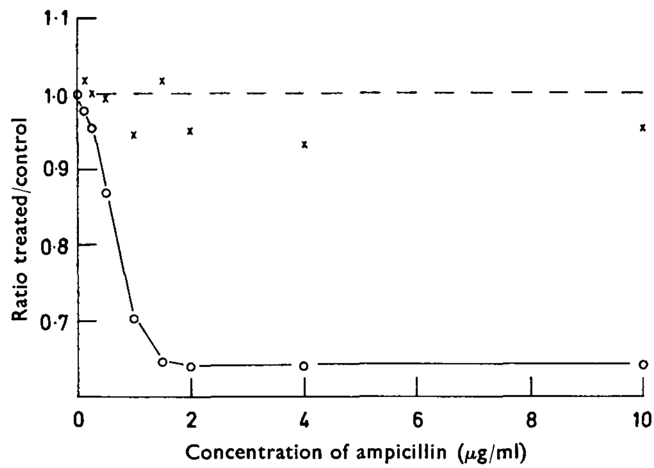

FIG. 2. The ratios of the total cellular ^{14}C-content and total cell counts of ampicillin treated cultures to those of an untreated control. All measurements were made at an absorbance of 0·200. × Carbon content, ○ total cell counts.

Fig. 2 and Table 1 show the results obtained when cells were treated with ampicillin. No change in the rate of increase in absorbance occurred with any of the ampicillin concentrations used and as the relative growth rate was consequently unaltered, the results are expressed as a function of ampicillin concentration. The total cell counts in the treated cultures were lower than in the control, decreasing rapidly with increasing concentrations of ampicillin, reaching a minimum at a concentration of 1·5μg/ml and then remaining constant. These results were reflected by changes in cell size and ^{14}C-content/cell, both increased with increasing concentrations of ampicillin reaching a maximum at 1·5μg/ml. At no concentration was any decrease in their values observed.

Microscopical examination of partially inhibited cultures revealed no evidence of cell clumping with any of the antibacterial agents used.

Discussion

During the normal exponential growth of bacteria the rates of cellular division and of increase in cell mass are approximately equal and the mean cell size consequently remains virtually constant. Although it has been shown that the average size of cells is dependent on their rate of growth (Schaechter, Maaloe & Kjeldgaard, 1958) and that changes in the environment can lead to changes in cell size (Kjeldgaard, Maaloe & Schaechter, 1958), the mechanisms by which the balance between division and increase in mass is maintained are not yet understood. Several antibacterial agents have been shown to produce morphological changes in growing cultures (Duguid & Wilkinson, 1961; Dean & Hinshelwood, 1966) but studies of their relative effects upon division and growth have been almost entirely restricted to observations of swelling or of filament formation as evidence of preferential inhibition of division. Few, if any, reports of a decrease in cell size have previously been made.

The magnitude of any size changes which occur during imbalanced growth will depend not only upon the relative rates of division and mass increase, but also upon the total increase in cell mass which occurs. Thus in any quantitative investigation of size changes in partially inhibited cultures, a constant increase in total cell mass should be allowed after the agent has been added. In this paper a doubling in absorbance from 0·100 to 0·200 has been used as a measure of this constant amount of growth. The validity of absorbance measurements for determining total cell mass has been shown from theoretical considerations by Koch (1961) and is apparently confirmed by our observations that both partially inhibited and uninhibited cultures of the same absorbance contain the same amounts of cellular carbon. The results presented in Figs 1–3 are expressed using relative growth rate as the abscissa. This scale enables all rates of growth from that characteristic of a normal uninhibited culture, to no growth at all, to be shown: it also allows results from antibacterial agents acting at widely different concentrations to be directly compared.

All four antibacterial agents examined upset the balance between division and mass increase when added to cultures of *E. coli*. At low concentrations of tetracycline, and to a lesser extent phenol and chloramphenicol, the observed decrease in cell size indicates that the increase in mass is being inhibited more than cellular division. At higher concentrations the opposite effects occur. The remarkable similarity in the inhibitory patterns produced by these different agents suggests that some common factor must exist in the mechanism by which they exert their effects upon division and growth. Possibly at low concentrations, when there is little penetration of these agents into the cells, the decrease in cell size results from a slowing of growth due to some inhibition of enzymes at the cell surface, or other accessible sites, with no corresponding inhibition at the internal sites of crosswall formation. At the higher concentrations when more penetration will have occurred, the increase in size may be due either to a particularly high sensitivity of crosswall synthesis to inhibition or may simply result from the fact that to maintain a balanced supply of nutrients at a decreased rate of growth, a lower surface area to volume ratio can be tolerated. Ampicillin has virtually no effect upon the rate of increase in mass even at concentrations six times as high as those required for maximal effect upon cell division. With this agent, which has a highly specific action upon cell wall synthesis and particularly upon crosswall formation, no decrease in cell size was expected or observed even in the lowest concentrations used.

If the imbalance between division and mass increase, which can occur in partially inhibited cultures, continued indefinitely, the changes in cell size would become increasingly marked with the passage of time. In the case of cells which are becoming larger, such a continual change in size would be possible although perhaps unlikely as there is no theoretical limit to the maximum size of an individual cell. In conditions where the cell size is decreasing, however, a limit must be imposed upon the extent of change which can occur as a minimum possible cell size must exist! When this stage is reached a new balance between division and mass increase may be attained maintaining the cell size at this minimum value. In any studies using antibacterial agents which can cause size changes such as those described above, great caution should be exercised in converting results obtained in terms of cell number into terms of cell mass as any conclusions drawn after such a procedure may well be invalid.

Acknowledgement. We are grateful to Mrs. Sheena Kaye for technical assistance.

References

Dean, A. C. R. & Hinshelwood, C. (1966). *Growth, Function and Regulation in Bacterial cells.* Oxford: Clarendon Press.

Duguid, J. P. & Wilkinson, J. F. (1961). Environmentally induced changes in bacterial morphology. In *Microbial Reaction to Environment.* Symp. Soc. gen. Microbiol., 11, 69–99.

Koch, A. L. (1961). *Biochim. biophys. Acta,* **51**, 429–441.

Kjeldgaard, N. O., Maaloe, O. & Schaechter, M. (1958). *J. gen. Microbiol.,* **19**, 607–616.

Rye, R. M. & Wiseman, D. (1966). *J. Pharm. Pharmac.,* **18**, *Suppl.,* 114S–118S.

Rye, R. M. & Wiseman, D. (1967). *Ibid.,* **19**, *Suppl.,* 4S–8S.

Schaechter, M. Maaloe, O. & Kjeldgaard, N. O. (1958). *J. gen. Microbiol.,* **19**, 592–606.