Analysis of the resistance mediated by several R-factors to Tetracycline and Minocycline

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SUMMARY

The resistance levels conferred by the T-determinants in four R-factors to Tetracycline and Minocycline in cells of *Escherichia coli* K 12, before and after induction of maximum resistance by treatment with sub-inhibitory concentrations of the drugs, are measured by simple growth-and-challenge tests. The effect of a plasmid T_K which confers tetracycline resistance on its host *Klebsiella aerogenes* is tested in the same way. The five T-determinants fall into a high-level and a low-level group for resistance, the former giving 3- to 4-fold higher resistance in both induced and uninduced cells than the latter. The T-determinants all confer much lower resistance to Minocycline (a tetracycline molecule modified at the C-6 and C-7 positions) than to Tetracycline. The main cause of this difference is that cells carrying a T-determinant exclude Minocycline much less efficiently than Tetracycline, but in addition Minocycline is less effective than Tetracycline in inducing increased resistance. These results are discussed in the light of a model put forward to explain the inducible nature of R-factor resistance to the tetracyclines.

INTRODUCTION

Resistance to the tetracyclines in coliform bacteria, mediated by R-factors carrying a T-determinant,† appears to be the result of a specific decrease in the permeability of the bacterial cell membrane to this group of antibiotics. Sensitive cells accumulate tetracycline actively from the medium by a permease-like mechanism which is energy-dependent and leads to an internal concentration greatly in excess of that in the medium. Resistant cells carrying a T-determinant show a low level of resistance which is sharply increased by a few minutes of growth in a sub-inhibitory concentration of tetracycline. This induction of increased resistance requires protein synthesis. The evidence for these statements is given by Franklin & Godfrey (1965) and Franklin (1967), who also summarize earlier work.

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† Abbreviations and notes: we shall use Tc or Tetracycline (capital T) for this antibiotic, and tetracycline(s) with lower case initial for the group of related drugs. These include Minocycline (Mc), Chlortetracycline and Oxytetracycline. T-determinant is used here for the genes causing resistance to tetracyclines in an R-factor or other plasmid.

The studies referred to have characterized the behaviour of resistant strains by measuring their ability to accumulate tetracycline or to synthesize proteins under different conditions of pretreatment and challenge with the drug. In this paper we show that simple growth tests provide a good measure of the resistance levels of uninduced and induced R-factor-carrying cells. This method is used to analyse the resistance characteristics of five different T-determinants to the two antibiotics Tetracycline (Tc) and Minocycline (Mc).

Minocycline is a semi-synthetic variant of the tetracycline molecule (7-dimethyl-amino-6-demethyl-6-deoxytetracycline) which has been found to have much greater potency than other tetracyclines against R-factor-carrying bacteria (Jarolmen, Hewel & Kain, 1970). Mc has also shown increased potency against other bacteria including tetracycline-resistant strains of *Staphylococcus aureus* (Redin, 1966; Blackwood & English, 1970).

MATERIALS AND METHODS

Bacterial strains. The host strain for tests on R-factors was *Escherichia coli* K 12 RE 26 (strain 711 from R. Clowes) $F^- ProA^- Trp^- His^- Tsx^r LacY^- Str^s$. Tests were also performed on the plasmid T_K, causing resistance to tetracycline (Reeve, 1970) in its host strain *Klebsiella aerogenes* V9A (Reeve & Braithwaite, 1970), using a sub-line of V9A which had lost the plasmid as control.

R-factors. The four R-factors tested are listed in Table 1, which gives their characteristics and the numbers assigned to them by Naomi Datta, who supplied them. They all originated from strains of *Salmonella typhimurium*. The presence of a particular plasmid in a bacterial strain will be indicated by parentheses, e.g. RE 26 (R 57).

Table 1. *Source of T-determinants*

R factor or plasmid	Markers
R 46	AST Su fi^-
R 57	ST Su fi^-
R 82	ST Su fi^+
R 199	T Su fi^-
T_K	T

A, S, T, and Su indicate determinants giving resistance to ampicillin, streptomycin, tetracyclines and sulphonamides, respectively. fi^+: ability to inhibit fertility of F-factors. Plasmid T_K carries no sex factor.

Media. Broth was L-Broth, containing 10 g Difco Bacto Tryptone, 5 g Difco Yeast Extract, 5 g NaCl and 1 g glucose per litre distilled water; Nutrient Agar contained 8 g Difco Nutrient Broth granules, 5 g NaCl and 15 g Difco Bacto Agar per litre H_2O; MacConkey Agar was Oxoid MacConkey Agar No. 3.

Antibiotics. Tetracycline HCl (trade name Achromycin) and Minocycline, in powder form, were gifts from Cyanamid of Great Britain and Lederle Laboratories.

MIPC. The minimal inhibitory plate concentration of antibiotic was measured as described by Reeve (1968). A fully grown broth culture of each strain was diluted 10^{-2} and loopfuls were streaked on MacConkey agar plates containing successive concentrations of antibiotic. The MIPC was taken as the concentration of drug permitting thin confluent growth after overnight incubation at 37 °C.

Other methods. These are described in figure legends or text.

RESULTS

Table 2 gives the MIPC of the four R-factors, in RE 26, and T_K in its host *Klebsiella*, together with those of the uninfected host strains, to both Tc and Mc. These estimates are necessarily approximate, but it is clear that, while sensitive bacteria are at least as resistant to Mc as to Tc, a T-determinant confers much higher resistance to Tc than to Mc in all cases, roughly 10 times as much Tc as Mc being required to give the same degree of inhibition. T_K gives the same high level of resistance in *Klebsiella* as R 57 and R 82 give in *E. coli*, while R 46 and R 199 only produce a much lower level of resistance.

Table 2. *Approximate MIPC's of strains tested against Tc and Mc*

Bacterial strain	R-factor or T-plasmid	MIPC (μg/ml)	
		Tc	Mc
RE 26	—	1	2
	R 57	120	10
	R 82	160	10
	R 46	40	2
	R 199	40	2
V9A	—	1	2
V9A	T_K	160	10

Simple growth tests were used to measure the levels of resistance conferred by a T-determinant in uninduced and induced cells, as illustrated in Fig. 1. This shows V 9 A carrying T_K and growing in broth at 37 °C. Four samples were started from an overnight culture at time − 30 min, and two of them received a small inducing dose of the antibiotic at time 0; 15 min later one induced and one uninduced culture received a challenge dose of the drug, and growth was measured at intervals from time 0 by optical density (OD) readings at 550 mμ. The inducing and challenge doses were in this case 2 and 40 μg/ml of Tc. The inducing dose alone had no detectable effect on growth rate, which continued logarithmically, while a challenge dose added to uninduced cells caused a rapid decline in growth rate, which then continued for some time at about 20 % of normal. Cells induced and then challenged grew nearly as fast as the controls (at 82 % of the rate of cells receiving the inducing dose alone). This graph brings out clearly the marked effect of induction on the level of resistance expressed.

One question which arises is whether any of the increase in optical density of treated cells could be the result of absorption of Tc and not cell growth, since
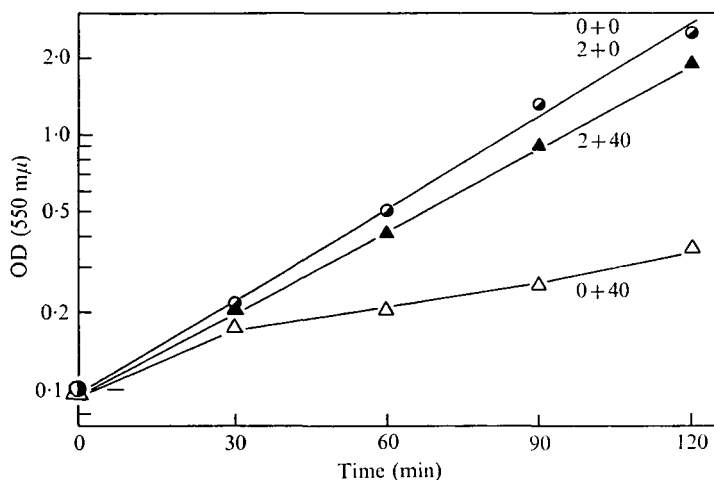
Fig. 1. Effect of induction and challenge with Tc on V9A(T_K) growing in broth. Black symbols: cultures induced with 2 μg/ml Tc at time 0. White symbols: un-induced cultures. Triangles: cultures challenged with 40 μg/ml Tc at time +15 min. Circles: unchallenged cultures.

Izaki & Arima (1963) found that *E. coli* incubated in a high concentration of Oxytetracycline rapidly increased in optical density due to absorption, although the effect was less marked with resistant than with sensitive cells. To test this possibility, K 12 carrying R 57 was induced with 2 μg/ml Tc and challenged with 80 μg/ml Tc, and both OD and viable count were measured at intervals (Fig. 2). It is clear that a rise in viable count always accompanied a rise in optical density, and there is no sign of any absorption effect on the latter index. The two indices of growth do not show identical trends, but the differences are just what would be expected from the fact that cell size in broth-grown cultures increases in early log phase and decreases again as the bacteria approach the end of log phase growth. Cells challenged with 80 μg/ml Tc without previous induction maintained an almost constant cell mass (OD) but declined slowly in viable count (by 70 % during the 105 min following challenge), suggesting that Tc caused a gradual loss of colony-forming ability without cell lysis.

We can thus assume that the OD trends in Figs. 1 and 2 are the result of growth, and the rates of OD increase after different treatments may be used to separate total resistance into a basal and an inducible fraction. Since gradual induction of high-level resistance may occur when a rather small challenge dose is given to uninduced cells, growth rate was measured during the short period from 15 to 75 min after challenge. The OD was read at 30, 60 and 90 min after induction, and the linear regression coefficient of log OD against time was used to calculate the growth rate in units of number of doublings in cell mass per hour.

Before looking at the resistance profiles of the five T-determinants to the two antibiotics, it is necessary to examine the relation between inducing dose and level of induction. Fig. 3 summarizes the results of a number of tests of the type
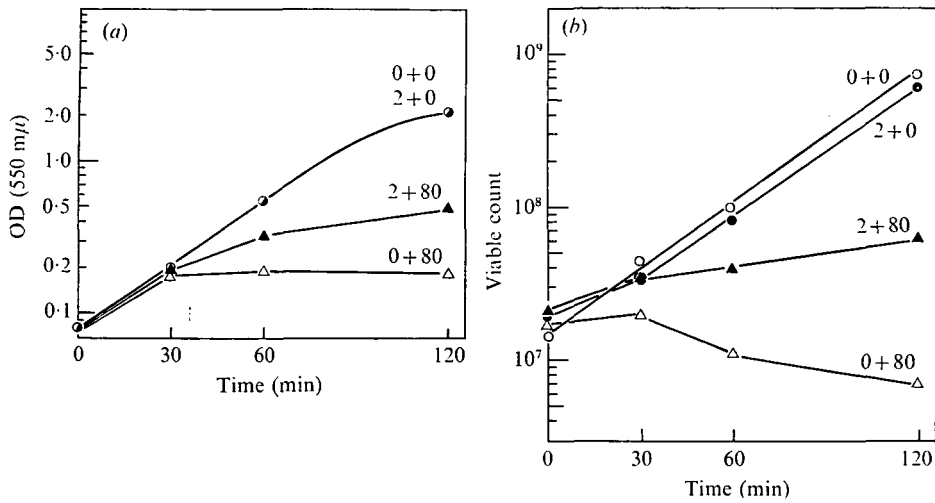
Fig. 2. Effect of induction and challenge with Tc on RE 26 (R 57) growing in broth. Challenge dose 80 μg/ml, otherwise symbols and methods as Fig. 1. (a) Optical density, (b) viable count.
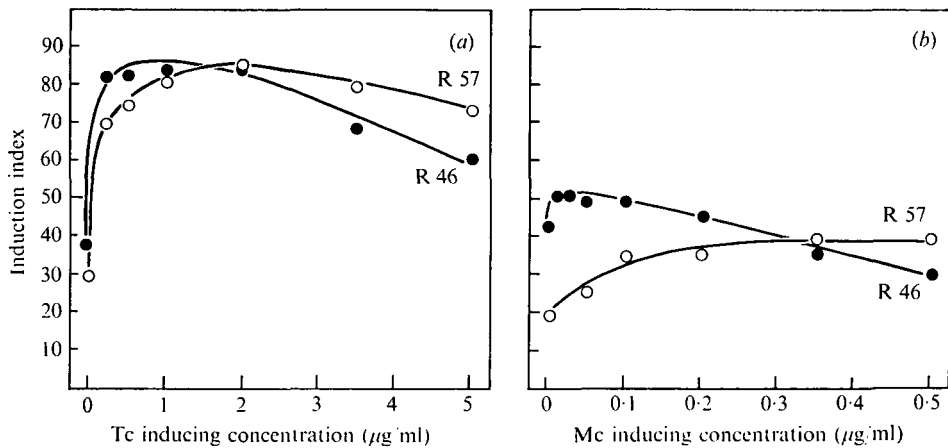

Fig. 3. Determination of optimal inducing concentrations of (a) Tc and (b) Mc. Induction index (vertical scale) is growth rate of cells induced and then challenged, calculated as % of growth rate of uninduced unchallenged cells. Horizontal scale is inducing concentration. White circles: tests on RE 26 (R 57) challenged with (a) 40 μg/ml Tc or (b) 5 μg/ml Mc. Black circles: tests on RE 26 (R 46) challenged with (a) 20 μg/ml Tc or (b) 0.5 μg/ml Mc. (a) Induction and challenge with Tc. (b) Induction and challenge with Mc.

illustrated in Fig. 1, and shows an index of induction efficiency plotted against the inducing dose. Suitable challenge doses were chosen to allow for the different resistance levels given by the two R-factors R 57 and R 46. The induction index is the percentage which the growth rate of challenged induced cells forms of that of unchallenged uninduced cells.
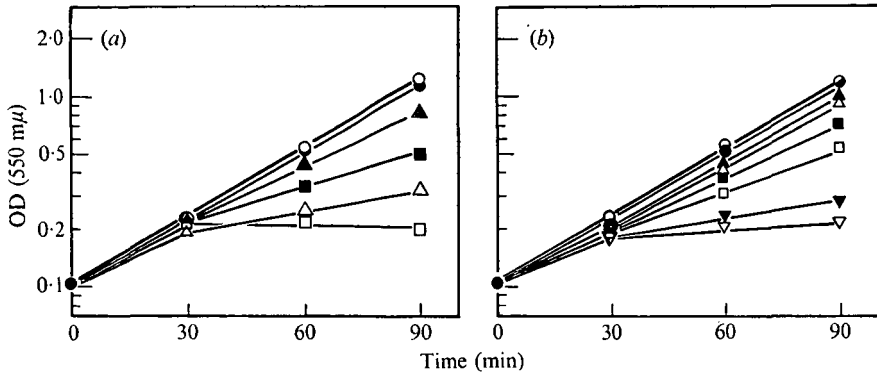
Fig. 4. Induction and challenge tests on RE 26 (R 57) with (a) Tc and (b) Mc. Methods as Fig. 1. Black symbols: cells induced with (a) $2 \mu g/ml$ Tc or (b) $0.1 \mu g/ml$ Mc. White symbols: uninduced cells. Circles: no challenge. Triangles: challenge with (a) $40 \mu g/ml$ Tc or (b) $1 \mu g/ml$ Mc. Squares: challenge with (a) $80 \mu g/ml$ Tc or (b) $2 \mu g/ml$ Mc. Inverted triangles: challenge with (b) $5 \mu g/ml$ Mc.
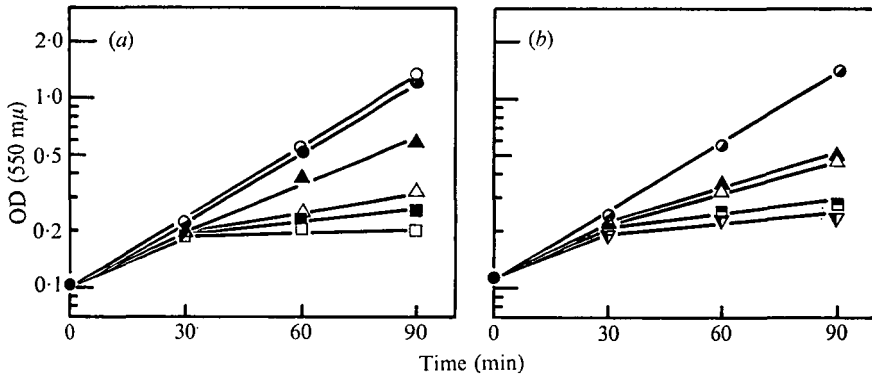

Fig. 5. Induction and challenge tests on RE 26 (R 46) with (a) Tc and (b) Mc. Methods as Fig. 1. Black symbols: cells induced with (a) $1 \mu g/ml$ Tc or (b) $0.025 \mu g/ml$ Mc. White symbols: uninduced cells. Circles: no challenge. Triangles: challenge with (a) $20 \mu g/ml$ Tc or (b) $1 \mu g/ml$ Mc. Squares: challenge with (a) $40 \mu g/ml$ Tc or (b) $2 \mu g/ml$ Mc. Inverted triangles: challenge with (b) $5 \mu g/ml$ Mc.

The curves are all rather flat-topped, showing a considerable range of effective inducing dose. Thus maximal induction of R 57 was obtained with $2 \mu g/ml$ Tc, but the range $1-4 \mu g/ml$ was almost equally effective, while $0.2-2 \mu g/ml$ Tc gave about equal induction efficiency with R 46. Very little induction by Mc was obtained, but the highest levels were within the ranges $0.1-0.5 \mu g/ml$ for R 57 and $0.01-0.1 \mu g/ml$ for R 46. Since the precise choice of inducing dose is not critical, we have chosen as standard for further tests: 2 and $1 \mu g/ml$ Tc and 0.1 and $0.025 \mu g/ml$ Mc, respectively, for the R-factors giving high and low levels of resistance as shown in Table 2.

Standard induction tests with Tc and Mc are given for R 57 in Fig. 4 and for R 46 in Fig. 5. Black and white circles show induced and uninduced cells growing
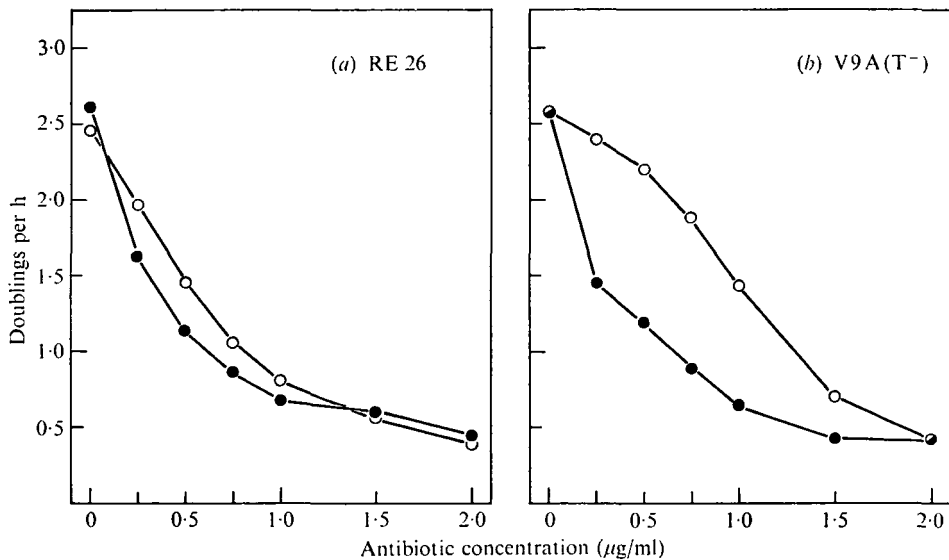
Fig. 6. Growth rate (doublings in cell mass per hr) of (*a*) RE 26 and (*b*) V9A(T⁻) in broth containing different concentrations of the antibiotic. ●, Growing in Tc; ○, growing in Mc.

without challenge, and it is clear that growth is scarcely if at all depressed by the inducing dose. Black and white triangles or squares show the effects of particular challenge doses on induced and uninduced cells. In each case the growth rate was approximately constant over the period 30–90 min.

From a number of tests of this kind we are able to plot a 'resistance profile' for induced and uninduced cells carrying a particular R-factor, showing the growth following challenge with different concentrations of antibiotic. However, the picture will not be complete without the profile of cells carrying no R-factor, since this will enable us to determine whether an R-factor confers any resistance on uninduced cells. No induction effect is found with sensitive cells (Franklin, 1967), and the growth rate was measured over the period 30–90 min, as in the induction tests, when the challenge dose was added at 15 min. The resistance profiles of RE 26 and V9A(T⁻) to Tc and Mc are given in Fig. 6. This shows that both strains are about equally sensitive to Tc and a little more resistant to Mc, the *Klebsiella* strain being clearly more resistant then *E. coli* to Mc.

We can now complete the resistance profiles of the R-factor carrying strains, and these are given in Figs. 7–9 for R 57, R 82 and T_K, and in Figs. 10 and 11 for the two factors with lower resistance levels, R 46 and R 199. Study of these profiles brings out the following points.

In the case of the three high-level determinants (Figs. 7–9) it is clear that:

(1) Uninduced cells carrying the determinant are much more resistant to both antibiotics than cells carrying no determinant – hence these determinants give a high basal level of activity in the repressed state.
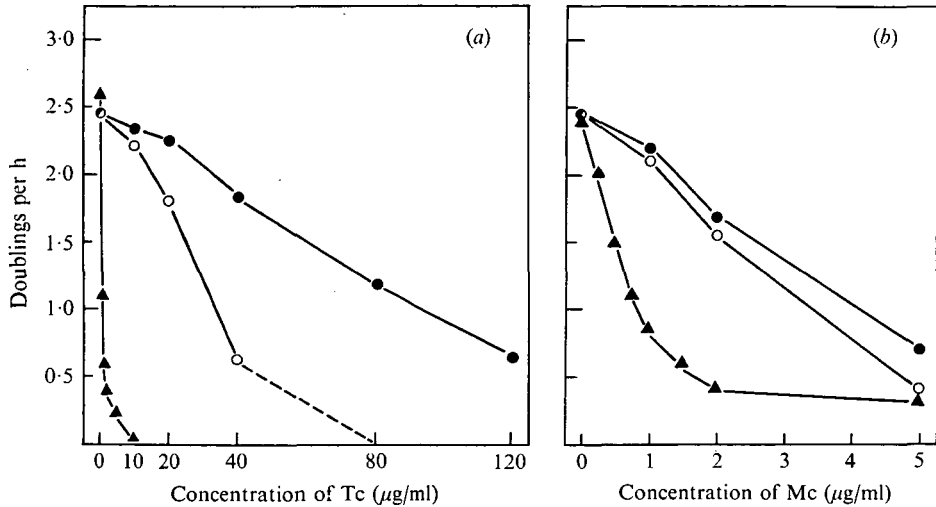
Fig. 7. Resistance profiles of RE 26 (R 57) for (a) Tc and (b) Mc. Vertical scale: no. of doublings in cell mass per h. ●, Induced R$^+$ cells; ○, uninduced R$^+$ cells; ▲, R$^-$ cells.
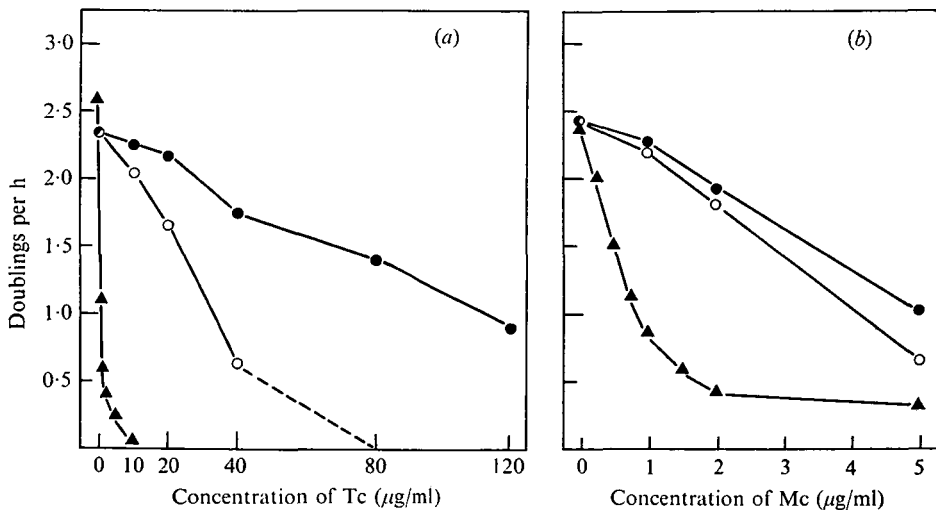

Fig. 8. Resistance profiles of RE 26 (R 82) for (a) Tc and (b) Mc. Symbols as Fig. 7.

(2) Previous induction gives a very striking increase in resistance to Tc and a rather small increase in resistance to Mc, indicating that either Mc is a poor inducer or induction has very little effect on the ability of Mc to penetrate the cell.

(3) Although sensitive cells are a little more resistant to Mc than to Tc, cells carrying an R-factor are very much more resistant to Tc than to Mc in the un-induced as well as in the induced state (note the different horizontal scales for Tc and Mc). Therefore, the differences in resistance to the two antibiotics cannot be mainly the result of their different induction abilities.
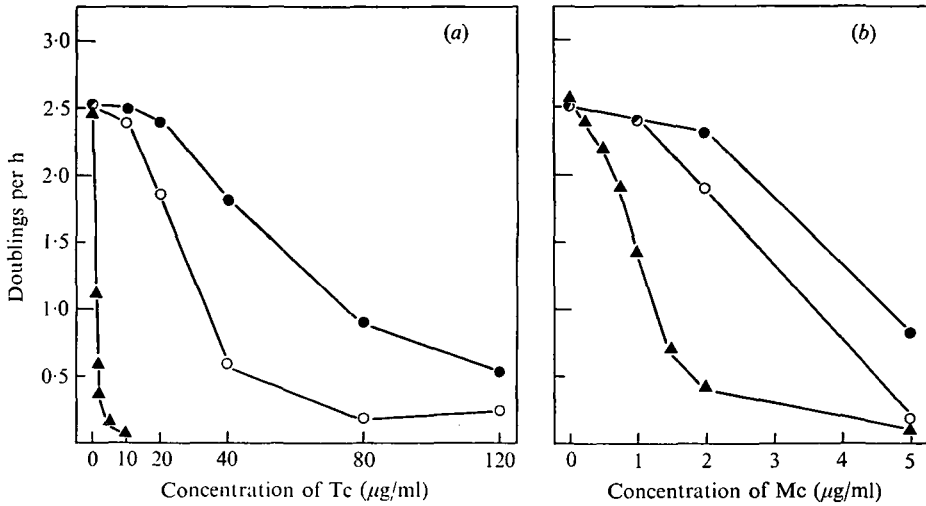
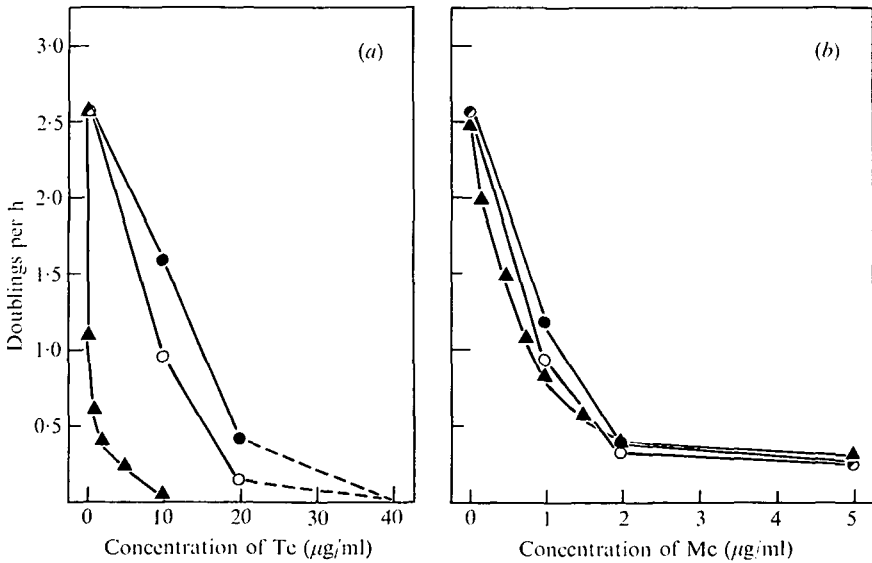Fig. 9. Resistance profiles of V9A (T_k) to (a) Tc and (b) Mc. Symbols as Fig. 7.

Fig. 10. Resistance profiles of RE 26 (R 46) to (a) Tc and (b) Mc.
Symbols as Fig. 7.

(4) R 57 and R 82 have almost identical profiles in *E. coli*, which are closely similar to that of T_K in its *Klebsiella* host.

The two factors R 46 and R 199 (Figs. 10, 11) give much lower levels of resistance than the other group, but still cause a marked increase in the resistance of un-induced cells to Tc, compared to sensitive bacteria, while induction by Tc causes an appreciable further increase. Tested against Mc, however, there is a barely detectable effect of the R-factor in either uninduced or induced cells.
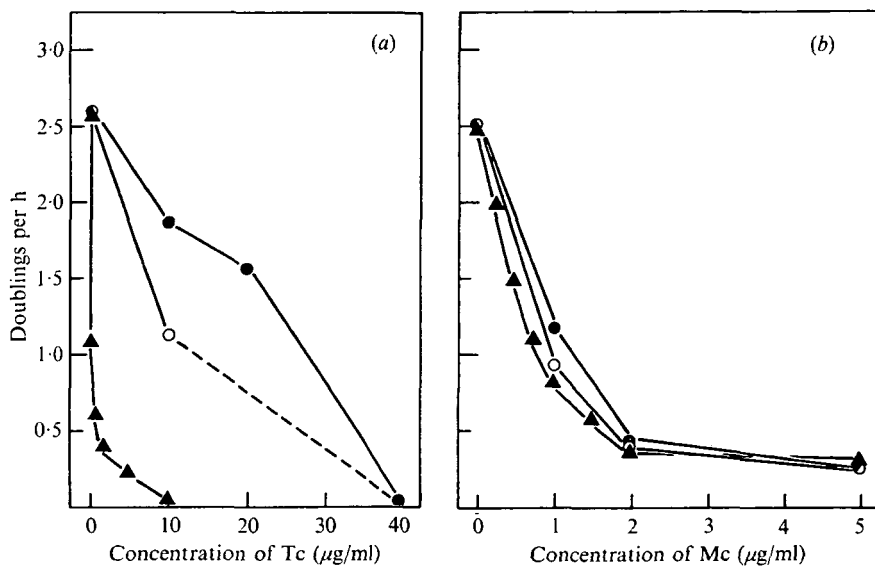
Fig. 11. Resistance profiles of RE 26 (R 199) to (a) Tc and (b) Mc.
Symbols as Fig. 7.
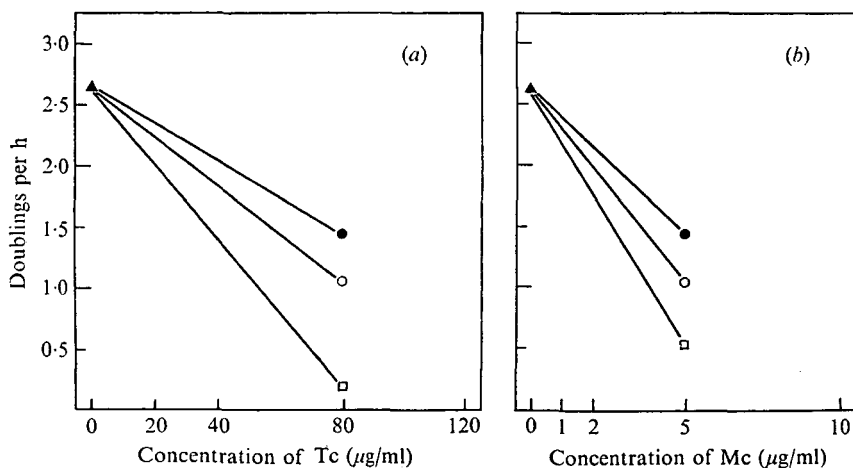
Fig. 12. Effects of induction by Tc and Mc on growth rate of RE 26 (R 57) in
(a) 80 μg/ml Tc and (b) 5 μg/ml Mc. ●, Cells induced by 2 μg/ml Tc; ○, cells in-
duced by 0·1 μg/ml Mc; □, uninduced cells.

Figs. 7–9 suggest that Mc is a poor inducer of increased resistance above the
basal level, and the induction abilities of Mc and Tc on cells carrying R 57 are
compared in Fig. 12. For this test cells are induced with the optimum inducer
dose of each antibiotic and then challenged with 80 μg/ml of Tc or 5 μg/ml of Mc
and the effects on growth rate calculated as before. Tc-induced cells grow some
40 % faster than Mc-induced cells after challenge with either antibiotic, indicating

Table 3. *Analysis of R-factor resistance to Tc and Mc*

(a) Antibiotic concentrations giving 50% reduction in growth rate

Strain	Plasmid	Tc (μg/ml)		Mc (μg/ml)	
		U	I	U	I
V9A	—	0·40	—	1·10	—
	T_K	29·5	66	3·1	4·2
RE 26	—	0·43	—	0·60	—
	R 57	29·0	75	2·9	3·6
	R 82	29·0	99	3·5	4·2
	R 46	7·5	12·5	0·7	1·0
	R 199	9·0	24	0·8	1·0

(b) Resistance ratios

Plasmid	Tc		Mc	
	U/S*	I/U	U/S	I/U
T_K	74	2·2	2·8	1·4
R 57	67	2·6	4·8	1·2
R 82	67	3·4	5·8	1·2
R 46	17	1·7	1·2	1·4
R 199	21	2·7	1·3	1·2

* S is antibiotic concentration giving 50% reduction in growth rate of bacteria not carrying a plasmid.

that Tc is much better than Mc as an inducer. However, induction with Tc still leaves cells carrying the T-determinant about 16 times as sensitive to Mc as to Tc, and it thus appears that Mc is more effective than Tc against R-factor carrying cells for two reasons: (1) it is unable to induce maximum resistance and (2) it is much less effectively excluded from the cell in which maximum resistance has been induced.

A numerical measure of the resistance levels of sensitive, uninduced and induced cells carrying the various T-determinants, based on Figs. 6–11, is presented in Table 3(a), resistance being measured as the concentration of antibiotic needed to reduce growth rate by 50%. Table 3(b) gives the relative increase in resistance of uninduced R+ cells compared with the sensitive strain (ratio U/S) and the additional effect of induction by the same drug (ratio I/U). Of particular interest is the fact that the induction ratios (I/U) are about the same for all determinants tested with a particular antibiotic, while the main difference between the five determinants occurs in the ratio U/S, which is 3–4 times as great for T_K, R 57 and R 82 as for the two low-level determinants R 46 and R 199. This relationship holds true for both antibiotics. In other words, the main difference between the high- and low-level determinants is that the former give a 3- to 4-fold greater resistance than the latter in both uninduced and induced cells, in the case of both antibiotics.

DISCUSSION

The inducible nature of R-factor mediated resistance to the tetracyclines is most simply explained by the following hypothesis, based on that put forward by Franklin (1967) and elaborated by Franklin & Cook (1971). In sensitive cells a constitutive permease system of unknown function accumulates tetracyclines actively from the external medium. The product of the R-factor resistance gene modifies the sites of this permease in the cell membrane so as to reduce substantially their ability to take up the drug – presumably by reducing the affinity of the permease membrane protein for the antibiotic molecule. However, a second R-factor gene represses the transcription of the resistance gene unless the repressor product is inactivated by combination with tetracycline, so that induction by a sub-inhibitory concentration of the antibiotic is necessary for full expression of resistance. Challenge of uninduced cells with a high concentration of drug would inhibit protein synthesis and so prevent manufacture of the protein responsible for full resistance. By analogy with induction of the lactose operon, rapid induction of maximum resistance by tetracycline would be expected with this system, as was indeed found by Franklin (1967).

An interesting corollary of this hypothesis is that it should be possible to select single-step chromosomal mutations which give high-level resistance in the absence of an R-factor, by either inactivating the permease or modifying it in the same way as the R-factor gene product is assumed to do. Neither class of mutation has been found (Reeve, 1968, and unpublished observations), suggesting that the permease may be an essential structural component of the cell membrane, whose mutational loss or modification would be lethal.

It should also be possible to select R-factor mutants which give fully expressed resistance constitutively, either because the repressor gene has been inactivated or because the resistance gene has been modified and is no longer repressible. One such mutant has been described by Franklin & Cook (1971), and further study of this class of mutants should make it possible to test the validity of the hypothesis put forward above. One problem in tests of this kind is that chromosomal mutations are easily selected which give a small increase in tetracycline resistance in sensitive cells but cause a large increase in the resistance conferred by an R-factor (Reeve, 1966). Such mutations are likely to mimic the effects of R-factor constitutive mutations and will make their analysis difficult. Most of the results given for their mutant strain by Franklin & Cook (1971) could in fact be explained as due to a chromosomal mutation.

The permease model outlined above appears to be quite consistent with published results and with the observations reported in this paper, in particular the striking differences in resistance level found for the two groups of R-factors and the different reactions shown by both groups to the two antibiotics Tc and Mc. Uninduced R$^+$ cells have a substantial basal level of activity of the T-determinant, indicating that repression of the resistance gene in the absence of induction is far from complete. The high-level factors (T$_K$, R 57 and R 82) give a 3- to 4-fold

greater resistance than the low-level factors in both induced and uninduced cells. This is most easily explained if the gene product of the high-level factors has a higher affinity for the permease sites in the cell membrane – or otherwise modifies these sites more effectively in terms of reducing tetracycline permeability. An alternative hypothesis is that the two groups of factor differ only in the number of R-factor copies maintained per cell, but it seems unlikely that this would lead to proportional differences in the resistance of induced and uninduced cells.

The second major difference found was that between the activities of Mc and Tc. Mc is both less efficient than Tc as an inducer of increased resistance and (by inference from the relative resistance levels of R^+ and R^- cells to the two antibiotics) is also much less effectively excluded from both the induced and the uninduced cell. These facts can be explained on the permease hypothesis if Mc has less affinity than Tc for the repressor substance (hence it is a poor inducer), and if the induced change in the permease responsible for high-level resistance has much less effect on the affinity of Mc than of Tc for the permease, so that Mc is taken up more rapidly than Tc in resistant cells. This second difference appears to be responsible for most of the increased effectiveness of Mc compared with Tc.

Using both *in vivo* tests on mice and *in vitro* tests, Jarolmen *et al.* (1970) found Mc to be much more effective than Tc or Chlortetracycline against R-factor carrying strains of *Salmonella choleraesuis* and *Salmonella typhimurium*. Minocycline has also been reported to be more effective than Tc against a number of other bacteria, both *in vitro* and *in vivo* (Redin, 1966; Blackwood & English, 1970). These bacteria include tetracycline-resistant strains of *Staphylococcus aureus*, which appear to have a similar mechanism of resistance to that of R-factors, since increased resistance is induced by growth in a sub-inhibitory concentration of the antibiotic (Sompolinsky *et al.* 1970*a*, *b*). However, Mc does not appear to be more effective than other tetracyclines against sensitive *E. coli* and *Klebsiella*, as we have found, and it is of particular interest that *E. coli* and *S. choleraesuis* selected for multi-step chromosomal resistance to Chlortetracycline were actually a little more resistant to Mc than to Tc (Jarolmen *et al.* 1970). These observations may have a bearing on future prospects in this field. R-factor resistance developed during several years of use of Tc and the closely related substances Chlortetracycline (7-chloro-Tc) and Oxytetracycline (5-hydroxy-Tc), all of which appear to be very similar in their resistance spectra (Blackwood & English, 1970). Mc is just coming into use, and the possibility remains that large-scale use of this drug for a few years may lead to the evolution of R-factors and other resistance determinants which make their host cells more resistant to Mc than to the older tetracycline antibiotics.

REFERENCES

Blackwood, R. K. & English, A. R. (1970). Structure – activity relationships in the tetracycline series. *Advances in Applied Microbiology* **13**, 237–266.

Franklin, T. J. (1967). Resistance of *Escherichia coli* to tetracyclines. Changes in permeability to tetracyclines in *Escherichia coli* bearing transferable resistance factors. *Biochemical Journal* **105**, 371–378.

Franklin, T. J. & Godfrey, A. (1965). Resistance of *Eschericha coli* to Tetracyclines. *Biochemical Journal* **94**, 54–60.

Franklin, T. J. & Cook, J. M. (1971). R-factor with a mutation in the tetracycline resistance marker. *Nature* **229**, 273–274.

Izaki, K. & Arima, K. (1963). Disappearance of Oxytetracycline accumulation in the cells of multiple drug-resistant *Escherichia coli*. *Nature* **200**, 384–385.

Jarolmen, H., Hewel, D. & Kain, E. (1970). Activity of Minocycline against R-factor-carrying Enterobacteriaceae. *Infection and Immunity* **1**, 321–326.

Redin, G. S. (1966). Antibacterial activity in mice of Minocycline, a new Tetracycline. *Antimicrobial Agents and Chemotherapy* – 1966 (ed. G. L. Hobby), 371–376. American Society of Microbiology.

Reeve, E. C. R. (1966). Characteristics of some single step mutants to chloramphenicol resistance in *Escherichia coli* K 12 and their interactions with R-factor genes. *Genetical Research* **7**, 281–286.

Reeve, E. C. R. (1968). Genetic analysis of some mutations causing resistance to tetracycline in *Escherichia coli* K 12. *Genetical Research* **11**, 303–309.

Reeve, E. C. R. (1970). Transfer characteristics of two resistance determinants in a wild strain of *Klebsiella aerogenes* (V9A). *Genetical Research* **16**, 235–240.

Reeve, E. C. R. & Braithwaite, J. A. (1970). F_K-*lac*, an episome with unusual properties found in a wild strain of a *Klebsiella* species. *Nature* **228**, 162–164.

Sompolinsky, D., Krawitz, T., Zaidenzaig, Y. & Abramova, N. (1970a). Inducible resistance to tetracycline in *Staphylococcus aureus*. *Journal of General Microbiology* **62**, 341–349.

Sompolinsky, D., Zaidenzaig, T., Ziegler-Schlomowitz, R. & Abramova, N. (1970b). Mechanism of tetracycline resistance in *Staphylococcus aureus*. *Journal of General Microbiology* **62**, 351–362.