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Effects of chloramphenicol on *Chlamydia trachomatis* infection in neonatal conjunctivitis and in McCoy cell cultures

By D. HOBSON, D. STEFANIDIS

Department of Medical Microbiology, The University of Liverpool, Liverpool L69 3BX

E. REES and I. A. TAIT

Department of Venereology, Royal Liverpool Hospital, Liverpool L7 8XW

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SUMMARY

It was found that 26 of 127 infants with chlamydial conjunctivitis had previously received chloramphenicol eye drops. This treatment had delayed the onset and reduced the degree of oedema, congestion and discharge compared with infected infants with no 'first-line' chemotherapy, but eye swabs remained positive in 22 (85%) of the chloramphenicol treated infants.

The problems of designing laboratory tests which might accurately forecast the clinical value, or lack of value of antibiotics in chlamydial infections were investigated. The minimum inhibitory concentration (MIC) of chloramphenicol, against *Chlamydia trachomatis* in McCoy cell cultures varied widely according to the antibiotic preparation used, the timing and duration of its exhibition in relation to the single step growth cycle of *C. trachomatis*, and, in particular, the method of examination of infected tissue cultures for residual chlamydial growth after incubation with chloramphenicol.

INTRODUCTION

Conjunctivitis in newborn infants is a common result of infection with *Chlamydia trachomatis* derived from the infected genital tract of the mother (Rees *et al.* 1977*a*). On clinical grounds, it cannot reliably be differentiated from conjunctivitis caused by a wide range of bacteria e.g. staphylococci, streptococci, Gram negative intestinal bacteria or *Neisseria gonorrhoeae*. Thus, it is often the standard procedure in maternity units and general practice to begin chemotherapy immediately with chloramphenicol eye drops and to continue at least until the results of microbiological investigation of eye swabs have been reported. Many infants are only referred, with their mothers, to special clinics such as ours in Liverpool for further opinion after such prior treatment. Hence it is important to know whether chloramphenicol eye drops alone can cure chlamydial infection or might suppress it sufficiently to cause subsequent problems in clinical or laboratory diagnosis.

Our early experience (Rees *et al.* 1977*b*) with a small number of such infants was that prior treatment with chloramphenicol gave little clinical improvement and that *C. trachomatis* could usually be isolated from the eyes after treatment, whereas treatment with tetracycline eye ointment alone or with systemic

erythromycin gave rapid clinical improvement, and eye swabs became chlamydia-negative in the early follow-up period. One obvious explanation of these poor results is that the sensitivity of chlamydia to chloramphenicol is too low for effective concentrations to be attainable *in vivo*. The minimum inhibitory concentration (MIC) against various laboratory-adapted strains of *C. trachomatis* in chick embryos or in tissue culture has been estimated to be as high as 4–10 μg/ml (Kuo, Wang & Grayston, 1977; Treharne *et al.* 1977; Oriel & Ridgeway, 1982). However these results were obtained with various compounds such as chloramphenicol sodium succinate, the *in vitro* activity of which against bacteria is known to be considerably less than that of chloramphenicol itself (Garrod, Lambert & O'Grady, 1981). Such reports may have limited relevance to the therapy of infants with chlamydial conjunctivitis, where it is customary to introduce eye drops containing 5000 μg/ml of pure chloramphenicol base directly to the site of infection at four hourly intervals. Thus the discrepancy between clinical and laboratory evidence on the efficacy of chloramphenicol against *C. trachomatis* may be even greater than has been appreciated hitherto. The purpose of the present study is to examine both of these aspects in more detail in a larger group of infants and to determine whether antibiotic MIC tests against *C. trachomatis* in tissue culture can provide a reasonable measure of the benefit to be expected in clinical practice.

MATERIALS AND METHODS

Patients

The infants were a selected series of cases of conjunctivitis referred mainly because no bacterial cause had been identified, or because of failure to respond to routine treatment, or latterly as part of the initial microbiological investigation of neonatal conjunctivitis. Swabs from the eyes and throats of infants and from the cervix and urethra of the mothers were taken and examined as described previously (Rees *et al.* 1981; Davies *et al.* 1978). All clinical examination of infants was by a standardized procedure by only two experienced clinicians (ER and IAT) throughout. The degree of oedema of the eyelids and conjunctiva, the amount of inflammation (congestion) and mucopurulent discharge from the eyes was given a numerical score from 0 to 4, indicating nil, mild, moderate, severe or very severe, respectively. The age of the infant at the onset of conjunctivitis and upon first referral to ER or IAT was recorded.

Chlamydial cultures

Procedures for isolation and quantitation of *C. trachomatis* from clinical specimens in coverslip cultures of cycloheximide-treted McCoy cells have been described (Davies *et al.* 1978; Hobson *et al.* 1980). The origin of BK strain (*C. trachomatis* serotype D/E) and methods for its propagation and titration are as described earlier (Karayiannis & Hobson, 1981*a*).

Antibiotic titrations

Chloramphenicol sodium succinate (Parke-Davis) was used in preliminary experiments, only where stated in the text. This was freshly dissolved from the manufacturer's vials in sterile distilled water for each experiment and working

dilutions of 5000 μg/ml and below were made in tissue culture growth medium (GM) and added in volumes of 0·1 ml to each McCoy coverslip culture (MCC) infected with chlamydia to give the required final concentration.

In all other experiments pure chloramphenicol base was used in the form of paediatric eye drops (Smith & Nephew, Ltd.) containing 5000 μg/ml of antibiotic in distilled water without preservative or other additive. A fresh vial was used for each experiment, and dilutions and additions to MCC were as above. A standard preparation of pure base (Parke-Davis) in powder form was also obtained (by courtesy of Professor A. Percival) and gave similar results throughout.

The antibiotic MIC test procedure adopted was substantially that of Johnson & Hobson (1977) except for the use of cycloheximide-treated cultures of McCoy cells instead of replicating cells in the post-infective period. Preliminary experiments showed that the antichlamydial effect of chloramphenicol was similar in both types of culture but it was easier to detect chlamydial inclusions in those treated with cycloheximide.

For continuous incubation with chloramphenicol, MCC were inoculated with a dilution of BK strain calculated to yield 2000–4000 inclusions per coverslip, immediately after adding the antibiotic in final concentrations ranging from 10–0·01 μg/ml; they were centrifuged immediately to achieve chlamydial adsorption and incubated for 48 h at 35 °C without further change of medium.

In other experiments, the addition of chloramphenicol was delayed in order to determine its effect on chlamydia which had already established infection in McCoy cells and had begun to replicate. MCC were infected with BK strains and incubated as above for 18 h when, without further change of medium, chloramphenicol dilutions were added and incubation was continued for a further 30 h.

Conversely, to determine whether the effect of chloramphenicol was reversible, MCC were infected with BK strain and immediately inoculated with chloramphenicol dilutions and incubated as above. After 18 h incubation, half of these MCC and infected control cultures without chloramphenicol were removed, the medium was taken off and replaced with fresh medium without chloramphenicol after several washes in fresh medium to remove residual traces of the antibiotic. These MCC were then incubated for a further 30 h before examination. In all experiments 2–4 MCC were used at each chloramphenicol dilution and in chloramphenicol-free controls, and were stained by Giemsa after incubation.

Estimation of chlamydial growth

In initial experiments the MIC of chloramphenicol was taken as the lowest concentration in which no chlamydial inclusions could be seen by light or dark-ground microscopy at 400× magnification. In all later experiments the number of inclusions per whole coverslip was counted at each antibiotic concentration and the results were expressed as a percentage of the count in control cultures without chloramphenicol. The 95% confidence limits of this counting procedure in replicate coverslips has been shown to be of the order of ±23·5% (Karayiannis & Hobson, 1981*b*). This procedure can only be expected to reveal chlamydial inclusions which are > 2–3 mm diameter and which are fully mature, containing a closely packed array of deeply staining elementary bodies (EB) and a few or no lightly-staining residual intermediate forms (reticulate bodies). Under

normal conditions after 48 h incubation at 35 °C the appearance to be expected is that shown in Plate 1A. The diameter of such inclusions was estimated by eyepiece micrometer as described by Bushell & Hobson (1978).

In order to determine whether this normal development of chlamydial inclusions might be replaced by abnormal or retarded growth during incubation with chloramphenicol, all coverslips were re-examined by light microscopy with the oil immersion lens (1000× magnification). Small abortive inclusions (< 1 μm diameter) of the type shown in Plate 1B and subsequently referred to as tiny inclusions were seen, in addition to or instead of normal inclusions. The chlamydial count at 1000× magnification represents the total number of inclusions (normal plus tiny) per whole coverslip expressed as a percentage of the total count at this magnification by light microscopy in chloramphenicol-free control cultures. The differences in counts of tiny inclusions in replicate coverslips showed similar confidence limits to those for normal inclusions.

RESULTS

Clinical findings

Out of a total of 451 neonates with conjunctivitis referred to us over the last five years, *C. trachomatis* was isolated from the eyes in 112 cases. In another 15, although eye swabs were negative, *C. trachomatis* was recovered either from the infant's throat or from the mother's cervix or both on our first examination. Some of these infants had received topical or systemic antiobiotics before the first eye swab was taken. In the 127 proven or strongly-presumptive cases of chlamydial conjunctivitis, 81 had been given no antibiotic or only neomycin eye drops, and eye swabs were chlamydia positive in 78 (96%) of these cases. However, only 12 (60%) of 20 babies given even an inadequate course of tetracycline eye ointment or systemic or local penicillin for 2–7 days before referral gave positive eye swabs. In contrast, *C. trachomatis* was isolated from the eye swabs of 22 (85%) of 26 infants who had already been given chloramphenicol eye drops for varying periods. Of the four infants who were chlamydia-negative and remained so on extensive follow-up investigation after discontinuing the chloramphenicol, one had received treatment for only 2 days, two for 7–10 days and one for 15 days. Of the 22 persistently chlamydia-positive infants, four had received chloramphenicol eye drops for 7–10 days and three for 11–13 days and only two had been treated for less than three days. In most cases the drops had been instilled 4-hourly under trained nursing supervision.

Clinical and laboratory findings in the 78 infants with chlamydia-positive eye swabs who had received no prior chemotherapy, or neomycin eye drops only, were compared with those in the 22 infants with positive eye cultures despite prior chloramphenicol (Table 1). All had probably been infected at birth, and the reported mean day of onset was similar in both groups. However, the mean time of referral to us was 6·3 days later in the chloramphenicol-treated than in the untreated babies, which suggests that the antibiotic had retarded the clinical course of the disease, in that it took significantly longer for those in charge of their primary care to become aware that the conjunctivitis was progressing to the point where further medical advice needed to be sought. However, on referral, the infants

Table 1. *A comparison of the findings on first examination of* 100 *infants with persisting* C. trachomatis *conjunctivitis in* (*a*) 78 *cases with no prior chemotherapy and* (*b*) 22 *cases with prior treatment with chloramphenicol eye drops*

		'First-line' prior treatment	
		Nil	Chloramphenicol
Number		78	22
Mean age at onset (days)		6·2	6·6
Mean age on referral (days)		10·6	16·9
Chlamydial inclusion count*	range	2 to ⩽ 50000	1–9870
	mean	7333	1895
Eye signs:			
Eyelid oedema (max = 4)		1·9	0·9
Mucosal oedema		2·3	1·4
Congestion		2·5	1·6
Discharge		2·1	1·5
Total score (max = 16)		8·8	5·4

* Counts per whole McCoy coverslip culture (two per specimen) inoculated with a standardized amount of material from eye swabs.

given chloramphenicol were less severely affected than the untreated babies in terms of the degree of palpebral or mucosal oedema or of conjunctival congestion or discharge. Moreover the chlamydial inclusion counts in McCoy cell cultures inoculated with eye swabs from treated infants were much lower (mean, 1895; range, 1–9870) than in untreated infants (mean, 7333, range 2 to ⩽ 50000).

In all 112 infants with chlamydia-positive eye swabs, there was rapid clinical improvement and eye swabs became negative on culture shortly after giving tetracycline eye ointment alone or with systemic erythromycin for 7–14 days, even if they had failed to be cured by prior 'first-line' chemotherapy. Subsequent follow-up showed that re-isolation of chlamydia from the eye or pharnyx was significantly less frequent with the combined treatment than with tetracycline alone.

Microbiological findings

MIC (0–48 *h*) *of chloramphenicol against* C. trachomatis. The laboratory BK strain produced normal autofluorescent inclusions well filled with EB (Fig. 1*a*) in the presence of 0·1 and 0·2 μg/ml chloramphenicol base but none was detected in the presence of 0·4 μg/ml or greater. The apparent MIC did not vary significantly with inocula of 20–20000 inclusion forming units (i.f.u.) per culture. In similar titrations with chloramphenicol sodium succinate the MIC was 8 μg/ml.

From seven infants and seven mothers, aliquots of the original swab suspension which had proven chlamydia-positive on culture yielded *C. trachomatis* again when reinoculated into McCoy cultures containing no antibiotic or 0·1 μg/ml chloramphenicol but no inclusions were detected with 0·2 μg/ml in three cases or with 0·4 μg/ml in 11 cases. In contrast, concentrations of 4–8 μg/ml of chloramphenicol sodium succinate were necessary to inhibit the growth of *C. trachomatis* in eight of these specimens tested in the same manner.

In all subsequent experiments a single pool of BK strain was used throughout to investigate further aspects of the MIC test with pure chloramphenicol base.

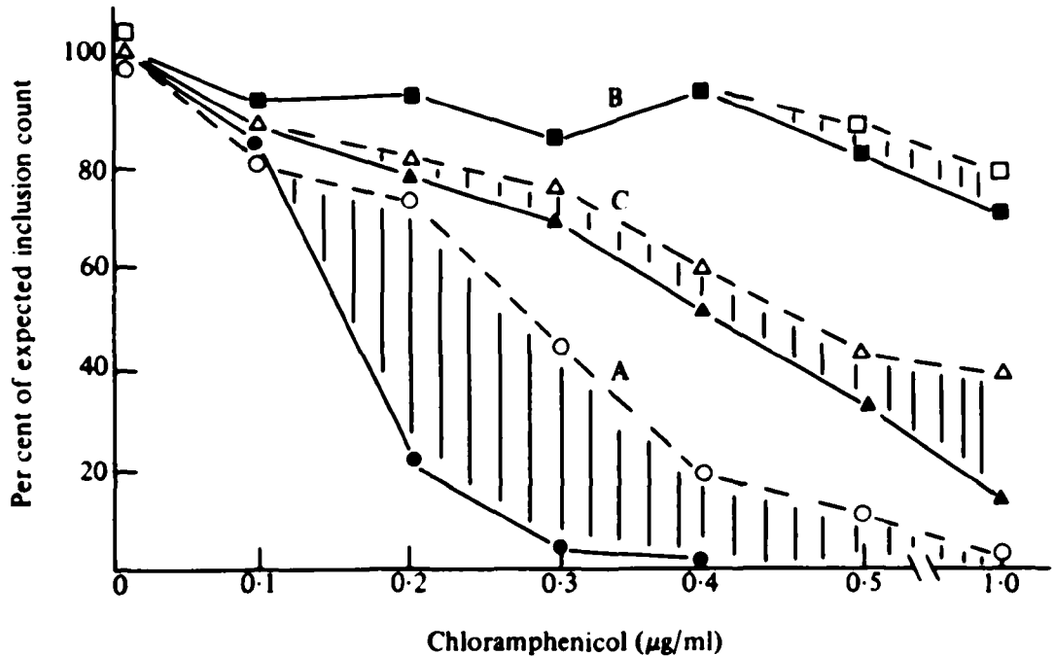

Fig. 1. The growth of *C. trachomatis* BK strain in McCoy cell cultures treated with chloramphenicol throughout or for only part of the chlamydial growth cycle. All cultures were incubated at 35 °C/48 h after infection with a constant inoculum of BK strain. In 14 control cultures without chloramphenicol, the mean inclusion count per coverslip culture, 3002 (range, 2134–3718). Each experimental point is the mean of 2–4 coverslip counts. The symbols indicate: A (●—● and ○—○) chloramphenicol present throughout infection. B (■—■ and □—□) chloramphenicol present 0–18 h but removed during incubation 18–48 h. C (▲—▲ and △—△) no chloramphenicol 0–18 h incubation but chloramphenicol present from 18–48 h incubation. Solid symbols: ●, ■, ▲, count of mature inclusions only at 400 × magnification dark-ground microscopy. Open symbols: ○, □, △, total count of mature plus tiny inclusions at 1000 × magnification by oil immersion light microscopy.

Quantitative dose–response relationships in the MIC test. A constant dilution of BK strain was inoculated into McCoy cultures to which serial dilutions of chloramphenicol had been freshly added, and the antibiotic remained throughout the 48 h incubation period. The percentage of the BK inoculum able to produce normal inclusions (as shown in Plate 1A) was 82 % of that in control cultures (mean, 2652, range, 2134–3095) in the presence of 0·1 μg/ml but fell to only 20 % with 0·2 μg/ml chloramphenicol (Fig. 1A), and only occasional inclusions could be detected at 0·3 μg/ml or greater by dark-ground or light microscopy at 400 × magnification. The mean diameter of the inclusions also fell progressively, from 16 μm in control cultures to 10 μm in 0·1 μg/ml, 7 μm in 0·2 μg/ml and only 2–3 μm in the few inclusions (< 1 % of control count) at 0·3 μg/ml chloramphenicol. It thus seemed possible that even smaller inclusions, not detectable at 400 × magnification, might still have been formed in the presence of chloramphenicol. Accordingly, all the cultures were re-examined under oil-immersion bright light microscopy at 1000 × magnification, which revealed numbers of tiny (⩽ 1 μm) inclusions (as shown in Plate 1B) containing only a few pale staining particles resembling reticulate bodies and occasional EB. These were in addition to normal well-filled inclusions in cultures with 0·1 and 0·2 μg/ml, or instead of normal inclusions with 0·3, 0·4 and 0·5 μg/ml, but none were detectable with 1 μg/ml chloramphenicol (Fig. 1A).

Tiny inclusions were not seen in control infected cultures without chloramphenicol nor in uninfected cultures treated with chloramphenicol alone, nor in infected cultures with chloramphenicol in concentrations which did not reduce the normal inclusion count significantly. Their number increased as a percentage of the total count with increasing concentration of chloramphenicol. The total number of tiny inclusions and their size decreased and the degree of abnormality increased progressively with increasing chloramphenicol concentration. Thus it seemed unlikely that the tiny forms were spontaneously late-starting or early-aborting infective centres or artefacts occurring independently of exposure to antibiotic. Their presence, responsible for the difference between inclusion counts at 1000 × and 400 × magnification, suggested that intermediate doses of chloramphenicol merely gave a gross slowing of inclusion development by a substantial portion (45–60 %) of the infective inoculum rather than abolishing growth entirely.

The effect of continued incubation. In cultures where incubation was continued for a further 48 h (96 h total), the size of the inclusions had increased (mean diameter, 22 μm) in control cultures and in those with 0·1 μg/ml antibiotic, and many had burst from the McCoy cells, scattering elementary bodies over the monolayer. Consequently the inclusion count had fallen to 20 % of that at 48 h. In cultures with 0·2 μg/ml antibiotic the inclusion count had fallen only by 45 % from the count at 48 h, and the mean diameter of the remaining inclusions was only 13 μm. With 0·3 μg/ml chloramphenicol, where few mature inclusions had been seen at 48 h, there were now 96 (3 % of the original inoculum) with a mean diameter of 8 μm. In these and in cultures with 0·4 and 0·5 μg/ml of antibiotic, tiny inclusions were still detectable, but the total count at 1000 × magnification was now only 50–75 % of the count at 48 h. There was still no evidence of infection in the continued presence of 1 μg/ml chloramphenicol.

In replicates of the above cultures, the medium containing chloramphenicol was removed from infected cultures after 48 h, and replaced by fresh medium without antibiotics before a further 48 h incubation. Chlamydial growth which had been inhibited in the presence of antibiotic was resumed after its removal; even with 1 μg/ml chloramphenicol, normal well-filled inclusions, equivalent to 37 % of the original inoculum, were found. These were very variable in size with mean diameter of only 14 μm, compared with 23 μm in control antibiotic-free cultures, probably as a result of the delayed start to their growth.

The effect of earlier removal of chloramphenicol. The inhibitory effect was significantly reduced in cultures where serial dilutions of the antibiotic were added at the time of chlamydial infection but were removed 18 h later, with incubation then continuing for a total of 48 h (Fig. 1 B). In control cultures without antibiotic the mean inclusion count was 3564 (range, 3102–3718) but even in cultures given an early pulsed exposure to 1 μg/ml of chloramphenicol, 69 % of this chlamydial inoculum yielded inclusions which were of normal well-filled appearance, although smaller (mean diameter, 9 μm) than those in control cultures (mean diameter, 15 μm). Tiny abnormal inclusions were detectable in small numbers only in cultures treated with 1 μg/ml chloramphenicol.

The effect of delayed addition of chloramphenicol. When infected cultures were incubated for 18 h before adding chloramphenicol and then re-incubated for a further 30 h after adding serial dilutions of the antibiotic, many of the infective

centres had been able to continue growing to form normal mature inclusions (mean diameter, 11–15 μm) in all concentrations of chloramphenicol (Fig. 1C). In cultures with 0·2 μg/ml, the count of mature inclusions was 78% of that in control cultures (mean, 2981, range, 2303–3453), but was only 32% after adding 0·5 μg/ml, and only 16% with 1 μg/ml chloramphenicol. Tiny inclusions accounted for a further 5% of the original inoculum in cultures exposed to 0·2 μg/ml, and for a further 26% in those treated with 1 μg/ml chloramphenicol. A large proportion of the original inoculum thus remains unaccounted for; presumably many of the early infective centres which would have been present after 18 h growth, when the antibiotic was added, had either still not reached visibility or had been digested or eliminated by the McCoy cell during chloramphenicol treatment.

DISCUSSION

Neither on clinical nor on microbiological grounds can the common practice of blind 'first-line' chemotherapy of neonatal conjunctivitis with chloramphenicol eye drops be recommended now that *C. trachomatis* is recognized to be a common cause of this condition. Chloramphenicol is unlikely to cure chlamydial conjunctivitis, but can delay and diminish clinical signs sufficiently to impair diagnosis, and can affect the degree of shedding of the infective agent, making laboratory confirmation slower and more difficult. Early and precise diagnosis of chlamydial neonatal conjunctivitis is essential, not only for prompt and correct treatment of the infant itself, but because it is often the first indicator that the mother must be infected and at risk of puerperal complications (Rees *et al.* 1977*b*).

The discrepancy between the poor clinical results with chloramphenicol and the apparent high sensitivity of *C. trachomatis* strains (even when obtained directly from the patient), illustrates some of the drawbacks in applying conventional MIC tests, which were designed primarily for determining antibiotic activity in simple media against free-living bacteria with rapid and multicyclic growth by simple binary fission, to the much more complex situation of *C. trachomatis* growing within tissue culture cells.

Firstly, it is not easy to see whether the apparent end-point, i.e. the concentration of antibiotic beyond which there is failure of the organism to produce normal inclusions, well-filled with elementary bodies, is equivalent to a bacteriostatic or a bactericidal effect. Early arrested inclusions are very difficult to detect in cell monolayers. Their capacity to resume growth after prolonged exposure to antibiotic may be limited because the tissue cultures into which they were inoculated might decline in their capacity to support chlamydial growth with increasing age, either by exhaustion of nutrients, accumulation of toxic byproducts or by the acquisition of defence mechanisms, especially the mobilization of lysosomal enzymes. With the exception of the lymphogranuloma venereum (L1–3)serotypes, all other serotypes of *C. trachomatis* undergo only a single cycle of development in tissue culture. Thus, primary infective centres which might have formed in the presence of antibiotic could not subsequently produce secondary daughter inclusions in the original culture, and this further limits the ability to determine whether an antibiotic has only a bacteriostatic effect. Attempted passage to fresh cultures from treated infected cultures in which no mature inclusions are seen may not be helpful for

this purpose. Early infective centres which might have survived the antibiotic could be destroyed in the process of passage; the replicating reticulate bodies are by definition non-infective since they have no cell wall and cannot withstand osmotic shock on dispersal from the host cell (Manire, 1977). A further artificiality of the MIC test as conventionally applied to *C. trachomatis* in tissue culture is that the antibiotic is normally added from the onset of infection and remains present at high concentration throughout the whole synchronous single-step growth cycle. In the patient, in contrast, growth of *C. trachomatis* is neither synchronous nor single step and the antibiotic may be present in effective concentration for only a short part of the cycle of development of individual inclusions.

From the present experiments the clinical short-coming of chloramphenicol were not revealed by conventional methods of MIC testing usually applied to this organism, but could have been forecast more precisely (*a*) by searching for arrest at an early incomplete stage of growth, as here by oil-immersion microscopy, or probably better in future by specific staining for cycloplasmic chlamydial DNA or RNA, (*b*) by exposing the infected culture to antibiotic in early or late pulses over only part of the growth cycle, thus simulating the events of treating natural eye infections more closely.

Discrepancy between the MIC of chloramphenicol for *C. trachomatis* reported here, and the ten-fold or higher MIC reported previously, e.g. Kuo, Wang & Grayston (1977) seems to be mainly due to the choice of test substance. Chloramphenicol sodium succinate, which has often been used in such tests with chlamydia, has little or no antimicrobial activity *per se* (Garrod, Lambert & O'Grady, 1981). Clinically it can be used with success because it is broken down by esterases in the body to release active chloramphenicol base, but in bacteriological laboratories its use for MIC tests has long been discouraged because breakdown of the ester by hydrolysis in bacteriological media is slow and unreliable, and hence the sensitivity of organisms to chloramphenicol is grossly underestimated. From the present findings it also seems unlikely that the McCoy cell system can effectively release active chloramphenicol from the ester, and that the pure base which is used in paediatric eye drops should also be used for *in vitro* tests with chloramphenicol.

The interruption of chlamydial maturation produced by chloramphenicol gives abnormal tiny inclusions which are quite different in appearance from the abortive spheroplastic forms produced by penicillin (Johnson & Hobson, 1977) and other β lactam antibiotics (Hobson *et al.* 1982). The nature of this interruption and the point of the chlamydial development cycle at which chloramphenicol acts deserves further attention both as a probe to greater understanding of chlamydial growth mechanisms in tissue culture and as part of the search for improved antibiotics capable of chlamydicidal rather than chlamydistatic activity in clinical infections.

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REFERENCES

BUSHELL, A. C. & HOBSON, D. (1978). Effect of Cortisol on the growth of *Chlamydia trachomatis* in McCoy cells. *Infection and Immunity* **21**, 946–953.

DAVIES, J. A., REES, E., HOBSON, D. & KARAYIANNIS, P. (1978). Isolation of *Chlamydia trachomatis* from Bartholin's ducts. *British Journal of Venereal Diseases* **54**, 409–413.

GARROD, L. P., LAMBERT, H. P. & O'GRADY, F. (1981). *Antibiotic & Chemotherapy* 5th ed., p. 491. Edinburgh: Churchill Livingstone.

HOBSON, D., KARAYIANNIS, P., BYNG, R. E., REES, E. & TAIT, I. A. (1980). Quantitative aspects of chlamydial infection of the cervix. *British Journal of Venereal Diseases* **56**, 56–62.

HOBSON, D., LEE, N., BUSHELL, A. C. & WITHANA, N. (1982). The activity of β lactam antibiotics against *Chlamydia trachomatis* in McCoy cell cultures. In *Chlamydial Infections* (ed. P.-A. Mårdh, K. K. Holmes, J. D. Oriel, P. Piot & J. Schachter), pp. 249–252. Amsterdam: Elsevier.

JOHNSON, F. W. A. & HOBSON, D. (1977). The effect of penicillin on genital strains of *Chlamydia trachomatis* in tissue culture. *Journal of Antimicrobial Chemotherapy* **3**, 49–56.

KARAYIANNIS, P. & HOBSON, D. (1981*a*). The role of calf serum in the growth of *Chlamydia trachomatis* in McCoy cell cultures. *Journal of General Microbiology* **122**, 47–54.

KARAYIANNIS, P. & HOBSON, D. (1981*b*). Amino-acid requirements of a *Chlamydia trachomatis* genital strain in McCoy cell cultures. *Journal of Clinical Microbiology* **13**, 427–432.

KUO, C.-C., WANG, S.-P. & GRAYSTON, J. T. (1977). Antimicrobial activity of several antibiotics against *Chlamydia trachomatis* organisms in cell culture. *Antimicrobial Agents & Chemotherapy* **12**, 80–89.

MANIRE, G. P. (1977). Biological characteristics of chlamydiae. In *Nongonococcal Urethritis and Related Infections* (ed. D. Hobson & K. K. Holmes), pp. 167–175. Washington, D.C.: American Society of Microbiology.

ORIEL, J. D. & RIDGEWAY, G. L. (1982). Genital infections by *Chlamydia trachomatis*. Pp. 14–19. London: Edward Arnold.

REES, E., TAIT, I. A., HOBSON, D., BYNG, R. E. & JOHNSON, F. W. A. (1977*a*). Neonatal conjunctivitis caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. *British Journal of Venereal Diseases* **53**, 173–179.

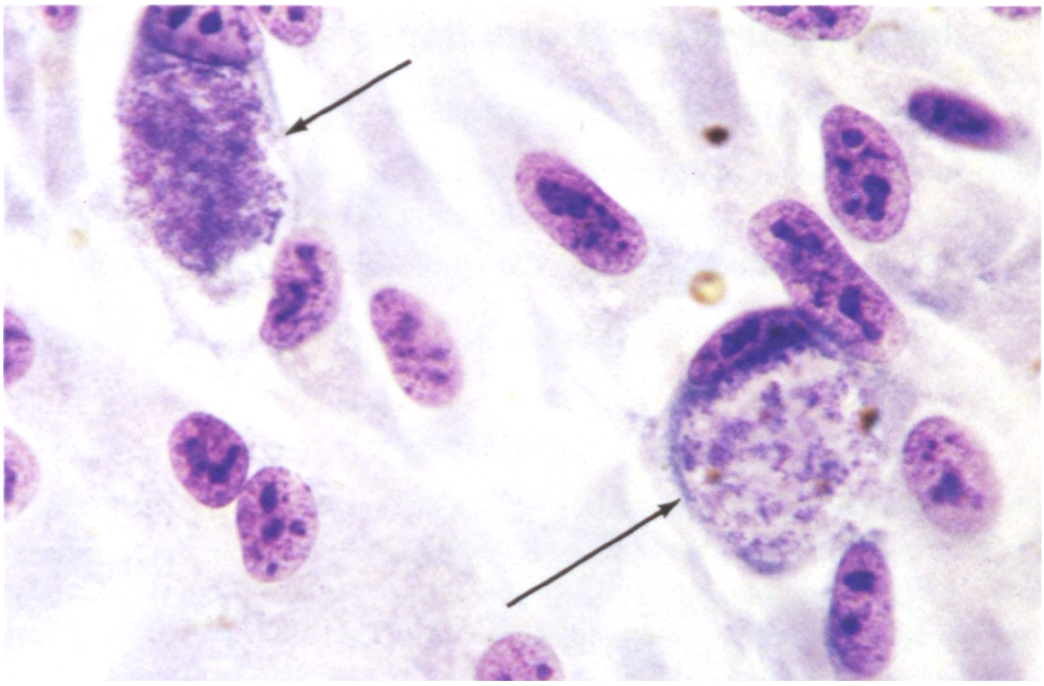
REES, E., TAIT, I. A., HOBSON, D. & JOHNSON, F. W. A. (1977*b*). Perinatal Chlamydial infection. In *Nongonococcal Urethritis and Related Infections* (ed. D. Hobson & K. K. Holmes), pp. 140–147. Washington, D.C.: American Society of Microbiology.

REES, E., TAIT, I. A., HOBSON, D., KARAYIANNIS, P. & LEE, N. (1981). Persistence of chlamydial infection after treatment for neonatal conjunctivitis. *Archives of Diseases in Childhood* **56**, 193–198.

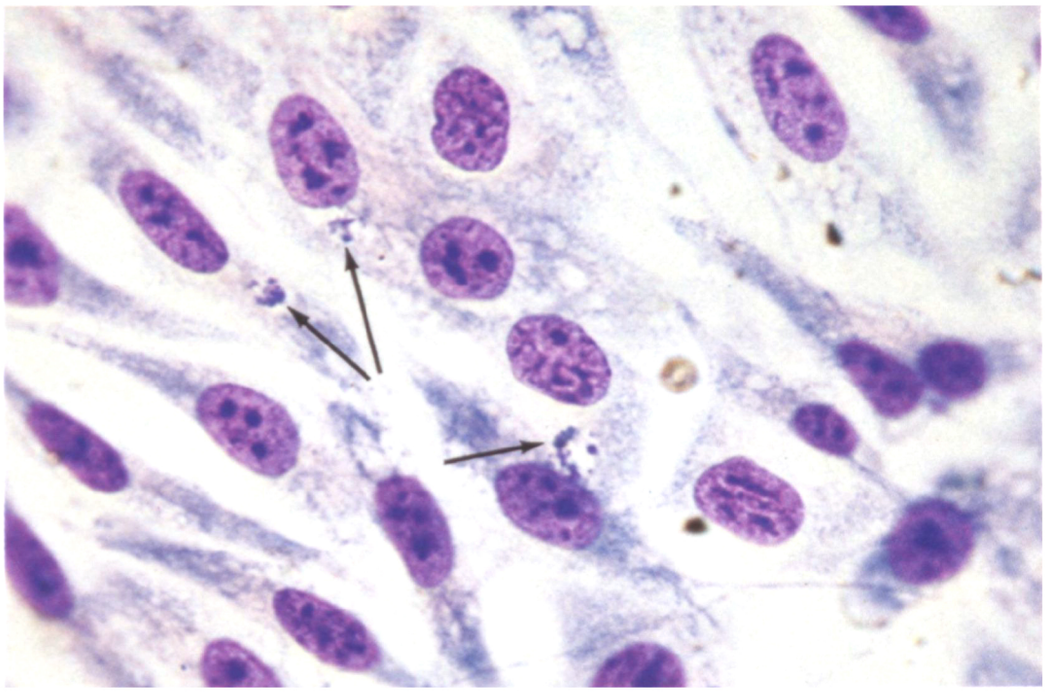
TREHARNE, J. D., DAY, J., YEO, C. K., JONES, B. R. & SQUIRES, S. (1977). Susceptibility of chlamydiae to chemotherapeutic agents. In *Nongonococcal Urethritis and Related Infections* (ed. D. Hobson & K. K. Holmes), pp. 214–222. Washington, D.C.: American Society of Microbiology.

EXPLANATION OF PLATE 1

The appearance of inclusions of *C. trachomatis* BK strain after 48 h incubation in McCoy cell tissue cultures. Giemsa-stained coverslip cultures of cycloheximide-treated McCoy cells. Oil immersion light field microscopy. Magnification 1000 ×. (A) Normal mature inclusions filled with elementary bodies in cultures without chloramphenicol. (B) Tiny abnormal inclusions in cultures incubated throughout with 0·5 μg/ml final concentration of chloramphenicol.



(*a*)



(*b*)

The appearance of inclusions of *C. trachomatis* BK strain after 48 h incubation in McCoy cell tissue cultures.*

(*a*) Normal mature inclusions (arrowed) filled with elementary bodies in cultures without chloramphenicol.

(*b*) Tiny abnormal inclusions (arrowed) in cultures incubated throughout with 0·5 μg/ml final concentration of chloramphenicol.

* Giemsa-stained coverslip cultures of cycloheximide-treated McCoy cells. Oil-immersion light-field microscopy. Magnification × 1000.

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