Uptake, Accumulation, and Egress of Erythromycin by Tissue Culture Cells of Human Origin

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The ability of erythromycin A base to penetrate and accumulate in tissue culture cells of human origin was investigated. The antibiotic was highly concentrated by early passage cells of normal bronchus, kidney, liver, lung, and skin and by cancer cells derived from breast, liver, and lung. Intracellular levels 4 to 12 times that of the extracellular milieu were obtained in both early-passage and transformed cells. The total quantity of erythromycin accumulated depended on the extracellular concentration of antibiotic, but the cellular/extracellular ratios were, for the most part, independent of the initial extracellular drug concentration. In all cell types tested, the accumulated antibiotic rapidly egressed when cells were incubated in antibiotic-free medium. Bioactivity assays demonstrated that the expelled drug was unmetabolized, fully active antibiotic. The concentration of erythromycin by a variety of human cell types probably accounts, in part, for the effectiveness of the antibiotic against intracellular parasites such as *Legionella* and *Chlamydia* spp.

Erythromycin has been widely, successfully, and safely used for over two decades as treatment for infections caused by susceptible pathogens. Although erythromycin has been recognized as an effective drug, investigators have been puzzled by the low and unpredictable levels in plasma that are usually observed after administration of the antibiotic. The paucity of drug in the vasa may be due to tissue accumulation, since distribution studies on tissue samples excised from dogs (14) and rats (15) have shown that erythromycin is concentrated well above blood levels in organs such as the liver, spleen, kidney, lung, and pancreas and in submaxillary and adrenal glands.

Infections attributed to *Legionella* spp., which are facultative intracellular bacteria, and *Chlamydia* spp., which are obligate intracellular parasites, are effectively treated with erythromycin (1). Both organisms are known to parasitize a large number of human cell types. The ability of erythromycin to control these organisms suggests that the antibiotic penetrates and exerts its action within the invaded host cell. Indeed, recent studies have shown that both human polymorphonuclear leukocytes (PMNs) (20, 23) and alveolar macrophages (8, 12) accumulate erythromycin at 15 to 24 times extracellular levels. Further, studies have shown that erythromycin assists human PMNs (20) and cultured guinea pig peritoneal macrophages (28) in the killing of phagocytosed *Legionella pneumophila*. All the foregoing indicates that erythromycin may be concentrated intracellularly by eukaryotic cells and suggests that this accumulation may be responsible for the aberrant levels in plasma usually observed after antibiotic administration.

The results presented in this study demonstrate that a variety of types of human tissue culture cells sequester erythromycin. We suspect that, after erythromycin intake, certain mammalian tissues serve as drug reservoirs which vastly influence antibiotic pharmacokinetics and bioactivity. We suggest that in cases in which an antibiotic is significantly accumulated and released by host cells, the traditional measurement of drug levels in serum or plasma may not be a reliable predictor of therapeutic effectiveness. The ability of erythromycin to penetrate and accumulate in many

eukaryotic cell types has important therapeutic implications, particularly with regard to obligate or facultative intracellular organisms or other microbes that may survive phagocytosis.

MATERIALS AND METHODS

Antibiotics. Ring-labeled [^{3}H]erythromycin A base was prepared by incubating [^{3}H]sodium propionate (New England Nuclear Corp., Boston, Mass.) with a high-yielding strain of the producing organism, *Streptomyces erythreus*. The resulting [^{3}H]erythromycin was extracted with methylene chloride and crystallized from acetonitrile. Further purification by high-pressure liquid chromatography on a C_{18} column with a mobile phase consisting of acetonitrile (1,350 ml), methanol (300 ml), 0.2 M ammonium formate (300 ml), water (1,050 ml), and ammonium hydroxide (0.6 ml) (pH 7.5 to 8.0) gave [^{3}H]erythromycin A base (specific activity, 19.5 mCi/mmol; >98% radio pure). The [^{3}H]erythromycin was dissolved in a minimal amount of methanol and diluted to 1 mg/ml with water. The solution was stored frozen in small vials at $-20°C$ until used. On the day of an experiment, vials were thawed and diluted to the desired concentration in the indicated medium. [^{14}C]benzylpenicillin (specific activity, 54 mCi/mmol), purchased from Amersham Corp., Arlington Heights, Ill., was dissolved in water immediately before use and utilized as described for erythromycin.

Tissue culture cells. Tissue culture media and supplements were purchased from GIBCO Laboratories, Grand Island, N.Y., and trypsin was that of Difco Laboratories, Detroit, Mich. The respective cell types were grown and harvested as indicated below. The early-passage normal human cells were cultured at 37°C in a 5% CO_2–95% air atmosphere, and the cancer cells were incubated at the same temperature in 100% air. After harvest all cells were washed twice with gentamicin-free growth medium and used immediately for accumulation studies. All manipulations were performed with solutions prewarmed to 37°C. Viable cells were determined by the erythrocin B dye exclusion method in a hemacytometer.

Normal human cells. (i) Skin cells. Human skin cells (CCD-48 SK CRL 1507), subculture 3, obtained from the American Type Culture Collection, Rockville, Md., were

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grown in RPMI 1640 medium supplemented with 25 mmol of HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer, 10% fetal bovine serum (FBS), and 25 mg of gentamicin per liter. Adherent cells were harvested with trypsin in phosphate-buffered saline.

(ii) Lung cells. Human diploid embryonic lung cells (WI-38), subculture 14, obtained from the American Type Culture Collection, were grown as described above for skin cells.

(iii) Kidney cells. Human diploid embryonic kidney cells (lot 0916), subculture 1, were those of Abbott Laboratories, North Chicago, Ill. Cells were grown in medium 199 with Earle salts supplemented with 10% FBS. Adherent cells were harvested with trypsin.

(iv) Foreskin cells. Human diploid foreskin cells (MRHF), subculture 24, obtained from M. A. Bioproducts, Walkersville, Md., were grown as described above for skin cells.

(v) Bronchus cells. Human diploid bronchus cells (CCD-14 Br CRL-203), subculture 5, obtained from the American Type Culture Collection, were grown as described above for skin cells.

(vi) Liver fibroblasts. Human diploid liver fibroblasts (AB-1), subculture 9, were those of Abbott Laboratories. Cells were grown in minimal essential medium supplemented with 10% FBS. Adherent cells were released with trypsin-EDTA.

Human cancer cells. (i) Lung carcinoma cells. Human lung carcinoma cells (A549), a continuous cell line obtained from the American Type Culture Collection, were grown in Dulbecco modified Eagle medium supplemented with 10% FBS and 25 mg of gentamicin per liter. Adherent cells were harvested with trypsin.

(ii) Breast cancer cells. Human breast cancer cells (MCF7), a continuous cell line, were grown in minimal essential medium supplemented with Hanks salts and 5% FBS. Attached cells were harvested as described above for lung carcinoma cells.

(iii) Liver cancer cells. Human liver cancer cells (Hutu 80), a continuous cell line obtained from the National Cancer Institute, Bethesda, Md., were grown in minimal essential medium supplemented with Earle salts and 10% FBS. Adherent cells were harvested with trypsin-EDTA.

Velocity gradient sedimentation assay. Freshly harvested cells were suspended to ca. 5×10^6 cells per ml in prewarmed, antibiotic-free growth medium containing the normal serum and buffer supplements. To alleviate adherence, we performed accumulation studies in siliconized, 25-ml, screw-capped Erlenmeyer flasks. Cells grown in RPMI 1640 medium were incubated in open flasks, whereas cells grown in other media were incubated in closed vessels that were occasionally gassed with 5% CO_2–95% air to maintain a pH of 7.3 to 7.8. After incubation for 15 min at 37°C with rotary shaking (150 rpm), the indicated quantity of radiolabeled antibiotics were added to the incubation mixtures. Agitation was continued at 37°C and, at intervals, 0.2-ml portions were removed and immediately subjected to the velocity gradient technique previously described (6, 12). Some cell types tended to aggregate during incubation, in which case the aggregates were broken up by gently drawing up and expelling the cell suspension several times through a Pasteur pipette before the test sample was removed. The velocity gradient technique rapidly separates the test cells containing associated intracellular antibiotic from unaccumulated drug which remains in the incubation medium. Intracellularly accumulated antibiotic was determined by a previously described method (12). The cell volume of each cell suspension was determined with tritiated water, and the intracel-

lular/extracellular distribution of antibiotic, expressed as the C/E ratio, was calculated (12).

The ability of cells to actively concentrate erythromycin was further evaluated with Formalin-killed cells. The cells were killed by treating a suspension (ca. 5×10^6 cells per ml) in growth medium minus FBS with 10% Formalin at 37°C for 30 min. The dead cells were sedimented, washed twice with growth medium, and resuspended to the original density in growth medium.

Measurement of egress of erythromycin. Egress of erythromycin was determined after incubation of the test cells in growth medium containing radiolabeled antibiotic for the time period indicated. After erythromycin accumulation, the cells were rapidly centrifuged and resuspended in prewarmed, antibiotic-free growth medium. The egress of erythromycin at the indicated times were determined by the velocity gradient technique described above.

Bioactivity of egressed erythromycin. The test cell suspensions (ca. 5×10^6 cells per ml) were allowed to accumulate erythromycin by incubation with rotary agitation (150 rpm) for 2 h at 37°C in growth medium containing 10 mg of [^{3}H]erythromycin A base per liter. The cells were sedimented by centrifugation and resuspended in prewarmed 0.1 M potassium phosphate buffer (pH 8.0). Incubation was then continued for 2 h at 37°C with rotary shaking (150 rpm). The cells were sedimented by centrifugation, and the radioactivity and bioactivity (agar diffusion assay against Sarcina luteus ATCC 9341 on Difco medium no. 11 with erythromycin A base dihydrate as the standard) of the supernatant was determined. The specific activity of the effluxed erythromycin was compared with that of the antibiotic before its incubation with the test cells.

RESULTS

The accumulation of erythromycin by various human-derived tissue culture cells is shown in Table 1. Both normal early-passage cells and continuous cancer line cells sequestered erythromycin at levels several-fold higher than external concentrations. Of the cell types tested, the highest accumulation occurred in early-passage foreskin cells, for which a C/E ratio of 12 was attained upon exposure to 10 mg of antibiotic per liter. Most of the cell types accumulated erythromycin at six to nine times the extracellular concentration, although normal liver and cancer liver cells gave lower values. Although the cells were incubated in the presence of [^{3}H]erythromycin for 2 h, most of the intracellular accumulation occurred during the first hour of exposure. All cell types treated with 2 to 20 mg of erythromycin per liter maintained ≥90% viability during the course of the incubation period.

In all cell types examined, the total quantity of erythromycin accumulated depended on the extracellular concentration of antibiotic. Although some slight differences in C/E ratios were observed when the extracellular antibiotic concentrations were varied, the disparity was less than 20% at the levels of erythromycin tested (2 to 10 mg/liter) after 1 h of accumulation. In most cases, higher initial concentrations of erythromycin gave slightly higher C/E ratios, although in some cell types there was virtually no difference between the C/E ratios obtained with varied initial drug concentrations. Cancer liver and lung cells were the only exceptions noted to the above. In these cell types, slightly lower C/E ratios were consistently observed as the initial extracellular antibiotic level was increased (Table 1).

When cells that had accumulated erythromycin were transferred to antibiotic-free medium, rapid egress of the

intracellular erythromycin occurred (Fig. 1 and Table 1). The rate of efflux usually reflected the kinetics of accumulation. Cells which rapidly concentrated antibiotic lost drug at a similar rate when the external milieu was replaced with antibiotic-free medium (Fig. 1). When cells accumulated erythromycin at a diminished rate, the kinetics of efflux were slower than in cells which rapidly concentrated antibiotic. In cells which rapidly accumulated antibiotic, egress continued until equilibrium was again established.

The extremely rapid exchange between extracellular and intracellular erythromycin was demonstrated by incubation of cells that had been preloaded with [³H]erythromycin with high levels of unlabeled antibiotic. For example, with normal lung cells, extracellular unlabeled erythromycin was added at a level of 100 mg/liter to cells that had previously reached maximal intracellular accumulation (C/E ratio, ca. 9.0) in the presence of 10 mg of [³H]erythromycin per liter. The accumulated [³H]erythromycin exited the cells at virtually the same rate as controls in which the extracellular level was initially reduced to zero (Fig. 1). Similar efflux kinetics were observed for all cell types tested. These data suggest that intracellularly concentrated erythromycin is not tightly bound to cellular components.

Cells held at 4°C did not accumulate or bind antibiotic (Table 2) or allow egress of previously concentrated eryth-
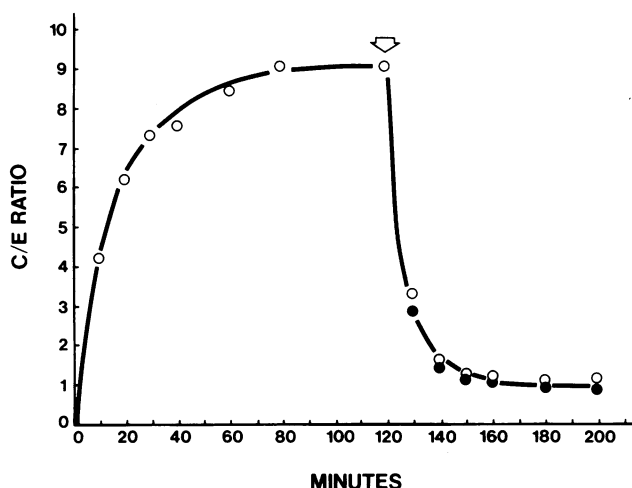

FIG. 1. Uptake, accumulation, and egress of [³H]erythromycin A by human diploid embryonic lung cells (WI-38). Cells were allowed to accumulate [³H]erythromycin (10 mg/liter) for 2 h. At the time indicated by the arrow, accumulation medium was rapidly removed and replaced with fresh erythromycin-free medium (O) or fresh medium containing 100 mg of unlabeled erythromycin per liter (●). Egress of erythromycin continued until equilibrium was again established. When the replacement medium contained 100 mg of unlabeled erythromycin per liter (10 times the concentration of the accumulation medium), the intracellular sequestered [³H]erythromycin egressed at a similar rate to that of the erythromycin-free replacement medium, thus demonstrating the fast exchange between intracellular and extracellular erythromycin.

TABLE 1. Accumulation and egress of [³H]erythromycin A by tissue culture cells of human origin[a]

Cell type	Initial erythromycin concn (mg/liter)	C/E ratio			
		Accumulation phase		Egress phase	
		60 min	120 min	20 min	40 min
Normal early passage					
Bronchus	2	7.2	8.7	3.1	1.6
	10	7.4	8.9	3.6	1.8
Foreskin	2	10.2	10.7	0.8	0.3
	10	11.8	12.0	0.8	0.3
Kidney	20	8.7	8.9	—[b]	—
Liver	2	5.6	5.0	1.6	1.0
	10	5.1	5.3	1.5	0.7
Lung	2	6.9	7.2	1.6	0.7
	10	8.3	9.3	1.6	0.8
Skin	2	8.8	9.2	4.7	2.1
	10	9.7	9.9	5.9	2.8
Cancer					
Breast	2	7.5	7.7	3.6	2.5
	10	6.5	7.7	3.1	2.1
Liver	2	4.0	5.4	1.4	1.1
	10	3.5	4.2	1.4	1.1
Lung	2	8.5	8.3	0.8	0.5
	10	7.6	7.3	0.6	0.3

[a] At designated times during the accumulation and egress phases, the concentrations of intracellular and extracellular erythromycin were determined by the velocity-gradient centrifugation method. Cells were allowed to accumulate [³H]erythromycin at the indicated concentrations. After accumulation for 2 h, egress of intracellular [³H]erythromycin was determined by rapidly collecting the cells by centrifugation and resuspending them in fresh antibiotic-free medium. For a complete description, see the text.
[b] —, Not determined.

romycin. Thus, when cells that had maximally sequestered erythromycin at 37°C were transferred to cold antibiotic-free medium, virtually no drug exited the cells upon incubation at 4°C. Binding of erythromycin to cells killed with Formalin varied depending on cell type (Table 2). Formalin-killed foreskin and kidney cells rapidly bound substantial amounts of erythromycin. In contrast, liver and breast cancer cells, treated in an identical manner, bound only miniscule quantities of erythromycin. With all cell types tested, binding was complete within 2 min and no additional antibiotic was bound with continued exposure to the drug. As the same cells at 4°C did not bind erythromycin, Formalin killing apparently altered the cellular surface, allowing antibiotic binding.

The polyfunctional erythromycin molecule is extremely sensitive to a variety of chemical and enzymatic degradations. To determine possible intracellular inactivation, we determined the specific activity of accumulated antibiotic after cellular expulsion. In these experiments, cells were allowed to maximally accumulate erythromycin, and after a suitable incubation period in an antibiotic-free milieu, the specific activity of the egressed drug was determined by measuring bioactivity and radioactivity. Since our erythromycin assay is standardized for solutions prepared in 0.1 M potassium phosphate buffer (pH 8.0), cells preloaded with radiolabeled antibiotic were transferred to this buffer for egress. Experiments indicated that, in most cases, egress was slightly slower in buffer than in the usual culture medium. The erythromycin released from all cell types was fully active, unmetabolized antibiotic (Table 3).

In contrast to the marked concentration of erythromycin, benzylpenicillin was not significantly accumulated by any cell type tested. In all cases the intracellular concentration of benzylpenicillin remained much lower than the extracellular

TABLE 2. Influence of temperature and cell viability on accumulation of [^{3}H]erythromycin A by human tissue culture cells

	C/E ratio for following cells[a]:											
	Normal early passage						Cancer					
Time of incubation (min)	Foreskin			Kidney			Breast			Liver		
	Viable		Dead (37°C, 10 mg/liter)	Viable		Dead (37°C, 10 mg/liter)	Viable		Dead (37°C, 5 mg/liter)	Viable		Dead (37°C, 10 mg/liter)
	37°C, 2 mg/liter	4°C, 10 mg/liter[b]		37°C, 5 mg/liter	4°C, 10 mg/liter[b]		37°C, 10 mg/liter	4°C, 5 mg/liter[b]		37°C, 2 mg/liter	4°C, 10 mg/liter[b]	
5	—[c]	0.34	12.78	2.73	0.18	3.88	2.38	0.42	1.46	1.97	0.41	0.24
10	6.62	0.35	12.63	4.13	0.21	4.27	3.49	0.23	1.57	3.38	0.37	0.37
20	7.91	0.48	11.68	5.36	0.57	3.66	4.59	0.26	1.42	3.99	0.41	0.41
30	8.77	0.49	9.38	6.02	0.21	3.80	5.43	0.37	1.20	5.24	0.45	0.45
40	9.55	0.50	8.98	6.60	0.21	3.61	—	0.35	1.56	4.98	0.69	0.69
60	8.97	0.45	7.33	5.97	—	3.97	6.48	0.31	1.21	5.93	0.48	0.48
80	9.21	0.46	—	6.31	—	3.89	7.67	0.23	1.49	5.88	0.50	0.50

[a] For a complete description, see the text.
[b] After harvest, cells were cooled to 4°C and maintained at this temperature during the course of the experiment.
[c] —, Not determined.

concentration (C/E ratio, 0.16 to 0.64), even after prolonged incubation.

DISCUSSION

Tissue distribution studies in dogs (14) and rats (15) with ^{14}C-labeled erythromycin have indicated that the antibiotic is concentrated in liver, spleen, kidney, lungs, pancreas, and submaxillary and adrenal glands at levels significantly above those in plasma. With a noninvasive procedure, a more recent study (27) in which ^{11}C-labeled erythromycin and positron emission tomography were used has shown that erythromycin rapidly penetrates and concentrates in human pulmonary tissue. These studies demonstrate that erythromycin accumulates in mammalian tissues at concentrations above levels in plasma, but the techniques used did not allow differentiation between intracellularly accumulated drug and antibiotic loaded in interstitial and other extravascular compartments.

The data presented here, showing the in vitro uptake and intracellular accumulation of erythromycin by a variety of human-derived tissue culture cells, strongly suggest that a similar concentration phenomenon occurs in mammalian tissues after antibiotic administration. Although anomalies associated with cells grown in tissue culture must be considered, the marked accumulation by several types of normal, early-passage cells lends further credence to this thesis.

Our findings suggest that mammalian organs and tissues, upon exposure to erythromycin, accumulate an intracellular reservoir of antibiotic from the vasa. In consonance with the tissue culture cell data, this reservoir of sequestered erythromycin would readily egress from the intracellular compartments as the extracellular level of antibiotic diminished. The released erythromycin would be unmetabolized, unaltered, fully active antibiotic.

Although the efficacy of erythromycin against a variety of infections caused by susceptible organisms has been demonstrated (1), the low and erratic levels in plasma that are usually observed after antibiotic intake has often concerned many clinicians. The blood level, however, is only one factor which must be considered when selecting an appropriate antibiotic therapy. To our knowledge, the concentration of erythromycin in plasma that is associated with therapeutic success has not been established, but the ability of the antibiotic to penetrate, accumulate, and exit eukaryotic cells must have both pharmacokinetic and antibacterial implications. We suspect that the extravascular localization of erythromycin may be more pertinent to the clinical efficacy, at least with some types of infection, than the actual levels in plasma, since active drug at the site of sepsis is the most important consideration.

For example, there is abundant evidence that some microorganisms survive or proliferate after phagocytosis by human cells (3, 10, 11, 13, 22). This frustration of the normal phagocytic-killing process must be of paramount importance in cases of persistent or relapsed infections. The intracellular location of the engulfed organisms protects them from antibiotics that are unable to penetrate and exert their action. In these cases, after termination of antibiotic therapy, the protected, intracellular organism may again fulminate into a renewed infection. The ability of erythromycin to penetrate and accumulate in a variety of eukaryotic cell types may prevent survival of sensitive phagocytosed organisms, thereby exerting profound effects on the course of an infection.

Phagocytosis, which is the ingestion of any particulate matter (e.g., bacteria) by cells, is common to all nucleated eukaryotic cells (4) and is not limited to leukocytes. Nucleated eukaryotic cells can be classified as either professional phagocytes (PMNs, monocytes, and tissue macrophages) or

TABLE 3. Comparison of the bioactivity of erythromycin A before accumulation and after egress from human tissue culture cells

| Cell type | Bioactivity of erythromycin (μg)[a] | |
	Before accumulation	After release
Normal early passage		
Bronchus	0.39	0.34
Foreskin	0.39	0.38
Kidney	0.28	0.25
Lung	0.33	0.34
Skin	0.39	0.39
Cancer		
Breast	0.41	0.45
Liver	0.28	0.28
Lung	0.28	0.28

[a] The bioactivity was that determined for 10^4 cpm of [^{3}H]erythromycin.

nonprofessional phagocytes (e.g., fibroblasts). The former group of cells normally circulates via the circulatory system and constitutes the cellular host defense; the latter group is usually fixed and does not circulate. Although the extent of cellular killing and the role of these latter cells in host defense remains obscure and undefined, nonprofessional phagocytes may influence the course of an infection.

Erythromycin, which is the antibiotic of choice for the treatment of Legionnaires disease (1), is thought to owe much of its effectiveness to its ability to penetrate, accumulate, and inhibit L. pneumophila ingested by PMNs (20). Legionnaires disease was first considered to be exclusively a pneumonic illness, and the intracellular nature of the infecting bacilli has been demonstrated in lung tissue. It is now clear that, in many hosts, the causative organism disseminates to extrathoracic organs. In some instances, infection without pulmonary involvement also occurs (18, 19). Biochemical abnormalities and direct-immunofluorescence techniques have revealed that the liver (24), spleen (25), kidney (25), peripheral lymph nodes (25), bone marrow (9, 25), brain (7), myocardium (26), and intestine (5) may be invaded. Direct evidence for the extrapulmonary involvement was confirmed when intracellular Legionella bacilli were demonstrated in liver sinusoidal lining cells (24) and kidney mesangial and endothelial cells (25) by electron microscopy.

The apparent propensity of erythromycin to accumulate not only in leukocytes but in nonprofessional phagocytes may account, in part, for the effectiveness of the antibiotic against Legionella spp. In this context, nonprofessional phagocytes would have to sequester erythromycin in the phagosomal compartment in concentrations sufficient to suppress the multiplication of intracellular Legionella organisms. At this point, we have no knowledge concerning the cellular distribution of the accumulated erythromycin. The concentrated erythromycin may not be evenly distributed throughout the cell but may be loaded only in certain cellular compartments. However, since erythromycin apparently accumulates in the phagosomes of human PMNs, as evidenced by the ability of the antibiotic to inhibit phagocytosed L. pneumophilia (20), it seems reasonable to extrapolate these findings to phagosomes of other eukaryotic cells. This leads us to suspect that all cells which concentrate erythromycin at high levels play a role in limiting the multiplication of intracellular Legionella spp. in the presence of therapeutic levels of antibiotics.

Chlamydia spp. are obligate intracellular parasites propagated in vitro in eukaryotic host cells. In their protected intracellular environment, only antibiotics that penetrate host cell membranes interfere with chlamydial growth (16). Chlamydia trachomatis has been implicated as the etiological agent in a considerable number of human male and female clinical infections (17). Although epithelial cells may be the primary host of C. trachomatis (21), the plethora of recognized and suspected clinical conditions suggests that these organisms are capable of invading a variety of cell types. Antibiotic therapy for chlamydioses usually consists of appropriate dosages of either erythromycin or tetracycline (1, 2). This suggests that these two antibiotics reach intracellular therapeutic levels in infected cells. Both antibiotics are known to penetrate and accumulate in human PMNs and alveolar macrophages (8, 20, 23). The ability of erythromycin to suppress Chlamydia infections further supports the thesis that the antibiotic penetrates and accumulates in a variety of human cell types in vivo.

In conclusion, this study indicates that erythromycin is highly concentrated by a variety of eukaryotic cell types,

and it seems likely that this intracellular drug reservoir must be of clinical importance. For many years a great deal of attention has been focused on obtaining erythromycin formulations which give predictably high and sustained antibiotic levels in plasma. These endeavors have not been entirely successful, although the resultant formulations are clinically efficacious. This indicates that, in the final analysis, high drug levels in blood may not be necessary for clinical efficacy and that, in fact, the unique ability of erythromycin to penetrate and accumulate intracellularly must be associated with its high therapeutic effectiveness.

ACKNOWLEDGMENTS

LITERATURE CITED

1. Abramowicz, M. (ed.). 1984. The choice of antimicrobial drugs. Med. Lett. 26:19–26.
2. Bergan, J. 1981. Biology of Chlamydia, p. 11–15. In P.-A. Mardh, B. R. Moller, and J. Paavonen (ed.), Chlamydia trachomatis in genital and related infections. Proceeding of the First Scandinavian Symposium on Chlamydia trachomatis. Kolle-Kolle, Denmark.
3. Easmon, E. S. J. 1979. The effect of antibiotic on the intracellular survival of Staphylococcus aureus in vitro. Br. J. Exp. Pathol. 60:24–28.
4. Edelson, P. J. 1982. Intracellular parasites and phagocytic cells: cell biology and pathophysiology. Rev. Infect. Dis. 4:124–135.
5. Foliani, J., T. F. Domenget, B. Hohn, G. Merignargues, and N. Bornstein. 1982. Maladie des legionnaires avec localisation digestive. Nouv. Presse Med. 11:2699–2702.
6. Freedman, M. H., M. C. Raff, and B. Gomperts. 1975. Induction of increased calcium uptake in mouse T lymphocytes by concanavalin A and its modulation by cyclic nucleotides. Nature (London) 12:378–382.
7. Gateil, J. M., J. M. Miro, M. Sasah, O. Ferrer, M. Rodriguez, and J. G. San Miguel. 1981. Legionella pneumophila antigen in brain. Lancet ii:202–203.
8. Hand, W. L., N. L. King-Thompson, and T. H. Steinberg. 1983. Interactions of antibiotics and phagocytes. J. Antimicrob. Chemother. 12(Suppl. C):1–11.
9. Hmayum, H. M., T. J. Bird, J. J. Daugirdas, R. C. Fruin, M. M. Shawky, and T. S. Ing. 1981. Legionnaires' disease bacillus in bone marrow. Can. Med. Assoc. J. 125:1084–1085.
10. Holmes, B., P. G. Quie, D. B. Windhorst, B. Pollara, and R. A. Good. 1966. Protection of phagocytized bacteria from the killing action of antibiotics. Nature (London) 210:1131–1132.
11. Horwitz, M. A. 1982. Phagocytosis of microorganisms. Rev. Infect. Dis. 4:104–123.
12. Johnson, J. D., W. L. Hand, J. B. Francis, N. L. King-Thompson, and R. W. Corwin. 1980. Antibiotic uptake by alveolar macrophages. J. Lab. Clin. Med. 95:429–439.
13. Lam, C., and G. E. Mathison. 1983. Effect of low intraphagolysosomal pH on antimicrobial activity of antibiotics against ingested staphylococci. J. Med. Microbiol. 16:309–316.
14. Lee, C.-C., R. C. Anderson, and K. K. Chen. 1953. The biliary and urinary excretion of erythromycin in dogs, p. 485. Antibiotic annual. Medical Encyclopedia, Inc., New York.
15. Lee, C.-C., R. C. Anderson, and K. K. Chen. 1956. Distribution and excretion of radioactivity in rats receiving N-methyl-C^{14}-erythromycin. J. Pharm. Exp. Ther. 117:265–273.

16. **Lycke, E.** 1981. Assaying antichlamydial drugs in vitro, p. 38–41. *In* P.-A. Mardh, B. R. Moller, and J. Paavonen (ed.), *Chlamydia trachomatis* in genital and related infections. Proceedings of the First Scandinavian Symposium of *Chlamydia trachomatis*. Kolle-Kolle, Denmark.

17. **Mardh, P.-A.** 1981. Medical chlamydiology—a position paper, p. 3–8. *In* P.-A. Mardh, B. R. Moller, and J. Paavonen (ed.), *Chlamydia trachomatis* in genital and related infections. Proceedings of the First Scandinavian Symposium on *Chlamydia trachomatis*. Kolle-Kolle, Denmark.

18. **McCabe, R. E., J. C. Baldwin, C. A. McGregor, D. G. Miller, and K. L. Vosti.** 1984. Prosthetic valve endocarditis caused by *Legionella pneumophila*. Ann. Intern. Med. **100**:525–527.

19. **Meyer, R. D.** 1983. Legionella infections: a review of five years of research. Rev. Infect. Dis. **5**:258–278.

20. **Miller, M. F., J. R. Martin, P. Johnson, J.T. Ulrich, E. J. Rdzok, and P. Billing.** 1984. Erythromycin uptake and accumulation by human polymorphonuclear leucocytes and efficacy in killing ingested *Legionella pneumophila*. J. Infect. Dis. **149**:714–718.

21. **Paavonen, J., and E. Vesterinen.** 1981. *Chlamydia trachomatis* in cervicitis and urethritis in women, p. 45–54. *In* P.-A. Mardh, B. R. Moller, and J. Paavonen (ed.), *Chlamydia trachomatis* in genital and related infections. Proceedings of the First Scandinavian Symposium on *Chlamydia trachomatis*. Kolle-Kolle, Denmark.

22. **Pesanti, E. L.** 1980. Protection of staphylococci ingested by macrophages from the bactericidal action of rifampin. Antimicrob. Agents Chemother. **18**:208–209.

23. **Prokesch, R. C., and W. L. Hand.** 1982. Antibiotic entry into human polymorphonuclear leukocytes. Antimicrob. Agents Chemother. **21**:373–380.

24. **Watts, J. C., M. D. Hicklin, B. M. Thomason, C. S. Callaway, and A. J. Levine.** 1980. Fatal pneumonia caused by *Legionella pneumophila*, serogroup 3: demonstration of the bacilli in extrathoracic organs. Ann. Intern. Med. **92**:186–188.

25. **Weisenburger, D. D., N. Rappaport, M. S. Ahlkwalia, R. Melvani, and E. D. Renner.** 1980. Legionnaires' disease. Am. J. Med. **69**:476–482.

26. **White, H. J., W. W. Felton, and N. C. Sun.** 1980. Extrapulmonary histopathologic manifestations of Legionnaires' disease. Arch. Pathol. Lab. Med. **104**:287–289.

27. **Wollmer, P., N. B. Pride, C. G. Rhodes, A. Sanders, V. W. Pike, A. J. Palmer, D. J. Silvester, and R. H. Liss.** 1982. Measurement of pulmonary erythromycin concentration in patients with lobar pneumonia by means of positron tomography. Lancet **ii**:1361–1364.

28. **Yoshida, S., and Y. Mizuguchi.** 1984. Antibiotic susceptibility of *Legionella pneumophila* Philadelphia-1 in cultured guinea pig peritoneal macrophages. J. Gen. Microbiol. **130**:901–906.