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Biotyping of *Chlamydia psittaci* Based on Inclusion Morphology and Response to Diethylaminoethyl-Dextran and Cycloheximide†

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Strains of *Chlamydia psittaci* from cattle, sheep, pigs, mice, guinea pigs, rabbits, cats, and parrots were subdivided based on their biological characteristics. Chlamydiae grown in the yolk sac of chicken embryos were used to infect L cell monolayers. The host cells were infected without further treatment or treated with diethylaminoethyl-dextran, cycloheximide, or both. The following criteria were used for biotyping the strains: the morphology of the inclusions and time after infection at which they appeared, the effect of chlamydial multiplication on the host cell cytoskeleton, and the change in the number of cells infected in response to diethylaminoethyl-dextran and cycloheximide. These properties were determined for 29 strains of *C. psittaci*. Based on the results, the strains were placed into eight biotypes.

The genus *Chlamydia* is divided into two species. Members of the species *C. trachomatis* deposit glycogen within the inclusion matrix and are sensitive to sulfonamides (12). Humans, exclusively, host *C. trachomatis* in nature. This species has been extensively serotyped (8), and its biological properties and growth characteristics in cultured cells are well established (2, 23). The second chlamydial species, *C. psittaci*, is described by the properties it lacks rather than by those it possesses. *C. psittaci* strains do not deposit glycogen within their inclusions and are, for the most part, resistant to sulfonamides. *C. psittaci* strains have been isolated from a wide range of avian and mammalian hosts, in which isolates produced both mucous membrane and systemic infections. Most studies of host-parasite relationships, biochemistry, and biological properties of *C. psittaci* have dealt with only a few strains. There have been several limited attempts to serotype *C. psittaci* (4, 13, 14), but an overall standardized system has not been established. The growth characteristics in cultured cells are known for only a few strains. Cell culture methods for isolation are rarely used, and *C. psittaci* infections are often diagnosed by using the group-specific complement-fixing antibody titer. It is important to further characterize and classify this heterogeneous group of pathogens. A necessary requisite is the establishment of stable, easily determined, biological properties that can be used as a basis for classification.

Diethylaminoethyl-dextran (DEAE-D) and cycloheximide treatments to the host cell can increase the number of inclusions formed by *C. psittaci* strains (6, 7). Previous studies indicated that the inclusion morphology and the response of isolates to DEAE-D and cycloheximide are properties that could be used to subdivide *C. psittaci* into biotypes (P. Spears, Ph.D. thesis, Colorado State University, Fort Collins, 1978). In the present study, we investigated the effect of DEAE-D and cycloheximide on the number of inclusions formed by a wide variety of *C. psittaci* isolates. The inclusion morphology and time at which inclusions appeared were determined in Giemsa-stained L-cell monolayers that had been infected with chlamydiae grown in the yolk sac of chicken embryos. Based on our observations, we divided the strains into eight biotypes.

MATERIALS AND METHODS

Chlamydial strains. The isolates of *C. psittaci* that were used are listed along with their origins in Table 1. Chlamydial strains were propagated in the yolk sacs of developing chicken embryos as previously described (19).

Growth and infection of L cells. Mouse L cells (L-929) were grown as monolayers, using Eagle minimal essential medium in Earle's base (K. C. Biological, Lenexa, Kans.). The growth medium was supplemented with 10% newborn calf serum, 500 μg of streptomycin sulfate per ml, and 75 μg of vancomycin per

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TABLE 1. *Isolates of C. psittaci and their origins*

Chlamydial strain	Serotype[a]	Host	Clinical finding	Specimen used for isolation	Reference/source
Biotype 1					
B577	1	Sheep	Abortion	Fetus	17
EBA-Colo	1	Cattle	Abortion	Placenta	Storz[b]
EBA-59-795	ND	Cattle	Abortion	Fetus	19
Fitz-65	1	Sheep	Abortion	Fetus	Storz[b]
LW-508	1	Cattle	Enteritis	Gut	Storz[b]
LX-206	1	Cattle	Enteritis	Gut	Storz[b]
MO-907	1	Sheep	Normal	Feces	15
Mouse Bab	ND	Mice	Normal	Lung	1
Mouse S	1	Mice	Normal	Lung	1
NGP-21/30	1	Guinea pig	Neonatal death	Pooled viscera	16
PHT-rabbit	1	Rabbit	Abortion	Fetus	Parker[c]
SVS-136	1	Cattle	Seminal vesiculitis	Semen	18
Biotype 2					
FC-Stra-12	2	Sheep	Conjunctivitis, polyarthritis	Blood	Storz[b]
FC-Stra-13	2	Sheep	Conjunctivitis, polyarthritis	Conjunctival scraping	Storz[b]
LW-613	2	Cattle	Polyarthritis	Joint fluid	22
LW-623	2	Cattle	Sporadic encephalomyelitis, polyarthritis	Gut	Storz[b]
LW-646	2	Sheep	Polyarthritis	Kidney	21
LW-679	ND	Sheep	Polyarthritis	Joint fluid	11
Biotype 3					
BED-1074	ND	Cattle	Diarrhea	Feces	Spears[d]
Cow H	1	Cattle	Normal	Feces	Storz[b]
66-P-130	1	Cattle	Normal	Feces	20
Biotype 4					
L-43	ND	Swine	Pericarditis, pleuritis	Lung	Kölbl[e]
L-71	ND	Swine	Polyarthritis	Joint fluid	Kölbl[e]
S-49	ND	Swine	Normal	Feces	Kölbl[e]
1710S	ND	Swine	Abortion	Vaginal discharge	Kölbl[e]
Biotype 5					
S-45	ND	Swine	Normal	Feces	Kölbl[e]
Biotype 6					
DD-34	ND	Parrot	Unknown		3, White[f]
Biotype 7					
FePn[g]	ND	Cat	Pneumonitis	Vaccine	Fromm[h]
Biotype 8					
Gp-ic[i]	ND	Guinea pig	Conjunctivitis	Conjunctiva	10, Murray[j]

[a] Determined by Schachter and co-workers (13, 14). ND, Not done.
[b] Isolated in the laboratory of J. Storz.
[c] Obtained from H. Parker (deceased), Montana State University Veterinary Research Laboratory, Bozeman.
[d] Isolated by P. Spears.
[e] Obtained from O. Kölbl, Federal Institute for Viral Diseases of Animals, Vienna, Austria.
[f] Obtained from L. White, Center for Disease Control, Atlanta, Georgia; originally called strain 4.
[g] Feline pneumonitis.
[h] Cultured from a vaccine produced by Fromm Laboratories, Grafton, Wis.
[i] Guinea pig inclusion conjunctivitis.
[j] Obtained from E. S. Murray (deceased), Harvard School of Public Health, Boston, Mass.

ml. Cells were planted in 60-mm petri dishes that contained six cover slips (9 by 22 mm) and were infected within 24 h of the planting time. Preparation of the inocula and treatment of the cells with DEAE-D and cycloheximide have been previously described (P. Spears, Ph.D. thesis). Briefly, inocula were prepared from freshly harvested, heavily infected yolk sacs. Monolayers were treated with 20 μg of DEAE-D per ml (Sigma Chemical Co.) in the buffer rinse used before infection. After infection, 2 μg of cycloheximide per ml (Sigma Chemical Co.) was added to the cell culture medium, which was minimal essential medium with 5% heat-inactivated fetal calf serum. The cultures were incubated at 37°C in 5% CO_2. Cover slips were

removed at selected intervals, fixed in Bouin's solution, and stained with Giemsa stain.

Observation of infected monolayers. Stained and mounted cover slips were observed at 400× and 1,000×. The number of infected cells in 50 fields from four separate areas of the cover slip (for a total of 200 fields) was counted at 1,000×. The area of the field was defined by a reticle in one of the oculars.

Criteria for definition of biotypes. Biotypes of *C. psittaci* were distinguished as follows. Giemsa-stained monolayers were examined at 18 to 20 h after infection to determine whether early inclusions had appeared by that time. Subsequently, cover slips were fixed and stained at 6- to 18-h intervals. The morphology of the early and mature inclusions was described, with attention to whether the inclusions had entire margins or were lobed or divided. Early inclusions were classified either as round if they were approximately circular, or as irregularly shaped. Infected cells were examined for changes in the shape of the cytoplasm, which would reflect alterations of the host cytoskeleton. The average number of infected cells in 50 fields in untreated monolayers was compared to that in monolayers treated with DEAE-D or cycloheximide or both. For this comparison, only preparations in which 10% or less of the untreated cells were infected were used.

RESULTS

The 29 isolates of *C. psittaci* were divided into eight biotypes. Four of the biotypes contained three or more strains, whereas the remaining biotypes contained one strain each. The inclusion morphology was most easily and accurately observed in cycloheximide-treated cells, or in other uncrowded monolayers in which the cytoplasm of the cells was large and spread out. Exceptions to the descriptions of the inclusion morphology could be found, but the descriptions given here are based on the appearance of the majority of the inclusions. The change in the number of cells infected which was caused by DEAE-D and cycloheximide treatment was averaged for biotypes 1 through 4 (Table 2). The infected cell counts in untreated and treated cells for biotypes 5 through 8, which each contained one strain, is given in Table 3. The characteristics of the eight biotypes are described below and summarized in Table 4.

Biotype 1. Chlamydial strains that cause abortions, genital tract infections, and inapparent infections were placed into biotype 1. These strains grew more slowly than other *C. psittaci*. Round or oval inclusions usually appeared around 30 h postinfection, but could be seen earlier if the monolayer was heavily infected (Fig. 1A). The compact inclusions maintained entire margins as they enlarged to fill the host cell cytoplasm (Fig. 1B). The lack of changes in the shape of the cytoplasm was notable (Fig. 1C). The host cell remained spread out on the glass until just before lysis. Many cells appeared to lyse without rounding up. DEAE-D treatment alone caused a slight variable change in the number of infected cells. An average threefold increase was seen in cycloheximide-treated cultures. Both treatments gave fourfold more inclusions (Table 2).

TABLE 2. *C. psittaci biotypes 1 through 4: change in the number of cells infected after treatment of host cells with DEAE-D and cycloheximide*

Biotype	Change after treatment with:[a]			
	Untreated	DEAE-D	Cycloheximide	Both
1[b]	1.0	1.4 (0.8–2.1)	2.8 (1.7–3.9)	3.8 (2.6–6.1)
2[c]	1.0	9.3 (4.9–14)	1.6 (1.2–2.6)	10.9 (6.2–17)
3[d]	1.0	3.2 (2.3–4.9)	1.5 (0.4–2.5)	3.8 (2.7–5.1)
4[e]	1.0	2.8 (2.3–3.8)	0.9 (0.7–1.1)	2.6 (2.2–3.4)

[a] Values indicate number of infected cells per 50 fields, divided by number of infected cells per 50 fields in untreated monolayers; the range of values is given in parentheses.
[b] Average of 7 strains.
[c] Average of 4 strains.
[d] Average of 3 strains.
[e] Average of 4 strains.

TABLE 3. *Infected cell counts of C. psittaci biotypes 5 through 8 in L cell monolayers treated with DEAE-D and cycloheximide*

Biotype	Count after treatment with:[a]			
	Untreated	DEAE-D	Cyclohexamide	Both
5 (S-45)	13	86	11	57
6 (DD-34)	40	4	52	23
7 (FePn)	57	49	94	81
8 (Gp-ic)	0	4	5	19

[a] Values indicate number of infected cells per 50 fields.

Biotype 2. *C. psittaci* isolates that cause polyarthritis, conjunctivitis, and encephalomyelitis in cattle and sheep were placed in biotype 2. Early inclusions were detected within 18 to 20 h as irregular patches of reticulate bodies near the nucleus (Fig. 2A). As the inclusions continued to develop, diffuse or patchy areas and more compact lobes were seen (Fig. 2B). Host cells rounded up well before the time of lysis (Fig. 2C). The nucleus of infected cells was deformed and displaced to the side as the inclusion grew. In young monolayers of dividing cells, many infected dikaryons were seen at 18 h (Fig. 2D). DEAE-D treatment caused an average of ninefold more infected cells. Cycloheximide treatment gave an increase of about 1.5-fold. Both treatments produced about 11 times as many infected cells per 50 fields as were present in untreated monolayers (Table 2).

Biotype 3. This biotype contained three isolates from fecal samples of cattle with intestinal

TABLE 4. *Summary of the biological properties of C. psittaci biotypes 1 through 8*[a]

Biotype	Morphology		Cytoskeletal changes in host cell	Response to treatment with:[b]			Other distinguishing properties
	Early inclusion	Mature inclusion		DEAE-D	Cycloheximide	Both	
1	Rn	C, E	S	±	+	+	Slow growth, inclusions appeared after 24 h post-infection; mature inclusions conformed closely to the shape of the host cell cytoplasm
2	Ir	D, L	RC	+	+	+	Many infected dikaryons in heavily infected, young, dividing monolayers; number of inclusions after DEAE-D treatment increased an average of 9 times, highest of all biotypes; yield of infectious progeny per infected cell decreased in treated cells[c]
3	Ir	L, D	S	+	+	+	Mature inclusions had large lobes and a perinuclear diffuse region
4	Ir	L, C	RC	+	±	+	Dividing infected cells commonly formed
5	Rn	C, E	S	+	−	+	Inclusions were more rigid; in heavily infected monolayers, many cells lysed before inclusions appeared and enlarged, pleomorphic forms were present; sulfadiazine sensitive[d]
6	Ir	L, C	S	−	+	−	Mature inclusions had light-staining network; inclusions grew close to the nucleus and could surround it; margin of the inclusions distal from the nucleus were rounded
7	Ir	L, C, E	S	−	+	+	Margins of the mature inclusions contract after fixation and staining, leaving an unstained border
8	Ir	D	S	+	+	+	Fast growing inclusions are full size before 24 h post-infection; very low infectivity in untreated cells; many defective forms in DEAE-D-treated monolayers

[a] C, Compact; D, diffuse, finely divided; E, entire margins; Ir, irregular and spreading; L, compact lobes; RC, rounded cells; Rn, round and compact; S, slight or no change until late in infection.

[b] Change in the number of infected cells in 50 fields; see Tables 2 and 3 for magnitude of the response.

[c] P. Spears, Ph.D. thesis.

[d] Determined by E. H. Stephenson.

infections. One of the host animals had diarrhea; the other two were clinically normal. Inclusions first appeared by 18 h as irregular patches of reticulate bodies or clusters of round patches (Fig. 3A). As inclusions enlarged, they developed one or more compact lobes and usually a perinuclear diffuse region. Large U-shaped lobes were seen in some inclusions (Fig. 3B). There was little rounding of infected cells until late in infection. Nuclei were deformed and displaced to the side of the cell. DEAE-D treatment caused about a threefold increase in the number of cells infected, and cycloheximide treatment caused a small, variable increase, up to twofold. The combination of both treatments caused about a fourfold increase in the infected cell count.

Biotype 4. Four porcine chlamydial isolates that were associated with various diseases were placed into biotype 4. Early inclusions were present at 18 h, in the form of irregular patches of chlamydiae. Older inclusions either had entire margins or one or more dense lobes along with a diffuse area (Fig. 4A). Infected cells were seen dividing, sometimes with the inclusion also dividing. These strains deformed the nucleus and caused the host cell to round up before lysis (Fig. 4B). There was about a 2.8-fold increase in the number of infected cells in DEAE-D-treated cells, but a slight decrease in the infected cell count in cycloheximide-treated cells. Monolayers that received both treatments had fewer infected cells than monolayers with DEAE-D alone (Table 2).

Biotype 5. The porcine isolate, S-45, which is sulfadiazine sensitive, was the only member of this biotype. Small round inclusions, sometimes several per cell, appeared within 18 h and en-

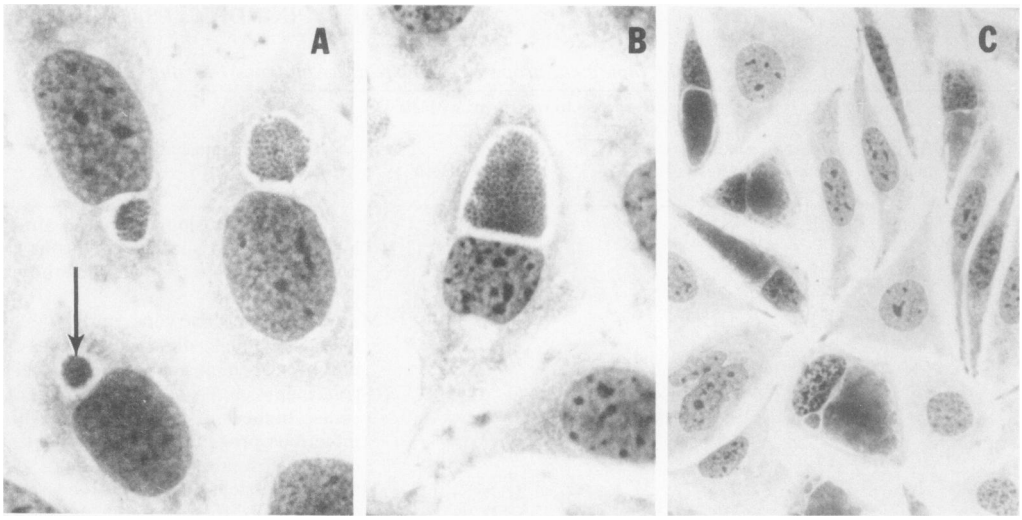

FIG. 1. *Inclusion morphology of C. psittaci biotype 1. (A) Early inclusions of biotype 1. The round or oval, compact inclusions (arrow) develop near the nucleus and enlarge maintaining an entire margin. 1,000×. (B) Developing inclusion of biotype 1. The inclusion has little effect on the shape of the host cell. The margin of the inclusion is entire. 1,000×. (A and B) Strain B577 in L cells treated with DEAE-D and cycloheximide, 40 h after infection. (C) Mature inclusions of biotype 1. The large inclusions fill the cytoplasm of the host cell without altering its shape. Strain NGP 21/30 in L cells treated with cycloheximide, 65 h after infection. 400×.*

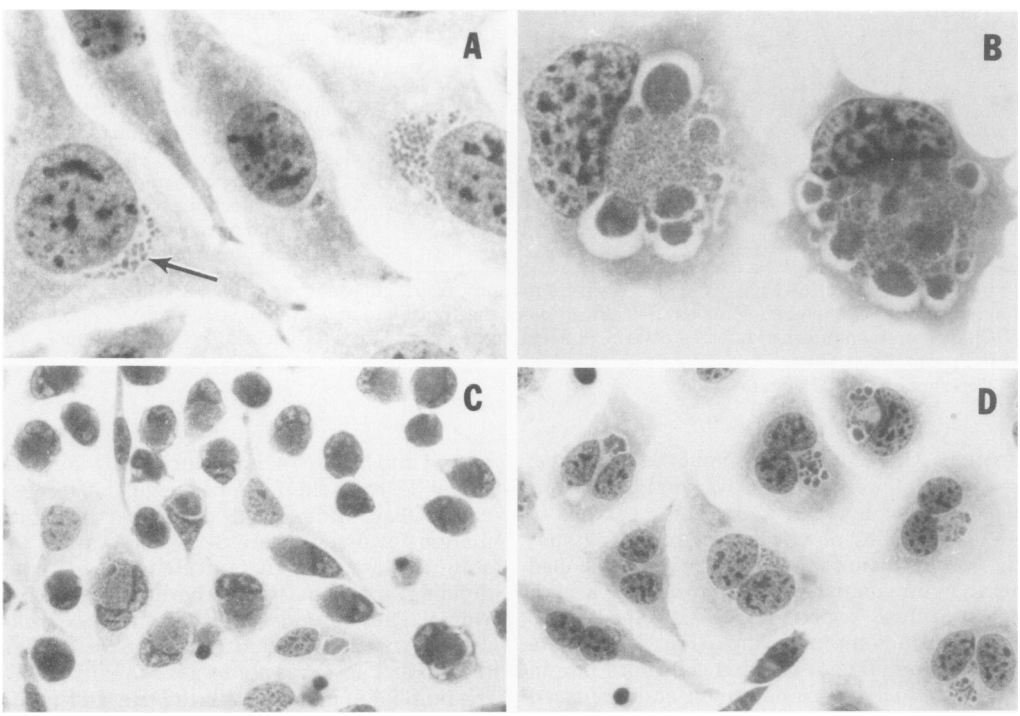

FIG. 2. *Inclusion morphology of C. psittaci biotype 2. (A) Early inclusions of biotype 2. The inclusions (arrow) have irregular shapes and are loosely spread. Strain LW-613 in L cells treated with DEAE-D, 18 h after infection. 1,000×. (B) Developing inclusions of biotype 2. Both diffuse areas and dense lobes are present. The host cells have begun to round up. Strain LW-679 in L cells treated with DEAE-D and cycloheximide, 24 h after infection. 1,000×. (C) Mature inclusions of biotype 2. The host cells are rounded up before the cell lyses. L cells treated with DEAE-D and cycloheximide, 50 h after infection with strain LW-613. 400×. (D) Infected dikaryons in an L cell monolayer 18 h after infection with strain LW-679. The binucleated cells were abundant in this DEAE-D-treated monolayer, but few were present in the monolayer treated with both DEAE-D and cycloheximide. 400×.*

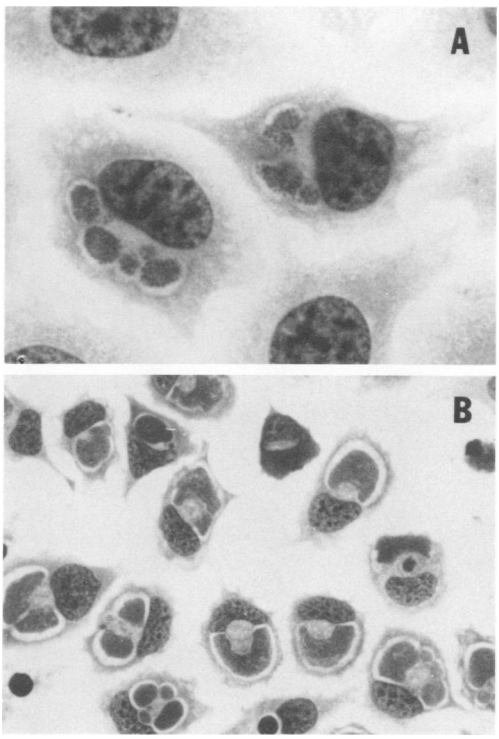

FIG. 3. *Inclusion morphology of C. psittaci biotype 3. (A) Early inclusions of biotype 3 appear as irregular or round patches of reticulate bodies. Strain Cow H in untreated L cells, 36 h after infection. 1,000×. (B) Mature inclusions of biotype 3. Large, densely packed lobes have developed, often leaving a perinuclear area that contains a few scattered chlamydial forms. Strain Cow H at 36 h after infection of L cells treated with DEAE-D and cycloheximide. 400×.*

larged to form round or oval inclusions that varied little with the shape of the host cytoplasm (Fig. 5A and B). Cell rounding only occurred late in the infectious cycle. There were enlarged, pleomorphic chlamydial forms present in many inclusions (Fig. 5C). A DEAE-D-treated monolayer had 6.5 times the number of inclusions as the untreated monolayer, but this is probably a low estimate since many of the DEAE-D-treated cells were killed within 18 h after infection, apparently without chlamydial multiplication (Fig. 5D). Cycloheximide treatment decreased the number of inclusions formed. The monolayer that received both treatments contained fewer inclusions than did the DEAE-D-treated monolayer, but also had many dead cells 18 h after infection (Table 2).

Biotype 6. This biotype contained one parrot isolate. Inclusions were present within 18 h and were either round or had a dense region with an adjacent diffuse area (Fig. 6A). As the inclusions developed, some had entire margins and others were lobed. Later the lobes enlarged and grew together, sometimes leaving a lightly stained, branched structure near the host cell nucleus (Fig. 6B). Some of the large inclusions grew surrounding the nucleus. Infected cells rounded only late in infection. DEAE-D treatment notably depressed inclusion formation, and cycloheximide treatment increased it slightly. Monolayers that were treated with both had fewer inclusions than did untreated monolayers (Table 2).

Biotype 7. A feline pneumonitis strain was placed in this biotype. Round or irregular inclusions were present by 18 h (Fig. 7A). As inclusions developed, they formed compact areas with entire margins. When the infected cells were fixed and stained, the chlamydial forms contracted from the border of the mature inclusions leaving a wide clear border and an irregular margin to the chlamydial inclusions (Fig. 7B). The inclusions conformed to the shape of the host cell cytoplasm and did not round up the cells. DEAE-D treatment caused a slight decrease in the number of inclusions. Cycloheximide treatment produced nearly a twofold in-

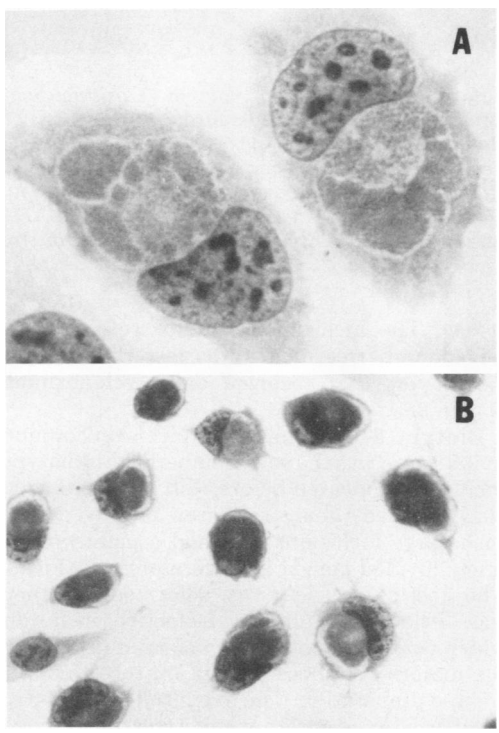

FIG. 4. *Inclusion morphology of C. psittaci biotype 4. (A) Developing inclusions of biotype 4. Dense lobes of the inclusion develop around a diffuse perinuclear area. Strain L-71 in L cells treated with DEAE-D, 24 h after infection. 1,000×. (B) Mature inclusions of biotype 4. The host cells are rounded up before lysis. Strain L-71 at 38 h after infection of L cells treated with DEAE-D. 1,000×.*

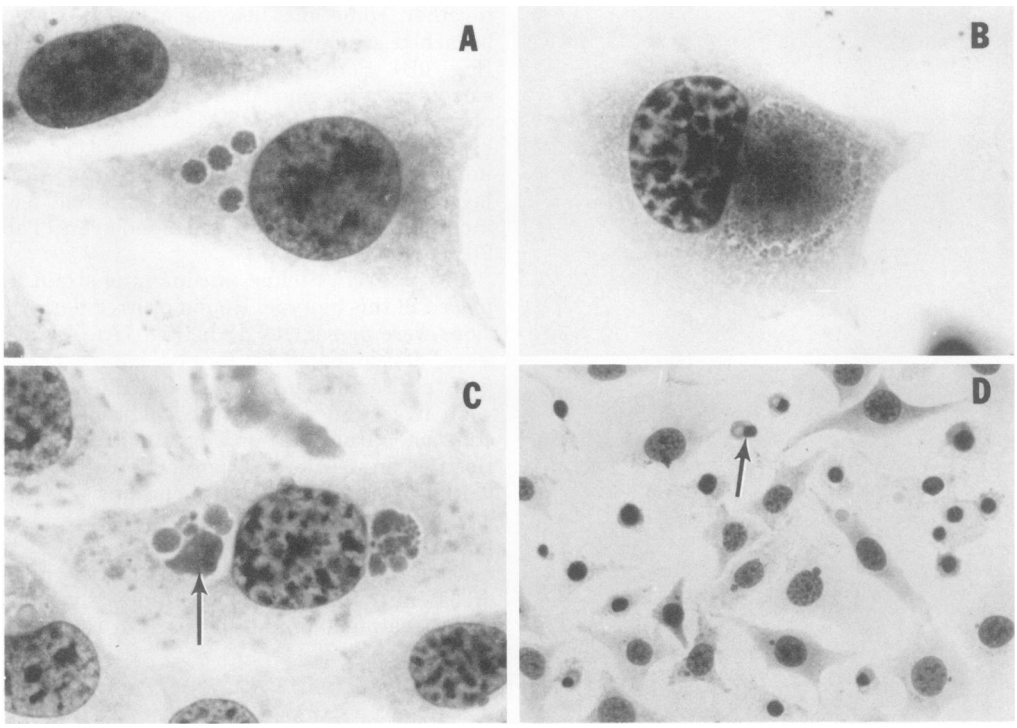

FIG. 5. *Inclusion morphology of C. psittaci biotype 5. (A) Early inclusions of biotype 5. Several round, regularly shaped inclusions. Strain S-45 at 18 h after infection of L cells treated with DEAE-D. 1,000×. (B) Mature inclusion of biotype 5. The inclusion has rounded, entire margins and does not conform closely with the shape of the cell. Strain S-45 at 38 h after infection of L cells treated with DEAE-D. 1,000×. (C) Enlarged chlamydial forms of biotype 5. Large, pleomorphic chlamydial forms (arrow) were characteristic of this biotype. Strain S-45 in L cells treated with DEAE-D, 18 h after infection. 1,000×. (D) Lysed cells 18 h after infection of a DEAE-D-treated monolayer with strain S-45. Although inclusions are still small, many cells have lysed (arrow). 250×.*

crease. The inclusion count for cells that received both treatments was less than that of monolayers that received only cycloheximide (Table 2).

Biotype 8. The guinea pig inclusion conjunctivitis strain was the sole member of this biotype. Inclusions appeared before 18 h and were irregularly shaped. This strain grew rapidly, and by 24 h, large inclusions that had a patchwork or finely divided appearance were present (Fig. 8). The host cell nucleus was deformed, but there was little cell rounding. Defective inclusions which contained only a few enlarged forms were the majority of those present on the DEAE-D-treated preparation. The magnitude of the effect of DEAE-D and cycloheximide treatments could not be estimated because of lack of inclusions in the untreated monolayer. Both of the treatments increased the number of inclusions formed (Table 2).

DISCUSSION

Since chlamydiae were divided into two groups based on their inclusion morphology (5), attempts to further subdivide *C. psittaci* by serotyping have been complicated by the presence of the group antigen, and the lack of easily performed, type-specific serology (4, 13, 14). Our observations of the inclusion morphology and response to DEAE-D and cycloheximide suggest that these biological properties are important and useful criteria for characterization and subdivision of *C. psittaci*. From this basis, more detailed molecular studies and serology can be done with an overall idea of the different groups of isolates with which one is dealing. Although inclusion morphology is a more subjective criterion, it was important to include this characteristic in biotyping because it demonstrated several unique biological properties not seen in the more objective inclusion counts of DEAE-D and cycloheximide-treated preparations. The use of both inclusion counts and morphology together was helpful to clearly distinguish all of the biotypes.

Several biological properties that warrant fur-

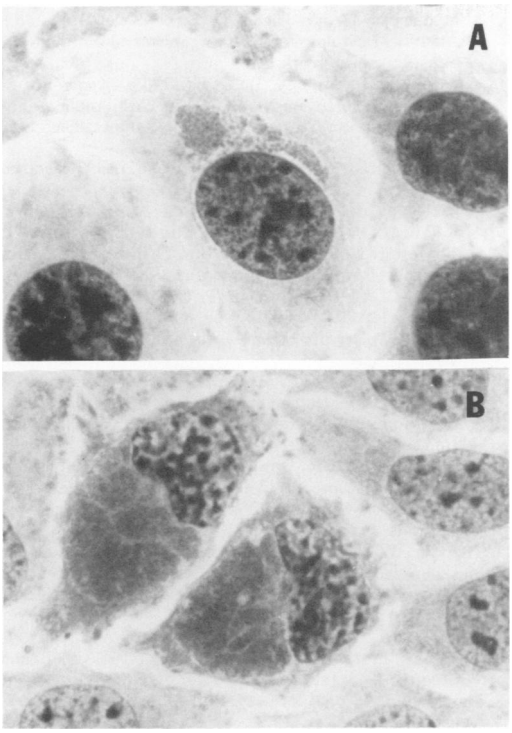

FIG. 6. *Inclusion morphology of C. psittaci biotype 6. (A) Early inclusion of biotype 6. An irregular spreading patch of chlamydiae is developing around the nucleus. Strain DD-34 at 20 h after infection of L cells treated with cycloheximide. 1,000×. (B) Mature inclusions of biotype 6. The lobes of the earlier inclusions have fused, leaving a branched, lightly stained network near the nucleus. Strain DD-34 at 38 h after infection of L cells treated with cycloheximide. 1,000×.*

ther investigation were detected in our study. The ability of the biotype 2 strains to cause dikaryon formation may be because these strains interfere with cytokinesis in a specific manner. The biotype 5 strain in infection of DEAE-D-treated cells caused more of a cytopathic effect that was similar to the immediate toxicity described by Moulder and co-workers (9) than did other *C. psittaci* biotypes. This could be because the uptake of the biotype 5 strain was greatly enhanced in DEAE-D-treated cells, or because of quantitative or qualitative differences in biochemical constituents of the biotype 5 elementary body. Biotypes 4 and 5 formed fewer inclusions in the presence of cycloheximide than in its absence, which may indicate that these strains, like the biotype 2 strain LW-613, require products of host cell protein synthesis. Strain LW-613 was previously shown to pro- duce fewer infectious progeny per infected cell in cycloheximide-treated cells than in untreated cells (P. Spears, Ph.D. thesis). Chlamydial strains with a range of responses to DEAE-D treatment of the host cell (e.g., biotype 2 versus biotype 6) could be helpful in studies of the primary interactions with host cells and analysis of the chlamydial surface components involved.

The properties used to subdivide *C. psittaci* into biotypes were apparently stable, since a biotype 2 strain that had been passed 55 times in chicken embryos behaved similarly to a strain passed four times. The biological properties of strains that were cultured in L cells on many occasions did not change. The influence of the host cell on the parameters used to define biotypes is not fully explored. Before attempting to characterize a new chlamydial isolate in a different cell culture system, investigators will need to observe the behavior of several isolates of known biotypes.

This proposed biotyping system needs to be expanded, especially to include more avian strains and isolates from respiratory infections. A system of standardized nomenclature for *C.*

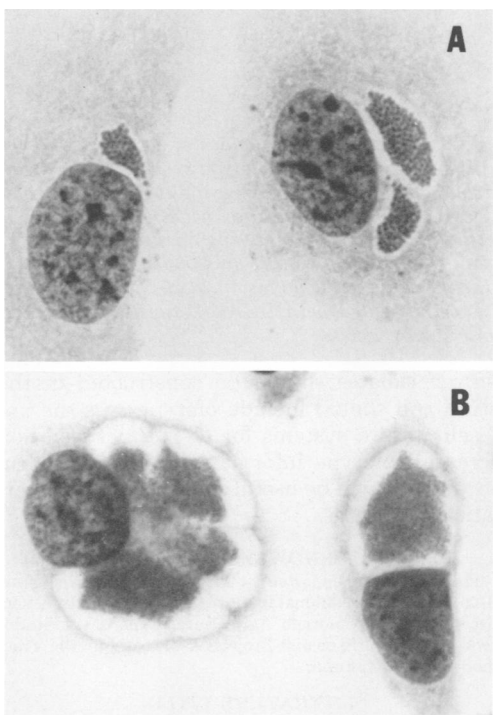

FIG. 7. *Inclusion morphology of C. psittaci biotype 7. (A) Early inclusions of biotype 7. A feline pneumonitis strain develops as irregular patches in L cells treated with DEAE-D and cycloheximide 18 h after infection. 1,000×. (B) Mature inclusions of biotype 7. After fixation and staining, there is a wide clear margin left between the cytoplasm and the chlamydial forms. Feline pneumonitis strain in L cells treated with DEAE-D and cycloheximide, 38 h after infection. 1,000×.*

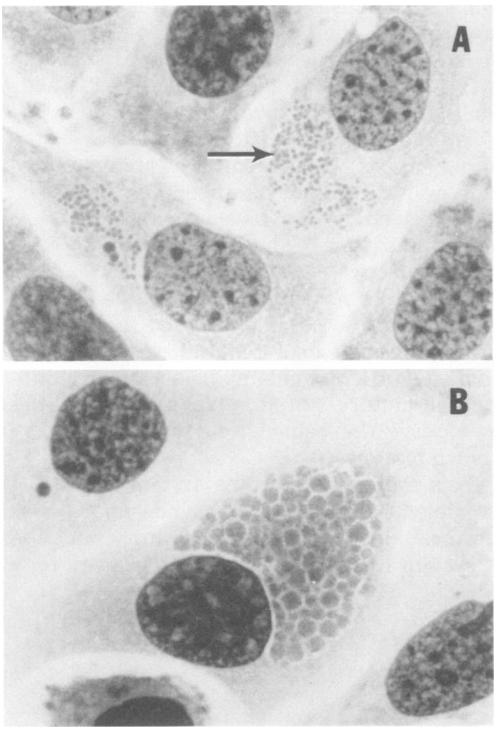

FIG. 8. *Inclusion morphology of C. psittaci biotype 8. (A) Early inclusion of biotype 8. Chlamydial forms (arrow) spread through the cytoplasm at 18 h after infection of L cells treated with DEAE-D and cycloheximide. Guinea pig inclusion conjunctivitis (Gp-ic) strain. 1,000×. (B) Mature inclusion of biotype 8. A large, finely divided inclusion at 25 h after infection of L cells treated with DEAE-D and cycloheximde. Gp-ic strain. 1,000×.*

psittaci isolates should be constructed in the future and should include biotypes. As the use of cell culture systems for *C. psittaci* isolation increases, biotype information about inclusion morphology will be useful in primary characterization of isolates.

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