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Studies on the Developmental Cycle of *Chlamydia trachomatis*: Isolation and Characterization of the Initial Bodies

BEZALEL GUTTER, YAEL ASHER, YAFA COHEN, AND YECHIEL BECKER

Laboratory for Molecular Virology, Department of Virology, Institute of Microbiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

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The initial bodies which develop in the inclusion bodies of trachoma agent (*Chlamydia trachomatis*) were separated from the infected cells nuclei and cytoplasmic components by zone centrifugation in sucrose gradients. The initial bodies are the site of the agent's ribonucleic acid synthesis and serve as precursors to the elementary bodies. The conversion of the initial bodies to elementary bodies is through a process which resembles binary fission. The effects of antibiotics on the development of the trachoma agent initial bodies revealed that rifampin prevented and hydroxyurea affected the formation of the initial bodies. Penicillin led to the formation of structures larger than the initial bodies.

Studies on the developmental cycle of trachoma agent, *Chlamydia trachomatis* (18), revealed that during the initial 24 h after infection the developing inclusion bodies in the cytoplasm of the host cells were devoid of infectivity (3, 6). During the subsequent 24 h the infectivity titer gradually increased, reaching maximal level at 48 h postinfection. Electron microscope studies made possible the correlation between the presence of initial bodies, 1 to 2 μm in diameter, in the inclusion bodies and the lack of infectivity (4, 11). The increase in infectivity titer was correlated with the gradual increase in the number of trachoma elementary bodies (EB), 0.3 μm in diameter. Recent studies by Kramer and Gordon (12) demonstrated that the initial bodies of *C. trachomatis* develop into structures which resemble prokaryotic cells in their ability to undergo binary fission prior to the formation of the EB. It was possible, therefore, to conclude that the initial bodies divide by binary fission and yield particles larger than EB, which shrink and become mature infectious EB. In these properties, *C. trachomatis* organisms resemble *C. psittaci* (14, 16, 17). Little is known about the molecular processes which occur in the initial bodies of *C. trachomatis* during their formation and transformation to EB. Recently, Gutter and Becker (10) reported that ribosomal ribonucleic acid (RNA) molecules are synthesized in the trachoma agent inclusion bodies, similarly to the RNA species reported by Tamura and Iwanga in *C. psittaci* reticulate bodies (19). The latter were purified, by Tamura, Matsumoto, and Higashi (20), by using centrifugation through a 30% sucrose solution and by density centrifugation in potassium tartrate (20-40%) gradients. In the present study, we attempted to purify *C. trachomatis* initial bodies, which were morphologically described (4, 12, 14) but not isolated. Use was made of the finding (2) that *C. trachomatis* develops in emetine-treated cells (9) under conditions which inhibit the host nucleic acids (7) and protein synthesis. The isolation of initial bodies from *C. trachomatis*-infected cells, using zone centrifugation, is reported.

MATERIALS AND METHODS

Trachoma agent and cells. The T'ang of trachoma agent (TRIC/ /IRC-PK-2/OT) was serially propagated in FL cell cultures (4) in vitro for more than 500 passages. The FL cells were infected with a suspension of EB (2) to enable the development of one inclusion body in each cell.

Labeling of infected and uninfected cells. Infected and control cells were treated for 2 h with 50 μg of emetine per ml (1, 8), labeled with 2 μCi of ^{14}C-algal protein hydrolysate per ml (specific activity 54 mCi/mmol of atom carbon; The Radiochemical Centre, Amersham, England) and 10 μCi of cytidine-5-^{3}H (specific activity 19.4 Ci/mmol; Nuclear Research Centre, Negev, Israel).

Centrifugation in sucrose gradients. Sucrose gradients (50 to 30%, wt/wt) were prepared in 0.01 M

tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, pH 7.2. A 0.5-ml cushion of 65% (wt/wt) sucrose was put at the bottom of the tube prior to the formation of the gradient. The samples were layered on the top of the gradients and centrifuged in a SW50 rotor of a Beckman preparative ultracentrifuge for 10 min at 22,000 rpm at 5 C.

Acrylamide gel electrophoresis of RNA. Samples to be analyzed by electrophoresis in acrylamide gel were treated with sodium dodecyl sulfate (SDS, 1% wt/vol) to permit free migration of RNA molecules (13). Sucrose was added to the viscous cell lysate, and 100-μliter samples were layered on polyacrylamide gels after shearing the deoxyribonucleic acid (DNA) on a Vortex mixer. Ethylene diacrylate (0.2% vol/vol) cross-linked polyacrylamide gels were used. The electrophoresis was carried out at 5 mA/gel as described by Bishop, Claybrook, and Spiegelman (5). Electrophoresis in 3% acrylamide gels was carried out using 8-cm-long gels per 5 h. The gels were frozen and sectioned into 1-mm slices, which were transferred to scintillation vials and dissolved overnight with 1.5 ml of ammonium hydroxide. Scintillation fluid (10 ml) was added to each vial, and the radioactivity was determined.

Harvest of infected cells. Prior to harvest, the cells were washed with two changes of buffer (0.01 M Tris-hydrochloride, pH 7.2) and scraped off the glass tubes with a pipette. A fine-drawn Pasteur pipette was used for the homogenization of the cells. The cell suspension was drawn and released numerous times through a fine capillary until all the cells were broken. The nuclei were spun in a refrigerated MSE centrifuge at 500 $\times$ g for 2 min. The supernatant fractions were removed and served for analysis. The nuclear pellets were also suspended in the same buffer.

Electron microscopy. The infected cells were washed with an isotonic salt solution and were fixed with glutaraldehyde (2.5%, vol/vol) for 2 h at 4 C. The glutaraldehyde was removed, and the cells were treated for 1 h with osmium. The latter was removed by subsequent washing, and the cells were dehydrated by passing through a series of alcohols at different concentrations and finally in propylene oxide. After the removal of propylene oxide, a solution containing propylene oxide and Spurr low-viscosity embedding medium (1) was added and the cells were incubated overnight at 4 C. The preparation was left at room temperature for half a day, fresh Spurr embedding solution was added, and the samples of cells were transferred to capsules. The cells were spun in the capsules and resuspended in fresh Spurr solution. The samples were incubated at 70 C for 8 h and then sectioned in a LKB ultramicrotome.

Samples containing purified elementary or initial bodies were also stained with phosphotungstic acid (2%, wt/vol).

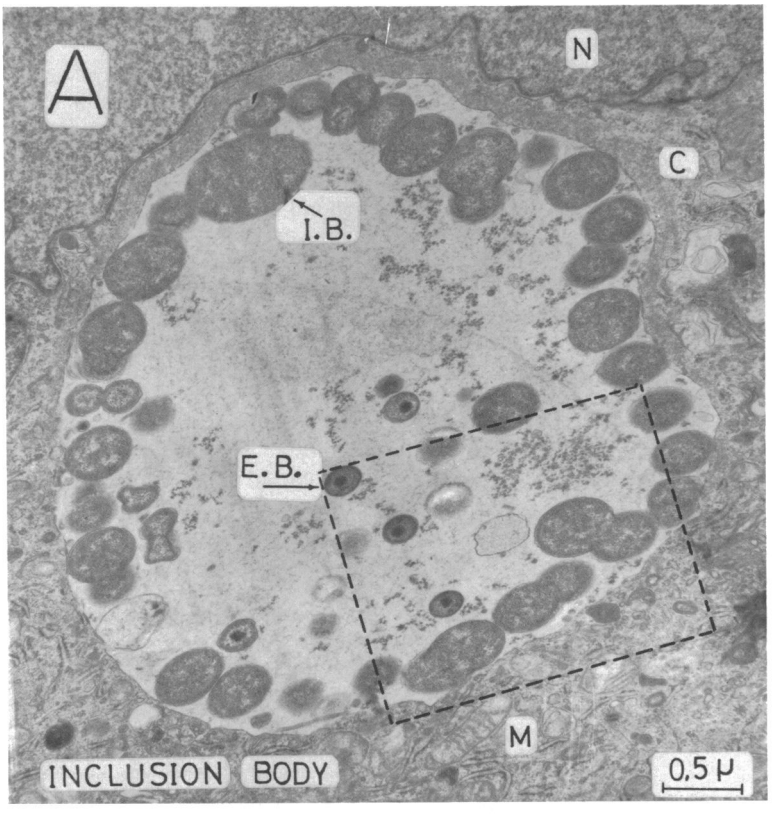
RESULTS

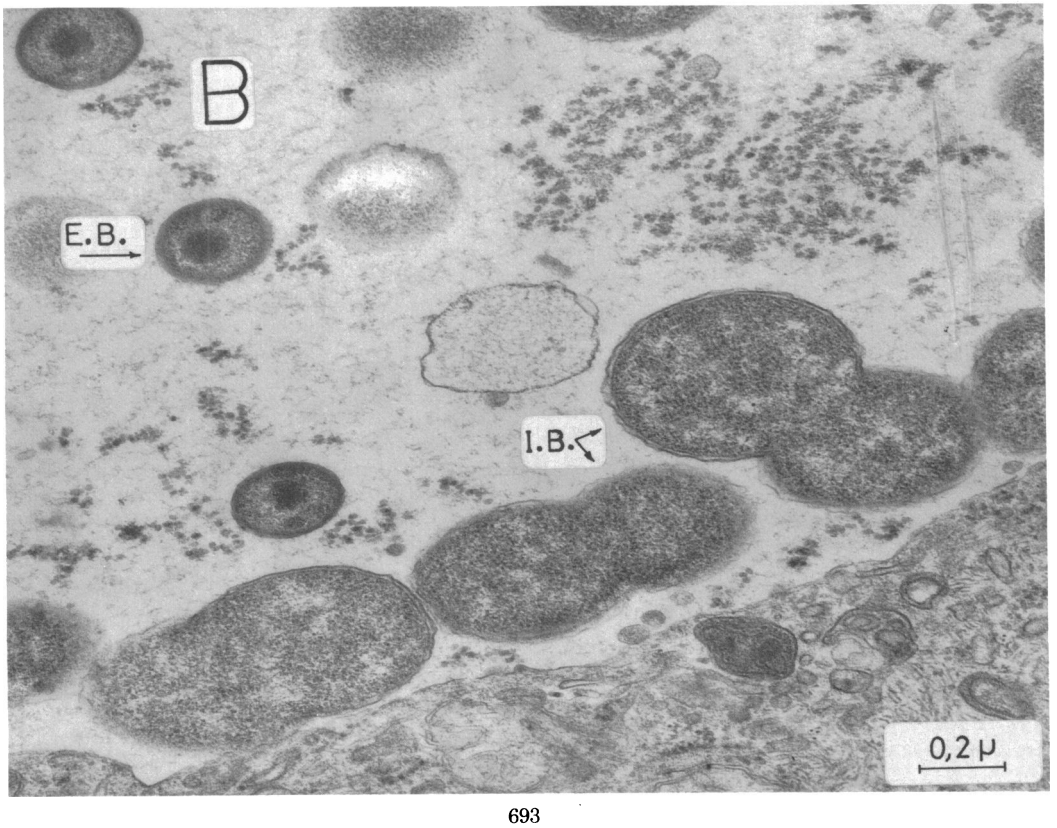
The typical inclusion body (Fig. 1) contains all the different morphological structures which are formed during the developmental cycle of the agent (4, 12). A more detailed study on the morphology of trachoma agent developmental cycle will be published elsewhere.

The presence of various developmental structures of the agent in one inclusion body clearly indicates that the development of trachoma agent is nonsynchronous. The inclusion body is a vacuole which is found in the cytoplasm of the infected cell. It has a distinct limiting membrane which differentiates between the cytoplasmic components and the agent's initial bodies (IB) and EB. The inclusion body exerts a pressure on the nucleus which is indented (Fig. 1A). Inside the inclusion body several structural elements can be seen, mostly around the vacuole membrane: (i) large round bodies with a diameter of 1 to 2 μm which are the agent's IB, (ii) IB which assume the form of dividing cells as can be seen at a larger magnification in Fig. 1B. The small particles present inside the initial bodies are probably the trachoma agent ribosomes. It is of interest that the initial bodies develop into the stage of division by enlarging their protoplasmic mass. It seems (Fig. 1B) that only one-half of the dividing initial body contains a distinct cell wall, whereas the second part has a cell membrane or an incomplete wall, and (iii) elementary bodies, 0.3 μm in diameter. The EB are in two distinct phases: the immature ones in which the nucleoid are distinct but the protoplasmic mass and the cell wall are still loose, and the mature EB with a dense nucleoid. In the present study the different developmental structures present in the trachoma inclusion bodies were isolated and studied.

Treatment of trachoma-infected cells with the alkaloid emetine was found to inhibit the synthesis of cellular proteins and also to affect the synthesis of cellular RNA (1, 8, 9). Under these conditions the synthesis of trachoma proteins and RNA was detected (1). To label the trachoma initial bodies, the infected cells, at 24 h postinfection, were labeled for 3 h with ^{3}H-cytidine and ^{14}C-algal protein hydrolysate. At the end of the labeling period one portion of the infected cells was scraped, homogenized, and analyzed by centrifugation in a sucrose

FIG. 1. *Electron microscopy of trachoma inclusion body. Infected cells were fixed with glutaraldehyde and embedded in Epon, and thin sections were prepared in an LKB ultramicrotome. The sections were stained with lead acetate and viewed in JEM 7A electron microscope. A, Section through a trachoma inclusion body containing initial (IB) and elementary bodies (EB) in different developmental stages. N, Cell nucleus; M, mitochondria. B, An enlargement of part of the inclusion body which contains developing initial bodies and elementary bodies.*


A
N
C
I.B.
E.B.
M
INCLUSION BODY
0,5 μ


B
E.B.
I.B.
0,2 μ

gradient. The rest of the infected cultures were carefully washed to remove the isotope and were reincubated up to 48 h in the absence or the presence of rifampin or chloramphenicol and then homogenized and analyzed by centrifugation in sucrose gradient. This was done to determine if the IB are the precursors of the EB and if RNA and protein synthesis are essential for the conversion of the IB to EB. Uninfected cells, similarly treated and labeled, were used as controls. The result of this experiment is presented in Fig. 2.

Centrifugation of the homogenate of uninfected cells in a sucrose gradient (Fig. 2A) showed that cellular radioactive nucleic acids sedimented to the lower part of the gradient. Due to the inhibitory effect of emetine on cellular protein synthesis, only a low background of radioactive proteins was found at the same position in the gradient. Electron microscope analysis revealed that intact nuclei sediment to the lower part of the sucrose gradient. Much more radioactivity in nucleic acids and proteins was found in the lower part of the sucrose gradient after centrifugation of homogenized infected cells. In addition to nuclei, particles which resembled the IB (Fig. 1) were also found. This result was taken to indicate that the trachoma IB band in the sucrose gradient in the position of the nuclei. Thus, the IB preparations are contaminated with the host cell nuclei.

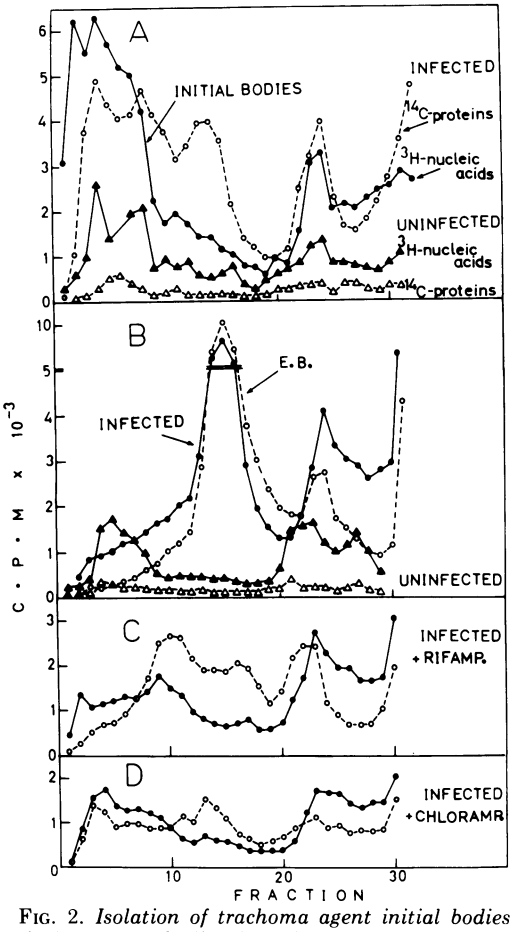


FIG. 2. *Isolation of trachoma agent initial bodies and elementary bodies from infected cells and the effect of rifampin and chloramphenicol on their development. Infected cells, treated with emetine immediately after infection, were labeled for 3 h at 26 h postinfection with ^{3}H-cytidine and ^{14}C-protein hydrolysate. Uninfected cells similarly treated were used as controls. At the end of the labeling period, the cultures were divided: part of the infected cells were harvested after careful washing, homogenized, and centrifuged in a sucrose gradient (A). Uninfected cells were similarly treated. The results of the different gradients were superimposed. The rest of the infected cultures were carefully washed and reincubated in fresh medium without the radioisotopes for an additional period of 22 h at 37 C. Uninfected cells were similarly treated and used as a control. To one part of the infected cultures rifampin (1 μg/ml) and, to another portion of the infected cultures, chloramphenicol (100 μg/ml) were added in the fresh medium. At 48 h postinfection the infected and uninfected, untreated cells were harvested and the elementary bodies progeny was isolated by centrifugation in a sucrose gradient. The two gradients were plotted in one panel (B). The rifampin-treated (C) and chloramphenicol-treated (C) infected cells were also harvested and analyzed by centrifugation in sucrose gradients. Symbols: ●——●, infected cells labeled with ^{3}H-cytidine; ○----○, infected cells labeled with ^{14}C-algal protein hydrolysate; ▲——▲, uninfected cells, labeled with ^{3}H-cytidine; △----△, uninfected cells labeled with ^{14}C-algal protein hydrolysate.*

Analysis of the infected cells, which were labeled for 3 h at 26 h postinfection and incubated for an additional period of 24 h in the absence of the isotope, is presented in Fig. 2B. It was found that the nuclei of uninfected cells band in the sucrose gradient (Fig. 2A). However, the initial bodies were converted into particles which banded in the gradient in the position of elementary bodies. All the radioactive nucleic acids and proteins which were present in the initial bodies (Fig. 2A) banded in the position of trachoma EB (Fig. 2B). This result is taken to indicate that the IB were converted during the developmental cycle to EB and served as their precursor.

To determine if the transformation of the trachoma IB to EB requires the synthesis of RNA and proteins, rifampin (1 μg/ml) and

chloramphenicol (100 μg/ml) were added to the labeled infected cultures immediately after the removal of the isotopes. It was found that in the presence of rifampin (Fig. 2C) and chloramphenicol (Fig. 2D) the formation of EB was inhibited and the IB were degraded and lost.

Previous studies on trachoma-infected cells demonstrated that 23S, 17.5S, and 16S trachoma-specific ribosomal RNA species were synthesized in the trachoma IB. The 17.5S RNA species was shown to be the precursor to the 16S ribosomal RNA (10). To determine if the initial bodies are the site of trachoma RNA synthesis the RNA species present in the initial bodies were studied by electrophoresis in acrylamide gels. Infected cells, untreated and rifampin-treated, were labeled with ^{3}H-cytidine for 1 h at 26 h after infection, and the cells were harvested, homogenized, and centrifuged in sucrose gradients to isolate the IB. The IB were obtained, and the RNA was analyzed. The IB were found to contain 23S, 17.5S, and 16S ribosomal RNA species (Fig. 3A). No such RNA species were found in rifampin-treated, infected cells (Fig. 3A). Incubation of trachoma-infected cells, labeled for 1 h with ^{3}H-cytidine for an additional period of 2 h in the absence of the isotope ("chase" experiment), resulted in the conversion of 17.5S RNA to 16S and an increase in the content of 23S RNA species (Fig. 3B). These results suggest that the IB is the site of RNA synthesis.

To achieve a better purification of the initial bodies, the homogenates of infected cells (labeled with ^{3}H-cytidine for 3 h, starting at 26 h postinfection) were centrifuged for 2 min at 1500 rpm to remove nuclei and unbroken cells. The radioactive RNA species present in the cytoplasmic (supernatant) fraction and in the nuclear fraction were analyzed by electrophoresis in acrylamide gels. It was found (Fig. 4) that most (70%) of the trachoma-specific radioactive RNA is present in the cytoplasmic fraction of the infected cells. No cellular RNA was found in the cytoplasmic fraction of uninfected cells (Fig. 4A), indicating that the contamination by cellular RNA was virtually eliminated. Trachoma-specific ribosomal RNA (about 30% of the total trachoma RNA) was also found in the pellet of the nuclei, indicating that one-third of the initial bodies are large and sedimented with the nuclei. Further studies are in progress to characterize these trachoma IB. In addition to the 23S, 17.5S, and 16S trachoma ribosomal RNA, a 32S RNA species was found in that fraction, most probably of cellular origin (Fig. 4B).

Trachoma IB were isolated from the cytoplasmic fraction of infected cells by centrifugation in sucrose gradients. Trachoma-infected cells were labeled with ^{3}H-cytidine for 3 h starting at 26 h postinfection; uninfected cells and infected cells which were treated with 10 μg of rifampin per ml served as controls. Part of the infected cells were washed to remove the isotope, reincubated until 48 h postinfection, ("chase" experiment). The cultures were harvested, and the cytoplasmic fractions were analyzed by centrifugation in sucrose gradients. The results (Fig. 5A) show that the trachoma IB band in the lower part of the sucrose gradient. Uninfected cells and rifampin-treated cells did not contain particles which banded at the position of the IB. It is of interest that several peaks of radioactively labeled IB were detected in the sucrose gradient (Fig. 5A) and radioactivity was also found at the top of the sucrose gradient. This might be due to damage of some IB. The results presented in Fig. 5B demonstrate that the IB were transformed into trachoma EB after incubation until 48 h, indicating that the initial bodies are the precursors to the EB. Some radioactivity was retained in the gradient at the position of the IB, probably due to IB which did not develop.

To characterize the trachoma IB, samples were removed from the different peaks which appeared in the sucrose gradients (Fig. 5A), and the RNA species in these particles were analyzed by electrophoresis in acrylamide gels (Fig. 6). The results presented in Fig. 6A and 6B reveal that 23S, 17.5S, and 16S RNA species were present in the heavy (peak A) and light (peak B) trachoma IB shown in Fig. 5A. The RNA present at the top of the sucrose gradient (Fig. 6C) is also trachoma-specific ribosomal RNA, suggesting that during the homogenization of the infected cells, trachoma IB are damaged and their content is retained at the top of the sucrose gradient. Analysis of the RNA species present in the trachoma EB (Fig. 5B) revealed the presence of 23S and 16S ribosomal RNA species which are present in mature EB (Fig. 6D).

The initial bodies present in the cytoplasmic fraction of infected cells which banded in the sucrose gradient (Fig. 5A) were removed and stained with phosphotungstic acid (Fig. 7). IB 2 μm in diameter (Fig. 7A) and IB in binary fission (Fig. 7B and C) were observed. The daughter cells which appear after the completion of the binary fission process are initially 1 μm in diameter (Fig. 7D). These particles become smaller (Fig. 7E) until they reach the size (0.3 μm) of the EB (Fig. 7F). These different trachoma particles are being studied.

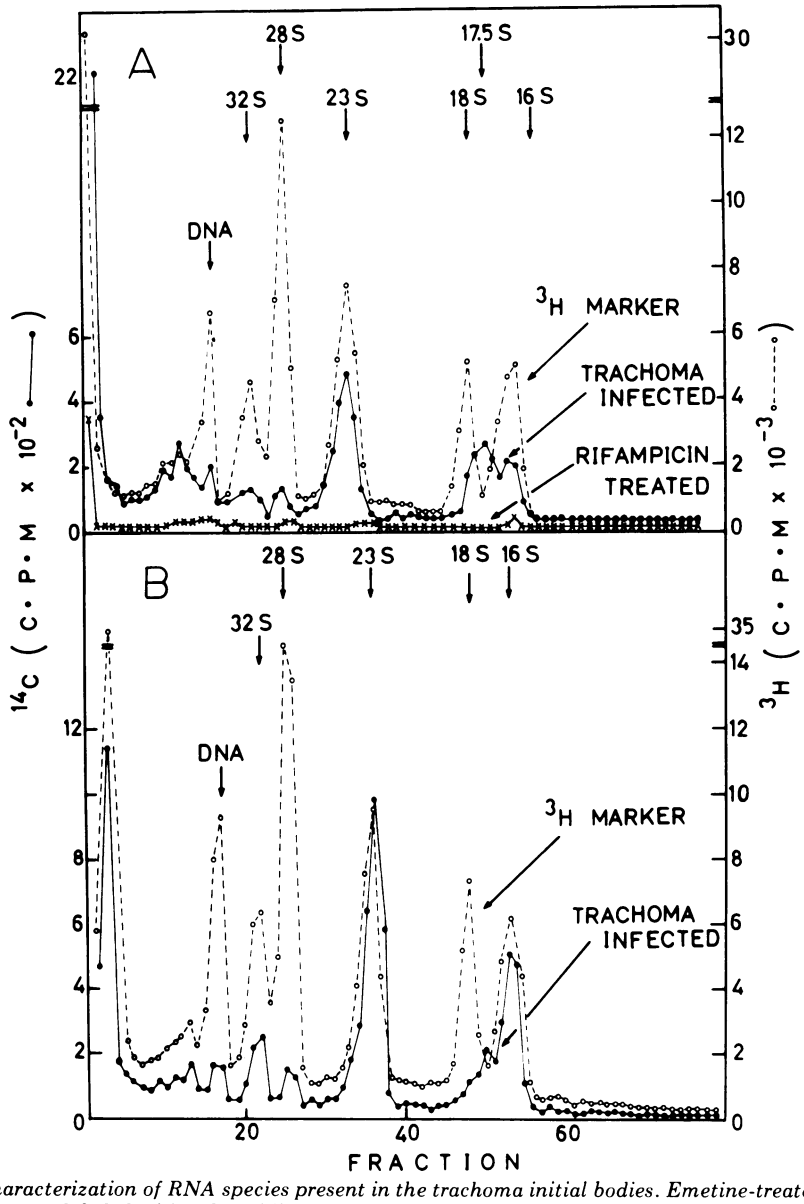


FIG. 3. *Characterization of RNA species present in the trachoma initial bodies. Emetine-treated, trachoma-infected cells were labeled with ^{14}C-cytidine for 60 min at 26 h after infection. Part of the infected cells were treated with rifampin (1 μg/ml); uninfected cells were similarly labeled. At the end of the labeling period, part of the untreated, infected cells were washed and reincubated at 37 C for an additional period of 2 h ("chase" experiment). The cultures were harvested and centrifuged into sucrose gradients. The initial bodies were removed from the sucrose gradient, and the RNA was extracted and analyzed by electrophoresis in acrylamide gels. A, Initial bodies labeled for 1 h in the absence (●——●) and presence (×- - - -×) of rifampin. The markers (O- - - -O) were a mixture of trachoma (16S and 23S) RNA and host cell RNA (18S, 28S, 32S). B, Analysis of the RNA species present in purified initial bodies labeled for 1 h and chased for 2 h. Symbols: O- - - -O, a mixture of ^{3}H-cytidine-labeled cellular and trachoma ribosomal RNA; ●——●, trachoma RNA labeled with ^{14}C-cytidine.*

The technique for the isolation of the trachoma IB was used to determine the effect of metabolic inhibitors on the developmental cycle of the agent. Sucrose gradient analysis of cytoplasmic fractions prepared from trachoma-infected cells, untreated and treated with rifampin, penicillin, or hydroxyurea, are presented in Fig. 8A. It can be seen that IB, heterogenous in

their size, were formed in the infected, untreated cells. Such particles did not form in the presence of rifampin (which inhibits RNA synthesis). A small amount of IB were formed in infected cells treated with hydroxyurea (5×10^{-4} M). Previous studies (4, 12) demonstrated that, in the absence of cell wall synthesis, due to penicillin treatment, large undifferentiated initial bodies appear in the trachoma inclusion bodies. These particles might have been sedimented together with the nuclei. Therefore, the nuclear fractions of the preparations which were analyzed in Fig. 8A were resuspended in buffer and analyzed by centrifugation in sucrose gradients (Fig. 8B). It was found that a large quantity of labeled particles which sedimented through the sucrose gradient to the bottom of the centrifuge tube were found in the nuclear fraction obtained from penicillin-treated cells. IB were also found in the nuclear fractions obtained from hydroxyurea-treated and untreated, infected cells. The chemical nature of these particles is still to be studied.

DISCUSSION

Electron microscopy of *C. trachomatis*-

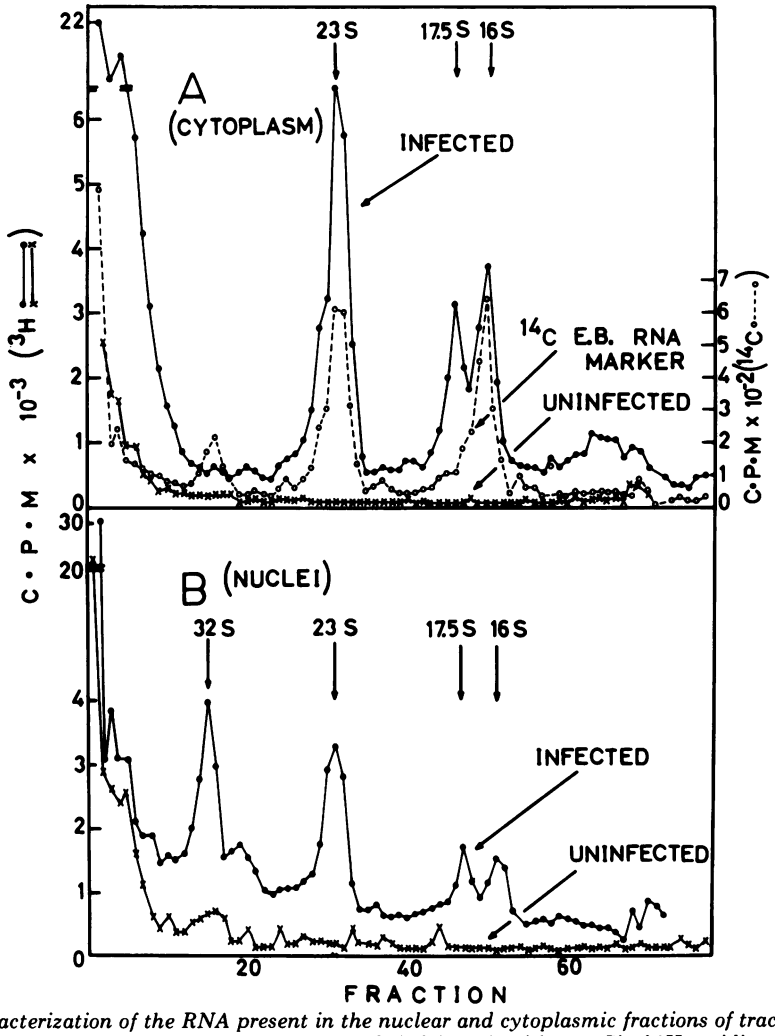


FIG. 4. *Characterization of the RNA present in the nuclear and cytoplasmic fractions of trachoma-infected cells. Trachoma-infected and uninfected cells were labeled for 3 h with 20 μCi of ^{3}H-cytidine per ml at 24 h postinfection. The cells were homogenized and the nuclei were centrifuged in a PR-2 refrigerated centrifuge at 1500 rpm. The supernatant fluid (cytoplasmic fraction) was removed, and the nuclei pellet was suspended in buffer. The two samples were treated with sodium dodecyl sulfate (1%, wt/vol) and analyzed by electrophoresis in acrylamide gels. ^{14}C-cytidine-labeled trachoma EB RNA was added to each sample and served as a marker. A, Cytoplasmic fraction; B, nuclear fraction. Symbols: ●——●, infected cells; ×——×, uninfected cells; O----O, EB ^{14}C-RNA marker.*

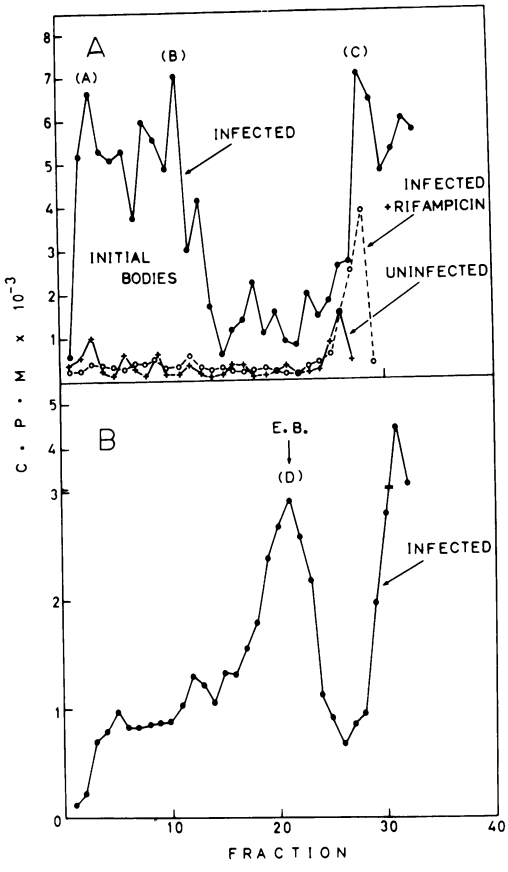


FIG. 5. *Isolation and purification of trachoma initial and elementary bodies from the cytoplasmic fractions of infected cells. Infected cells, untreated and treated with rifampin (10 μg/ml added immediately after infection) as well as uninfected cells were labeled with ^{3}H-cytidine for 3 h at 24 h postinfection. The cells were scraped off the glass and homogenized by drawing through a fine capillary. The homogenates were centrifuged for 2 min at 1500 rpm, and the supernatant fluids were removed and centrifuged into sucrose gradients. The results of three gradients are plotted in A. Part of the infected labeled cultures were washed and incubated at 37 C in fresh medium until 48 h postinfection. The cells were harvested, and the homogenates were centrifuged to remove nuclei. The supernatant fraction was centrifuged in a sucrose gradient (B). The radioactivity in the gradients was determined. Symbols: ●——●, trachoma-infected cells; O- - - -O,rifampin-treated, infected cells; ×- - - -×; uninfected cells. A, B, C, and D, indicate that samples were withdrawn from these fractions for RNA analysis.*

infected cells (Fig. 1 and references 4, 11, 12, and 15) revealed a nonsynchronous developmental cycle which is represented by IB heterogenous in size. These initial bodies are larger than the EB which were isolated and purified from cellular components by centrifugation in sucrose gradients (1). We decided, therefore, to use zone centrifugation in sucrose gradients for the isolation and purification of the different IB from the cytoplasm of the infected cells. This technique differs from the technique used by Tamura, Matsumoto, and Higashi (20) for the isolation of *C. psittaci* reticulate bodies from infected L cells. These authors used an initial sedimentation step of the reticulate bodies through a sucrose cushion and then centrifuged the pellet in a potassium tartarate density gradient. By this technique the reticulate bodies band at a density close to their own and the *C. psittaci* EB band at a higher density below the band of reticulate bodies. The technique used in the present study utilizes a milder procedure for the homogenization of the *C. trachomatis*-infected cells and a separation procedure based on sedimentation of the IB according to their size rather than to density. By this technique, large IB will sediment further into the sucrose gradient than the smaller IB and the *C. trachomatis* EB (Fig. 2). This technique made possible not only the isolation of the various forms of the initial bodies, but also their separation from the EB. Thus, it was possible to determine in the same gradient the relative content of each *C. trachomatis* structural component and to study the conversion of IB to EB.

The zone sedimentation in sucrose gradients provide a high degree of purification of *C. trachomatis* IB from cellular components. It was found that when infected and uninfected cells were gently homogenized and homogenates were centrifuged in the sucrose gradients (Fig. 2A), the nuclei banded in the gradients in the position of the IB. In the position of the EB in the gradient (Fig. 2B), no cellular contaminants were detected. To separate the IB from the nuclei, it was, therefore, essential to remove the nuclei prior to the analysis. Sedimentation of the nuclei removed about 20% of the initial bodies from the homogenate. These are most probably the very large IB. Afterwards, centrifugation of the supernatant fluids of the infected cells in sucrose gradients provided a separation between the various forms of the IB (Fig. 5A). Similar analysis of uninfected cells revealed that labeled cellular components did not band in the sucrose gradients, except at the top of the sucrose gradients (Fig. 5A). Electron microscopy of samples from different fractions of the gradient revealed only *C. trachomatis* IB. These studies were taken to indicate that pure preparations of *C. trachomatis* IB were obtained by zone centrifugation in sucrose gradients. This

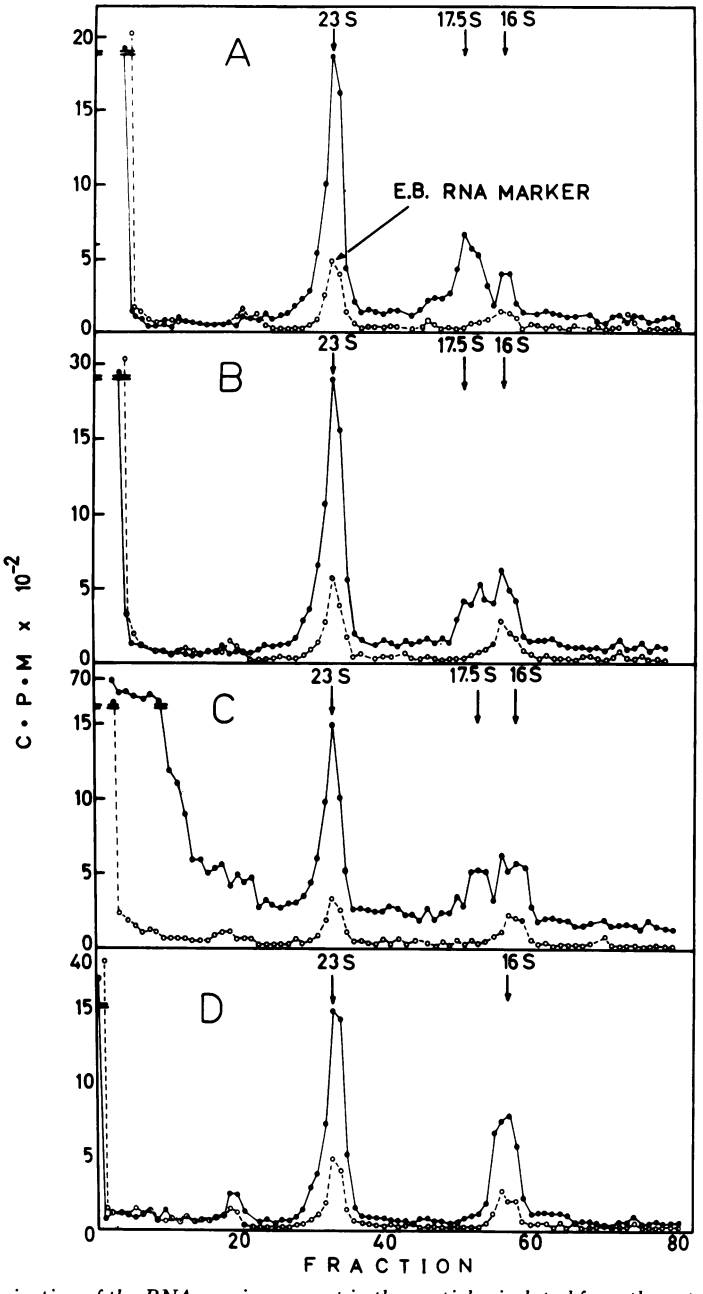


FIG. 6. *Characterization of the RNA species present in the particles isolated from the cytoplasmic fraction of the infected cells. From the gradient in Fig. 5, samples were removed (designated A, B, C, and D), and the RNA species in each was determined by electrophoresis in acrylamide gels. A, RNA from particles in tube 3 (initial bodies); B, RNA from particles in tube 12 (initial bodies); C, RNA present at the top of the sucrose gradient; D, RNA present in elementary bodies. Symbols: ●——●, ^{3}H-trachoma RNA; ○- - - -○, EB ^{14}C-RNA marker.*

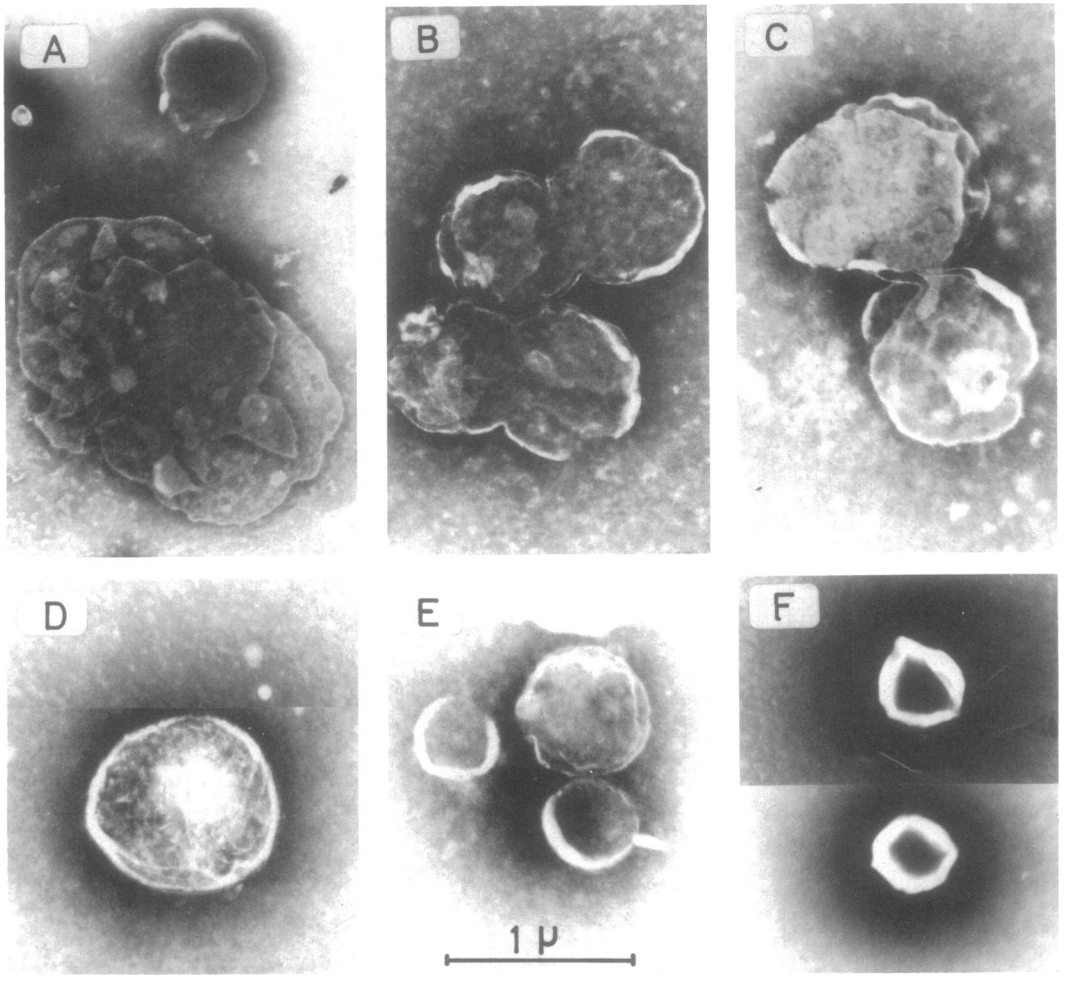


FIG. 7. *Electron microscopy of purified initial and elementary bodies. Initial bodies (A-E) from a cytoplasmic fraction of trachoma-infected cells were obtained from a sucrose gradient and stained with phosphotungstic acid. The preparations were viewed in a JEM 7A electron microscope. Purified trachoma EB were also used (F).*

procedure for the isolation of *C. trachomatis* IB could also be used for the isolation of *C. psittaci* reticulate bodies.

The development of the technique for the isolation of *C. trachomatis* IB made possible the study of the processes which take place in the developing inclusion bodies at different stages of the developmental cycle, the effect of antibiotics on these processes as well as the characterization of enzymatic processes in the IB. Most of these studies are still in progress and the utilization of the present technique for the study of two problems were presented in the present study: (i) the synthesis of *C. trachomatis* RNA in the initial bodies. It was possible to demonstrate that the 17.5*S* RNA precursor to the ribosomal 16*S* RNA species is synthesized and processed in the IB (Fig. 5 and 6) and, (ii) the effect of antibiotics on the development of *C. trachomatis* IB. We found that rifampin prevented and hydroxyurea affected the formation of IB, while large structural components of *C. trachomatis* were isolated from penicillin-treated cells. Such structures were already described in *C. psittaci*-infected cells (14) but were not yet isolated and characterized.

The isolation from infected cells of chlamydial IB by density (20) and zonal centrifugation as well as infectious EB by isopycnic gradient centrifugation in Renografin (7) provided techniques for the study of the various developmen-

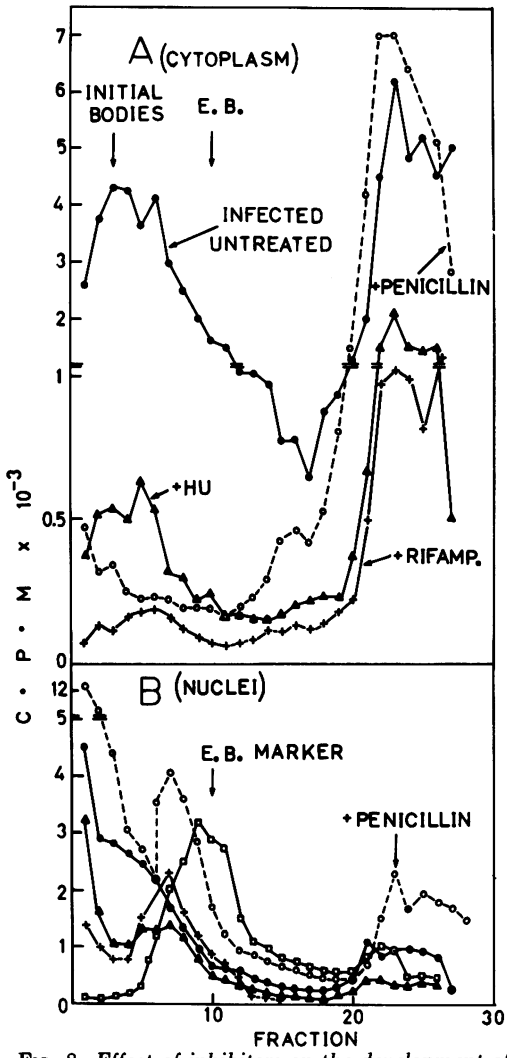


FIG. 8. *Effect of inhibitors on the development of trachoma initial bodies. Trachoma-infected cells were labeled with ^{3}H-cytidine for 4 h (starting at 24 h postinfection) in the absence or the presence of rifampin (1 μg/ml) hydroxyurea (5 × 10^{-4} M) or penicillin (100 μg/ml). The cells were harvested, homogenates were centrifuged to remove the nuclei, and the supernatant fractions of the four preparations were centrifuged on four different sucrose gradients and collected, and the radioactivity in each was determined. The results of the four gradients are plotted in panel A. The nuclei of each preparation were suspended in buffer and centrifuged on four sucrose gradients. The results of the four gradients are plotted in panel B. Purified trachoma EB were also centrifuged in a sucrose gradient to determine their position in the gradient after centrifugation. Symbols: ●——●, trachoma-infected, untreated cells; ▲——▲, infected, treated with hydroxyurea; O----O, infected, penicillin treated; ×——×, infected, rifampin treated; □——□, EB marker.*

tal forms of chlamydial agents. These techniques are essential for the studies on the molecular processes and enzymatic systems which function in the initial bodies during the developmental cycle of the agent.

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LITERATURE CITED

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