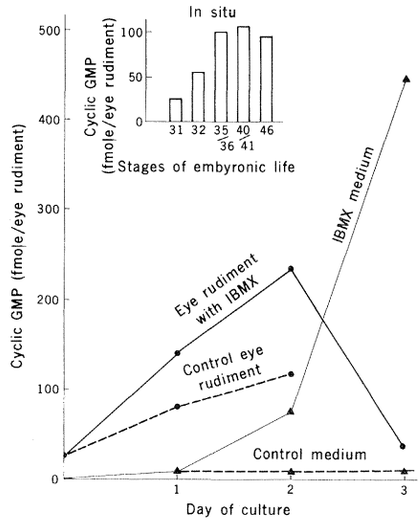

Fig. 2. Cyclic GMP content of eye rudiments and postincubation media displayed as a function of days in hanging-drop culture, with or without IBMX. After 1, 2, or 3 days of incubation, samples containing 20 to 40 eye rudiments cultured with or without $9 \times 10^{-4}M$ IBMX were frozen immediately upon collection, lyophilized, and extracted with 0.1*N* HCl. The acid extracts were lyophilized, reconstituted with sodium acetate buffer (50 m*M*, *p*H 6.2), serially diluted, and assayed for cyclic GMP by the radioimmunoassay (*8*). Values for the serially diluted samples were averaged and expressed per eye rudiment. Assay variation within a single sample was approximately 10 percent. Each value represents the mean of two samples of eye rudiments cultured separately, with intersample variation not exceeding 10 percent. The postincubation medium (with or without IBMX) was collected, lyophilized, and, after reconstitution with sodium acetate buffer, assayed directly for cyclic GMP. In order to distinguish the influence of eye rudiment maturation on cyclic GMP content in situ, rudiments were collected and processed for cyclic GMP assay without culturing (inset).

ment membranes in the space between the retina and pigment epithelium. Most of these disorganized photoreceptors remain viable at this time and only occasional pycnotic cells are encountered. Within some of the extruded photoreceptors after 2 days of culture, mitochondria are swollen and the outer mitochondria membranes have torturous, crenulated profiles. Other extruded photoreceptors contain mitochondria that are identical to those in control cultures. Altered mitochondria are more frequently encountered in rod photoreceptors than in cones. By day 3, most of the extruded photoreceptors are necrotic along with the few photoreceptors remaining in the outer retina (Fig. 1E). Cell death is selective for photoreceptors at concentrations of IBMX less than $10^{-3}M$ (Fig. 1F). Higher concentrations of IBMX result in cell death throughout the inner retinal layers as well.

The effectiveness of IBMX as an inhibitor of cyclic GMP phosphodiesterase was evaluated by measuring cyclic GMP in control and treated rudiments. Without IBMX there is an increase in cyclic GMP content of the control rudiments during the culture period (Fig. 2) which is similar to that observed in situ (Fig. 2 inset). The increase in cyclic GMP content between embryonic stages 31 and 42 probably reflects the growth and development of photoreceptor outer segments, which are rich in cyclic GMP (*3*). In the presence of $9 \times 10^{-4}M$ IBMX, cyclic GMP content in the eye rudiments increases about 80 percent above that of the control in day 1 of culture, and it becomes progressively greater than the control throughout day 2. During day 3, when photoreceptor cells are degenerating, the cyclic GMP content in the eye rudiments decreases. The concentration of cyclic GMP in the incubation medium increases during day 2 and becomes very high by day 3 (Fig. 2). This indicates that the photoreceptor cells release cyclic GMP into the medium when they degenerate.

These data show that the addition of a cyclic GMP phosphodiesterase inhibitor (IBMX) to the culture medium results in elevation of cyclic GMP in the rudiments and disorganization and death of the retinal photoreceptor cells.

It is proposed that cyclic GMP plays a role in dark-light adaptation or in the visual process (*4*). If cyclic GMP is associated with such a basic function of rod photoreceptor cells, then it is possible that inherited or acquired diseases result from errors in cyclic GMP metabolism. We have shown that in photoreceptor degeneration induced by IBMX in toads or by the *rd* gene in mice, cyclic GMP metabolism is abnormal before the cells show morphological abnormalities. In both disorders, the disruption in cyclic GMP metabolism results from a deficiency in cyclic GMP phosphodiesterase activity, and cyclic GMP content increases above normal before the photoreceptor cells degenerate. To our knowledge, this is the first simulation in normal retinas of an inherited retinal disease. Since both the IBMX-induced photoreceptor degeneration in toads and the gene-induced visual cell degeneration in *rd* mice are associated with a disruption in cyclic GMP metabolism, the etiology of these disorders may stem from the as yet undescribed role of cyclic GMP in the metabolism or function of the visual cells.

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Chlamydiae (with Phages), Mycoplasmas, and Rickettsiae in Chesapeake Bay Bivalves

Abstract. *Intracytoplasmic chlamydia-like organisms, some with phages, rickettsia-like organisms, and mycoplasma-like organisms have been found in clams and oysters from the Chesapeake Bay area by electron microscopy. None of these organisms have been previously detected in mollusks, nor have phages been previously observed in* Chlamydia *sp.*

Amorphous, basophilic, finely granular intracytoplasmic inclusions in digestive tubule cells of both hard clams, *Mercenaria mercenaria* (Fig. 1A), and soft clams, *Mya arenaria*, and in gut goblet cells of American oysters, *Crassostrea virginica*, from Chesapeake Bay or Chincoteague Bay, or both, were exam-

ined by transmission electron microscopy. Pleomorphic bodies representing the three life stages of chlamydia (*1*, *2*) were found in each inclusion from *Mercenaria mercenaria*: (i) large, round to oval, 400- to 900-nm, double membrane–bound, germinal initial bodies containing fine reticular strands of nucleic acid and a peripheral layer of ribosomes; (ii) lobulated, 400- to 600-nm, contracted intermediate bodies with a nucleoid core, peripheral ribosomes, and corrugated double plasma membrane; and (iii) small, round, dense, 200- to 300-nm infectious elementary bodies (Fig. 1B). Some chla-

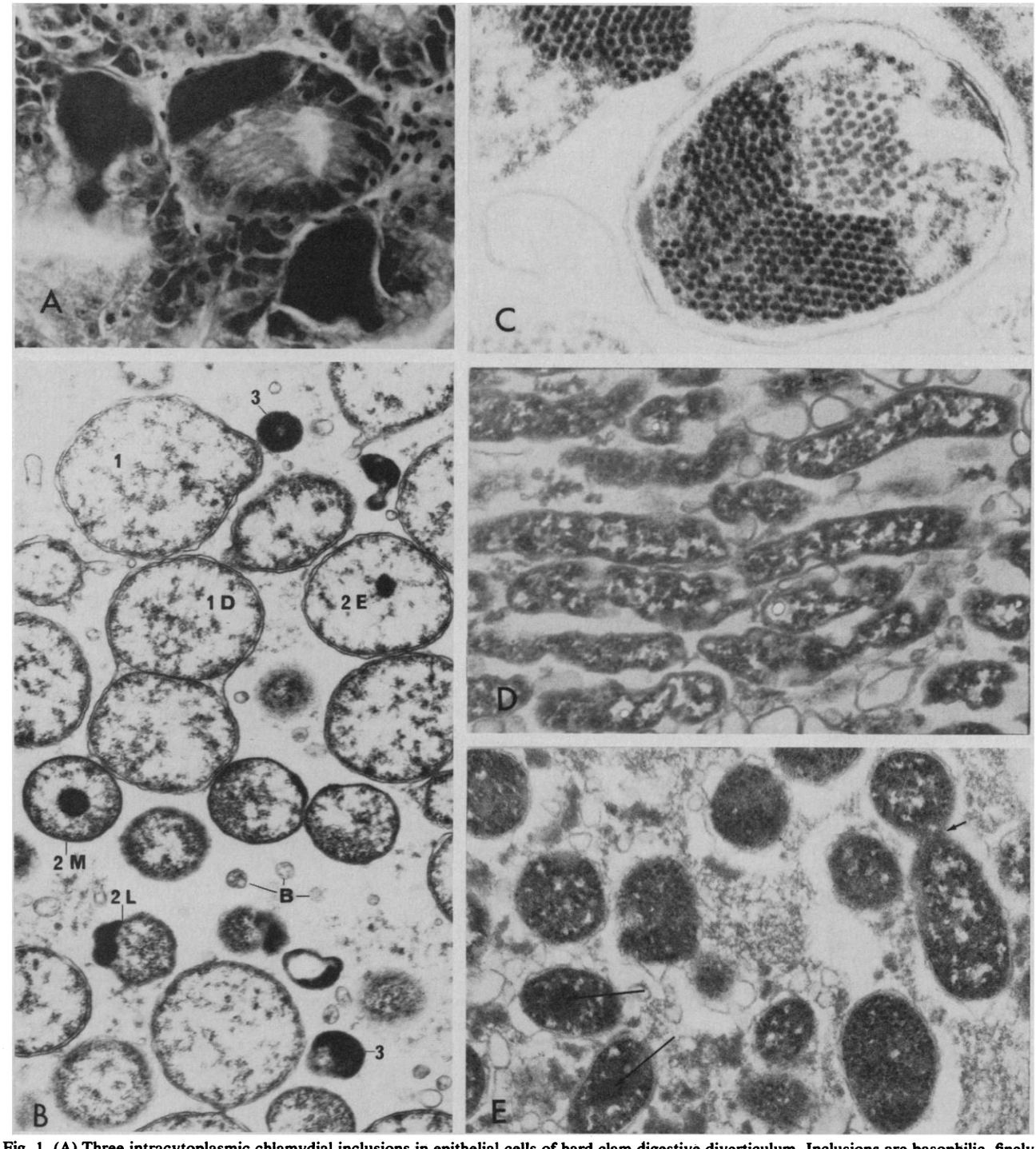

Fig. 1. (A) Three intracytoplasmic chlamydial inclusions in epithelial cells of hard clam digestive diverticulum. Inclusions are basophilic, finely granular, and irregular in shape and size. Section is 1.5 μm thick, embedded in Epon 820 resin, stained with hematoxylin and eosin (× 480). (B) Chlamydia, from a hard clam inclusion, showing all developmental stages: large reticulate initial bodies (1) including a pair of daughter cells still united by a cell membrane following binary fission (1D); intermediate bodies in early stage (2E), middle stage (2M), and late stage (2L) of nucleoid condensation; and small fully condensed elementary bodies (3). Small blebs (B), expelled from initial bodies, lie among the organisms. Two membranes are distinct on initial and intermediate bodies (× 28,000). (C) Icosahedral virus particles in crystalline arrays within greatly distended initial bodies of chlamydia from hard clams (× 40,000). (D) Curved and recurved rod-shaped, ribosome-rich, double membrane–bound, rickettsia-like organism with slightly rippled surfaces in inclusions from epithelial digestive diverticular cells of soft clams. Lucent vacuoles are apparent in the cytoplasm. Ghost cells lie among the organisms (× 28,000). (E) Round to oval, ribosome-rich, mycoplasma-like organism in inclusions from gut goblet cells of the American oyster. Cell division (short arrow) and dense bodies (long arrows) are demonstrated (× 51,000).

mydiae contained phage particles 50 nm in diameter in a crystal lattice array (Fig. 1C).

Organisms in digestive tubule inclusions of soft clams were ribosome-rich undulating rods measuring 300 by 2500 nm (Fig. 1D) characteristic of rickettsiae (*3*). Organisms in gut goblet cell inclusions in oysters were ribosome-rich, round to kidney bean–shaped bodies 250 to 350 nm in cross section and 400 to 1000 nm in length (Fig. 1E) characteristic of mycoplasmas (*4*).

Corroborative fluorescent antibody analyses remain to be carried out on fresh material. If verified, these findings may have far-reaching economic and public health significance. Serious diseases of humans and domestic animals are caused by organisms in all three of these groups, and all the species of bivalves found infected with these organisms are consumed raw. Psittacosis, an avian disease caused by a chlamydia that also infects humans, is known from numerous species of birds (*5, 6*), many of which are permanent or transitory residents of the Chesapeake Bay area. These observations raise the possibility that bivalves may be alternate hosts for zoonotic chlamydial, rickettsial, and mycoplasmal microorganisms. However, the discovery of a chlamydial phage suggests a potential mechanism by which pathogenic chlamydiae may be controlled.

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Latent Form of Scrapie Virus: A New Factor in Slow-Virus Disease

Abstract. *Scrapie is an unusual slow-virus disease of sheep which is very much like kuru and Creutzfeldt-Jakob disease, both fatal, slow neurological diseases of man. In mice, scrapie usually has an incubation period of about 6 months. Intraperitoneal inoculation of virus particles into newborn mice caused no disease, and there was no detectable virus replication for 1 year, but high titers of scrapie were present in the spleen and brain at 18 months. Virus replication occurred in mice injected from 4 days after birth by all inoculation routes, whether or not they were injected with scrapie virus on day 0. The results suggest that scrapie virus replicates peripherally only in thymocytes, which are not present in mice until a few days after birth. The latent state suggests that the comparable human diseases could appear in later life as a result of perinatal infection. In some respects these diseases resemble premature senility.*

Scrapie, an old and mysterious neurological disease of sheep, has received great attention as a classical example of slow-virus disease (*1*) and as a replicating agent of unusual smallness and stability that so far defies analysis or definition. It has been described as a subviral entity able to reproduce but possibly lacking the nucleic acid common to all known life forms (*2*), or as an agent comparable to a gene or provirus (*3*).

The importance of scrapie to man lies in the fact that it is the best known example of the spongiform encephalopathies, a group which includes two lethal diseases of the human brain, Creutzfeldt-Jakob disease and kuru, the "occupational" disease of the cannibals of New Guinea (*4*). The recent finding of cerebral amyloid deposits in murine scrapie suggests that there may be a virus cause for the similar amyloid plaques found in aged humans and in the premature senility of Alzheimer's disease and Down's syndrome (*5*). Thus the scrapie agent may offer clues to a cause of human aging.

Our results show that this agent can exist in a latent, undetectable form for many months and then return to its replicating infective state. This makes the provirus view more plausible. Newborn mice showed no disease or virus for about 1 year after they were injected intraperitoneally with scrapie virus, yet at 18 months their tissues contained vast numbers of infectious particles.

Albino Swiss mice of the Nya : NY-LAR (or Albany) strain were collected within 18 hours of birth, and three litters were inoculated intraperitoneally with 0.03 ml of brain tissue from mice infected with scrapie virus. The brain tissue was diluted 10^{-1} in tris-buffered 0.7 percent saline at *p*H 7.2. The inoculum contained $10^{6.1}$ LD_{50} (where LD_{50} is the dose that could kill 50 percent of the population) of scrapie virus (Compton strain) (*6*), determined by intracerebral mouse titration and the Reed and Muench end-point method (*7*). This large dose was used to encourage the possible induction of a state of immune tolerance to the scrapie agent in a manner analogous to that known to occur with lymphocytic choriomeningitis virus (*8*). Three other litters were similarly inoculated with ten times less virus ($10^{5.1}$ LD_{50}); sufficient additional litters were set aside for the same inoculation routine to be carried out on each of the following 5 days. A control group of three litters remained uninoculated. Three other litters that were inoculated with a 10^{-2} dilution of normal mouse brain in the same diluent remained well throughout the experiment (data not shown). In addition, 20 mice each weighing 8 to 10 g were inoculated intraperitoneally with each dose of scrapie virus as a positive control of mortality in adult animals. All male mice were removed from the experiment to eliminate the morbidity and mortality caused by fighting.

The results produced two main types of curves (Fig. 1). The control adult animals and older infants injected with scrapie virus showed a pattern of mortality beginning at 7 to 8 months, with a steep slope going up to 70 to 100 percent mortality by 10 months. The other curves showed a slow progressive increase in mortality until month 18.

Newborn animals responded to intraperitoneal injections of scrapie virus in a way similar to that of uninoculated controls: the expected clinical signs of scrapie and the expected mortality due to the disease were absent. In mice given high doses of virus on days 0 and 1 the signs of scrapie and mortality due to the disease were absent, whereas 18 animals injected on day 2 showed a 66 percent mortality by the tenth month, the incubation period relative to adults thus being extended 2 months. The animals injected