Legionnaires' Disease: Structural Characteristics of the Organism

Abstract. *The Legionnaires' disease organism was isolated from lung tissue taken from two fatalities of the Legionnaires' disease epidemic that occurred in Philadelphia during 1976. In yolk sac tissue the agent grew as a small coccobacillary microorganism, which was Gram variable and Giménez positive. Intracellular coccoid and bacillary forms, detected by electron microscopy, within and without vacuoles, underwent multiplication by septate binary fission. Some of the intracellular forms resembled obligate intracellular pathogens. On defined bacteriologic media, the organisms were predominantly bacillary. The organism conforms to the morphologic criteria of a prokaryocyte.*

An outbreak of a severe, febrile, respiratory illness (Legionnaires' disease) occurred in association with the State American Legion Convention held in Philadelphia during July 1976 (*1*). The Pennsylvania Department of Health, Bureau of Laboratories, isolated a microorganism from segments of lung tissue obtained at necropsy from two fatal cases of the outbreak (*2*), and we have studied the morphology that the isolates assume in cultures of yolk sac tissue and on bacteriologic media.

The two isolations were achieved by inoculation of suspensions of human lung tissue into guinea pigs and one passage of suspensions of infected guinea pig spleen into embryonated hens' eggs. The organism was passed in guinea pigs for 96 hours and in embryonated hens' eggs for 5 days. Yolk sac material that contained the Legionnaires' disease organism was incubated in both microaerophilic and anaerobic environments on Mueller-Hinton media supplemented with 1 percent Isovitalex and 1 percent

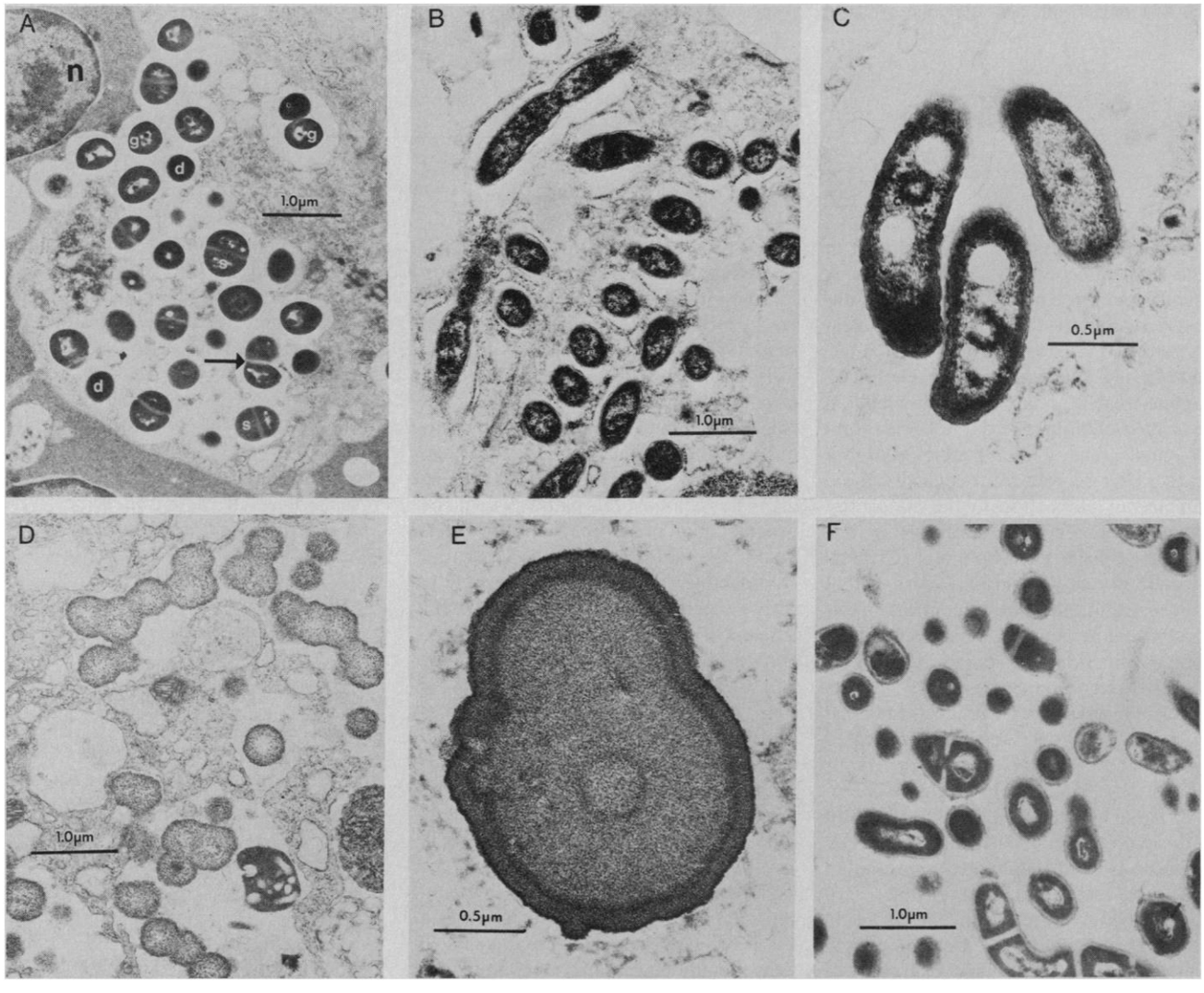

Fig. 1. (A) An electron micrograph of yolk sac cells illustrates replicating forms of the Legionnaires' disease organism, some of which are undergoing septate binary fission; *d*, dense body; *g*, gray body; *s*, septate body. The arrow points to a septate body which is forming two daughter cells; *n*, nucleus (lead citrate, uranyl acetate). (B) Stippled coccoid and bacillary forms are located within the cytoplasm of a yolk sac cell (lead citrate, uranyl acetate). (C) Three unstippled bacillary forms exhibit round electron-lucent zones and aggregates of electron opaque material that resemble nucleoids (lead citrate, uranyl acetate). (D) Electron micrograph of an infected yolk sac culture illustrates shadow forms that appear as isolated rounded structures and in fused chains (lead citrate, uranyl acetate). (E) Sporelike structure exhibits an osmophilic border and a grainy central zone (lead citrate, uranyl acetate). (F) The Legionnaires' disease organism, grown on bacteriologic media, exhibits pleomorphism and reproduction by septate binary fission (lead citrate, uranyl acetate).

0036-8075/78/0224-0896$00.50/0

hemoglobin (Baltimore Biological Laboratories). Control tests with diluent devoid of lung samples yielded no microorganisms in the yolk sac culture. To identify the presence of the Legionnaires' disease organism in culture, all isolates from yolk sac tissue and bacterial cultures were stained with Gram and Giménez reagents, and by an indirect immunofluorescent technique in which human convalescent serums that contained antibodies to the Legionnaires' disease organism were used. When tested with the convalescent serums, the microorganisms exhibited green fluorescence in a coccobacillary configuration. Samples taken from viable chick embryos and 7-day-old colonies grown on bacteriologic media were examined by electron microscopy. Specimens were fixed for 48 hours in 2.5 percent glutaraldehyde, postfixed with 1 percent osmium tetroxide, and processed for routine electron microscopy.

The organisms, when grown in yolk sacs, stained variably with the Gram stain. Giménez-stained smears disclosed intracellular pleomorphic cocci, coccobacilli, beaded bacilli, and infrequent filamentous bacilli. The particles ranged in size from 300 nm to 3.7 μm. By electron microscopy, the agent exhibited a complicated intracellular morphology which consisted of replicating coccoid forms (Fig. 1A), stippled coccoid and bacillary forms (Fig. 1B), unstippled bacillary forms (Fig. 1C), shadow forms (Fig. 1D), and sporelike structures (Fig. 1F). Replicating forms, those particles that underwent reproduction by septate binary fission, and stippled coccoid and bacillary forms, those particles which contained small intracytoplasmic granules that resembled ribosomes, were located primarily within vacuoles. The unstippled bacillary forms were usually found within the cytoplasm outside vacuoles, and, by contrast, contained round electron-lucent zones, coarse granules, and one or two nucleoids. In the vicinity of sporelike structures, shadow forms were numerous. It is possible that some of the intracellular structures that we observed may represent degenerating or necrotic organisms.

The organisms grown on bacteriologic media were predominantly bacillary; but coccoid, filamentous, and pleomorphic configurations were observed (Fig. 1F). The extracellular forms exhibited a paucity of cytoplasmic organelles and reproduction by septate binary fission; nuclear membranes and mitotic figures were absent. Several morphologic components that were detected in yolk sac material were not observed in samples taken from cell-free cultures.

The Legionnaires' disease organism conforms to the morphologic criteria of a prokaryocyte. The extracellular and intracellular growth exhibited by the microorganism are compatible with transmission to humans from a form that is native to the environment, possibly involving an animal reservoir. Moreover, the observation of sporelike structures suggests possible resistance to environmental adversity. Some of the intracellular cytoplasmic coccoid and bacillary structures are morphologically similar to obligate intracellular pathogens of the order Chlamydiales, the order Rickettsiales, and small bacterial bacilli (*3*). It is possible that the organism of Legionnaires' disease, with its ability to multiply intracellularly and extracellularly, is related to an environmental microbe from which the larger obligate intracellular pathogens have evolved.

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3 October 1977; revised 6 December 1977

Detection of Bityrosine in Cataractous Human Lens Protein

Abstract. *Bityrosine was isolated from the insoluble protein of human cataractous lenses. Identification was based on correspondence with synthetic bityrosine with respect to chromatography, fluorescence, and ultraviolet and mass spectra. It is suggested that the compound may form cross-links with polypeptide chains in old and cataractous lenses, causing significant alteration in native protein structure.*

The basis for recent interest in fluorescent components associated with human lens protein is due to the following observations. (i) With aging, there is a dramatic increase in nontryptophan (NT) protein-bound fluorescence (*1–3*); (ii) the fluorescence appears to be associated primarily with the high-molecular-weight (HMW), yellow, insoluble protein fractions in the inner region of the lens (*1, 2, 4*); (iii) the increase in fluorescence appears to be related to increases in HMW and insoluble protein (*1, 2, 4*); and (iv) the age related production of such protein has been linked to the development of certain types of senile cataract (*5*).

It is now apparent that there is a complex group of fluorescent components associated with lens proteins. Up to the present time only one such compound, a beta carboline (*6*), has been reported.

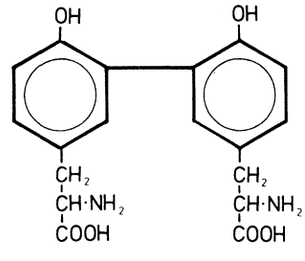

Fig. 1. Bityrosine.

Evidence has also been obtained in our laboratory suggesting the presence of anthranilic acid, a tryptophan degradation product (*7*). We now report the detection of bityrosine (Fig. 1) in the insoluble protein fraction of human cataractous lenses and confirm the unpublished suggestion of its presence in such preparations (*8*). This compound has previously only been found in connective tissue proteins (*9, 10*) such as collagen (*11*). In the lens such protein is found only in the capsular layer that surrounds the exterior of the tissue and represents approximately 1 percent of the lens weight. Nevertheless, in some experiments the capsule was eliminated before processing the lenses.

Human cataractous lenses were homogenized at 0°C in 0.01*M* tris buffer (*p*H 7.6), 0.1*M* KCl, 0.004*M* EDTA at a concentration of 1 g of lens per 10 ml of buffer and then centrifuged at 18,000*g* for 15 minutes at 4°C. After removing the supernatant, the white fraction of the precipitate was eliminated by additional washing. The remaining yellow precipitate was suspended in 0.1*M* KCl, succinylated, reduced, alkylated, and finally precipitated with acetone from an acidified solution. For general details, see Spector *et al.* (*2*). The lyophilized precipitate was suspended in 0.1*M* NH_4HCO_3 and digested in the dark with trypsin.

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