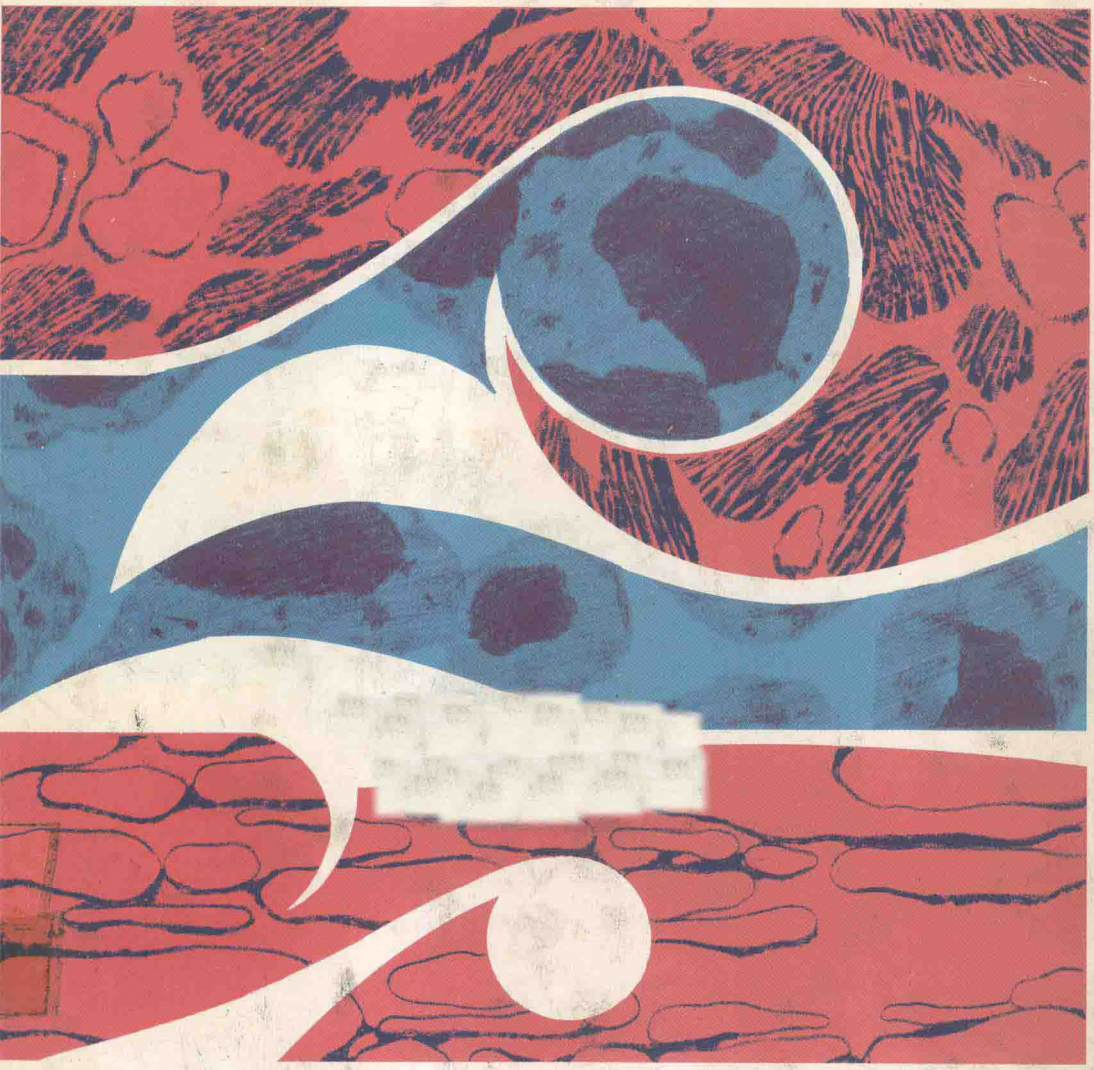
Julius Schachter
Chandler R. Dawson

HUMAN CHLAMYDIAL INFECTIONS



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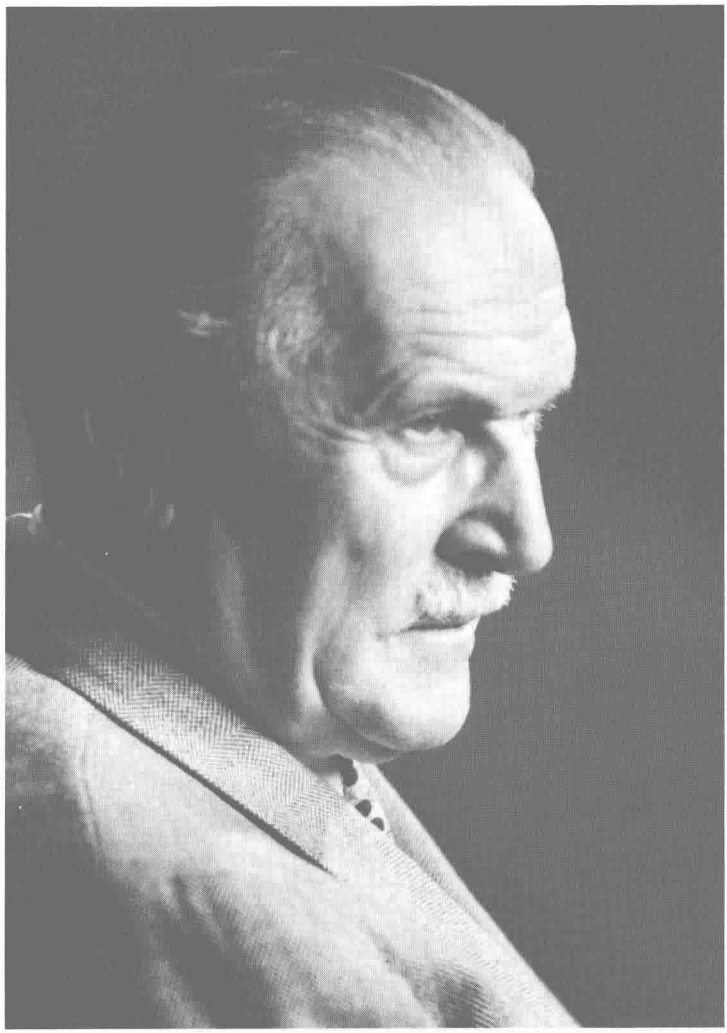

This book is dedicated to the memory of Karl F. Meyer (1884–1974). He led us here.

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ABBREVIATIONS

AHC	acute hemorrhagic conjunctivitis
AVU	arbitrary vaccine units
CF	complement-fixing (fixation)
CSD	cat-scratch disease
EB	elementary body
EKC	epidemic kerato-conjunctivitis
FA	fluorescent antibody
GC	gonorrhea
IC	inclusion conjunctivitis
ICN	inclusion conjunctivitis of the newborn (inclusion blennorrhea)
IDU (IUDR)	5-iodo 2-deoxyuridine
IgA, IgG, IgM	immunoglobulin classes
HSV	herpes simplex virus *(Herpesvirus hominis)*
LGV	lymphogranuloma venereum
Micro-IF	microimmunofluorescence
MRC	Medical Research Council
NAMRU	Naval Medical Research Unit
NDV	Newcastle disease virus
NGU	nongonococcal urethritis
PBS	phosphate buffered saline
PCF	pharyngoconjunctival fever
PEB	purified elementary body
PGU	postgonococcal urethritis
PID	pelvic inflammatory disease
PMN	polymorphonuclear neutrophil
RS	Reiter's syndrome
TPK	TRIC agent punctate keratoconjunctivitis
TRIC	trachoma–inclusion conjunctivitis
VD	venereal disease
WHO	World Health Organization
YS	yolk sac

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CONTENTS

1 Introduction

In this volume the authors cannot make any claim to performing a complete survey of the literature. Because of the ubiquitous nature of chlamydial parasites and the significance of the human diseases they cause, the list of references could easily number many thousands.

It should be obvious that each of the chapters in this book could well be the subject of its own volume. It was not our purpose to write an "Encyclopedia of the Chlamydiae" — nor would we be suitable authors in some areas. However, we have long felt the need for a single volume reviewing certain aspects of chlamydiae and their role in human disease. Therefore, our goal has been the preparation of a relatively concise review of our knowledge in this area to 1977. To keep the volume within manageable size, we have tried to discuss most of the major issues but have refrained from in-depth analysis of individual points. We leave this to those who may focus on single chlamydial diseases.

The book is designed to cover points of interest to clinicians, epidemiologists, and public health and laboratory workers. Because the emphasis is on human disease, microbiology and laboratory

diagnosis are discussed later. Realizing we must compete for the reader's time, we have written the text so that each chapter can be read independently; this is a reflection of the clinical spectrum of human chlamydial diseases. Obviously the ophthalmologist's major interest will be in the chapters dealing with eye disease, and the venereologist may be most interested in the chapters on lymphogranuloma venereum and other chlamydial infections of the genital tract; neither may be concerned with psittacosis. Therefore we must apologize to the person who has the interest and patience to read the whole book for the slight redundancy this has caused. The diagnostic procedures, discussed under specific diseases, are presented in the chapter on laboratory diagnosis in sufficient detail to allow the interested worker to implement the methods. Interpretation of results is also discussed; thus we hope the reader may benefit from our experience.

We feel that perhaps one of the most important things we can do is suggest additional reading for those students who may wish to delve further into selected aspects of chlamydial biology. Some of the references suggested here are presented because they cover areas beyond the scope of this particular volume; others are given because they represent the most complete, up-to-date reviews available. We have also included a number of papers published many years ago, because some of these observations are as significant today as they were when originally made. It is doubtful, for example, that there will be another psittacosis pandemic such as occurred in 1929 and 1930, but this does not diminish the value of the epidemiologic and pathologic studies done at that time. And, although trachoma is rapidly disappearing from some parts of the world, the disease still remains as the most important cause of preventable blindness in other regions.

For the historical aspects of psittacosis, the authors suggest the following: Bedson's Harben lectures (1959); and the recapitulation of the pandemic of 1929 and 1930 in England (Sturdee and Scott, 1930), in the United States (Armstrong, 1930), and in Europe and Argentina (Elkeles and Barros, 1931; Pfaffenberg, 1936; Barros, 1940). We recommend the monograph by Lillie (1933) for studies of the pathology of psittacosis. The Public Health Reports, Reprint No. 2580, reviewing the study of an epidemic outbreak of human psittacosis in Louisiana, is recommended as a complement to the studies of the 1929–1930 pandemic. For reviews of the physiology of chlamydiae we suggest the series of publications from Moulder: the two books *The Psittacosis Group as Bacteria* (1964) and *The Biochemistry of Intracellular Parasitism* (1962a); and the reviews "The Relation of the Psittacosis Group (Chlamydiae) to Bacteria

and Viruses" (1966) and "A Model for Studying the Biology of Parasitism" (1969). Weiss (1968) has also reviewed the physiology of these organisms, and Becker (1974) has written specifically on the biology of the trachoma agent. For a review of chlamydial infections with an emphasis on diseases in lower mammals, see Storz's *Chlamydia and Chlamydia-Induced Diseases* (1971); for reviews of the biology of the chlamydiae causing avian diseases, refer to the chapters by Meyer in the 1965 edition of *Diseases of Poultry* and by Page in the 1972 edition. For review of chemotherapy for chlamydial infections in general, see Jawetz (1969), and in the field of trachoma (Tarizzo and Nataf, 1970); this is, however, an area which needs much greater knowledge. For review of trachoma, the volumes by MacCallan (1936); by Bietti, Freyche, and Vozza (1962); and by Bietti and Werner (1967) are recommended. The field of chlamydial genital tract infections is not presented in any single book, but the most recent review is by Grayston and Wang (1975); the authors suggest that the interested reader refer to the 1964 and 1972 volumes of *The British Journal of Venereal Diseases* for a series of original articles, mainly by English workers, that review and present the then current status of chlamydial genital tract infections. Lymphogranuloma venereum (LGV) was reviewed by Sigel (1962).

For an interesting perspective on the evolution of research approaches to trachoma and oculogenital chlamydial infections, we recommend the three proceedings that have resulted from the international symposia held on these subjects in 1960, 1966, and 1970:

The biology of the trachoma agent. Proceedings of a conference, May 26–27, 1961. F.B. Gordon (ed.). *Ann. N.Y. Acad. Sci.* 98:382, 1962.

Trachoma and allied diseases. Proceedings of a conference, August 25–31, 1966. Sponsored by the Francis I. Proctor Foundation for Research in Ophthalmology and the Harvard School of Public Health. *Am. J. Ophthalmol.* 63:631, 1967.

Trachoma and related disorders caused by chlamydial agents. Proceedings of a symposium, August 17–20, 1970. R.L. Nichols (ed.). *Excerpta Medica,* International Congress Series No. 223, 586 pp., Amsterdam, 1971.

TAXONOMY

In recent years the chlamydiae have been known by a variety of names. Most important among these various epithets are *Bedsonia*

and *Miyagawanella*. The confusion was largely resolved by Page (1966), who reviewed the classification of this group of agents and suggested that the genus *Chlamydia* be used for all members of the psittacosis-lymphogranuloma venereum-trachoma group. He pointed out that this term had taxonomic validity over the various other proposals. The adoption of a single genus served to unite these organisms which have common antigenic and biological properties, and stressed their similarities rather than their differences. The name *Chlamydia* may be traced back to the original description by Halberstaedter and von Prowazek (1907a,b), who thought they were dealing with protozoan parasites and proposed the term *Chlamydozoaceae* for these "mantled animals" (von Prowazek, 1907). Unfortunately this is a misnomer (there is no mantle), and the derivative term is one which must be accepted because of priority in conformity with the systematics of bacterial nomenclature.

Differences in biological properties of various isolates within this genus had long been recognized. However, the first functional segregation was by Gordon and Quan (1965a), who studied 27 chlamydial isolates and divided them into two groups on the basis of inclusion morphology and the presence or absence of glycogen in the inclusion. The tight, compact inclusions containing glycogen were designated as group A, while diffuse inclusions which did not stain for glycogen (iodine stain) were designated group B. Lin and Moulder (1966) extended this grouping to include inhibition of growth by sulfadiazine, with all subgroup A strains being sensitive to the action of sulfadiazine and most (excepting only the psittacosis, 6 BC isolate) B strains being resistant. These authors also found that sensitivity to cycloserine was associated with group A isolates to a greater degree than with group B.

The morphologic criteria are not particularly useful, but the association of iodine-staining inclusions (glycogen) and sensitivity to sulfadiazine appear to be useful taxonomic tools. Sensitivity to sulfa alone will not give perfect discrimination between species because, in addition to the 6 BC strain, the Gleason strain and several recent chlamydial isolates have been shown to be sensitive to sulfadiazine, although they are clearly psittacosis strains and have glycogen negative inclusions. Thus, it would appear that the most useful distinction between these two groups is the ability to stain the inclusion with iodine in order to detect glycogen.

Page (1968) proposed that two species be recognized within the genus, and he utilized the terms *C. trachomatis* and *C. psittaci* to represent Gordon and Quan's groups A and B, respectively.

The following description of these species is taken from the eighth edition of *Bergey's Manual of Determinative Bacteriology* (Page, 1974) under the general heading "The Rickettsias":

Order II. Chlamydiales Storz and Page, 1971

Chla.my.di.a'les. M.L. n. *Chlamydia* type genus of the order; *-ales* ending to denote an order; M.L. fem. pl. n. *Chlamydiales* the *Chlamydia*.

Coccoid microorganisms whose obligately intracellular mode of multiplication is characterized by change of the small, rigid-walled infectious form of the organism (elementary body) into a larger, thin-walled, non-infectious form (initial body) that divides by fission. The developmental cycle is complete when daughter cells reorganize and condense to become elementary bodies which survive extracellularly to infect other host cells. Metabolically limited, Gram-negative parasites of vertebrates in which they may cause various diseases. Occasionally found in arthropods.

Family I. Chlamydiaceae Rake, 1957

Chla.my.di.a'ce.ae. M.L. fem. n. *Chlamydia* type genus of the family; *-aceae* ending to denote a family; M.L. fem. pl. n. *Chlamydiaceae* the *Chlamydia* family.

Coccoid microorganisms, 0.2–1.5 μm in diameter, which multiply only within the cytoplasm of host cells by a developmental cycle characterized by change of a small elementary body into a larger initial body that divides by fission. The cycle is complete when daughter cells reorganize and condense to become elementary bodies which survive extracellularly to infect other host cells. Elementary bodies contain compactly arranged nuclear material and ribosomes and are bounded by a rigid, trilaminar cell wall that is chemically similar to that of Gram-negative bacteria. Initial bodies contain amorphous nuclear material less electron-dense than that of elementary bodies, have thin, fragile cell walls and apparently are non-infectious. Metabolically limited, Gram-negative parasites of vertebrates in which they may cause various diseases. Occasionally found in arthropods. Cultivatable in yolk sac of chicken embryos. Sensitive to tetracycline antibiotics. Stain with aniline dyes.

Genus I. Chlamydia Jones, Rake and Stearns, 1945

Chla.my'di.a. Gr. fem. n. *chlamys, chlamydis* a cloak, M.L. fem. dim. n. *Chlamydia* a cloak.

Only two species are recognized. Type species: *Chlamydia trachomatis* (Busacca) Rake, 1957.

Key to the species of genus Chlamydia:

I. Forms compact microcolonies within cytoplasmic vesicles. Produces iodine-staining compounds within vesicle. Growth in the yolk sac of chicken embryos is inhibited by sodium sulfadiazine (1 mg/embryo).

1. *Chlamydia trachomatis*

II. Forms microcolonies within cytoplasmic vesicles which tend to rupture early in microcolony development, and the organisms in various stages of growth become distributed throughout the host cell cytoplasm. Does not produce iodine-staining compounds in vesicles. Growth in the yolk

sac of chicken embryos is not inhibited by sodium sulfadiazine (1 mg/embryo).

2. *Chlamydia psittaci*

Page's proposal has the merit of dividing the chlamydiae into two groups which can be differentiated by relatively simple biochemical tests. It suffers because organisms possessing a great diversity of biological and antigenic properties have been somewhat awkwardly compressed into each of these species. Easier methods of differentiating the agents will have to be developed before new species can be proposed. The current data suggest that there are differences readily demonstrable within either species, with the greatest diversity of biological properties in the species *C. psittaci*. Most members of the species *C. trachomatis* appear to present a spectrum of antigenic relationships and biological properties (with the singular exception of the markedly different mouse pneumonitis strain). That is, the lymphogranuloma venereum and trachoma-inclusion conjunctivitis strains are clearly very closely related. In spite of its drawbacks, this general system of nomenclature will be utilized here, largely because it has the general acceptance of the scientific community.

The chlamydiae for many years were included in the order Rickettsiales. Weiss (1968) showed that there are significant differences in the metabolic reactions of rickettsiae and chlamydiae. Among these differences are the metabolic independence of the rickettsiae with aerobic metabolic cycles resulting in energy production and including complete cytochrome systems; the chlamydiae, on the other hand, are essentially anaerobic, have no cytochrome system, do not produce ATP, and do not preferentially utilize glutamate as do the rickettsiae. Moulder (1964) had emphasized the unique nature of the chlamydial developmental cycle. Storz (1971) pointed out that this difference was probably sufficient to justify removing the chlamydiae from the Rickettsiales, and this proposal to establish a separate order Chlamydiales was formalized by Storz and Page (1971).

CHLAMYDIA AND *NEORICKETTSIA*

There is much literature, primarily French, on human and animal diseases associated with what are called neorickettsiae. Giroud (1969), who has been responsible for much of the original work in this area, has stated that he feels the terms neorickettsiae and bedsoniae (now chlamydiae) are essentially synonymous, and that these strains have the same biological properties. It is clear that at least some neorickettsial strains are chlamydiae, but it is not clear that all of them are. At any rate, it has been very difficult to determine from

the literature and simple strain designations which neorickettsiae really are chlamydiae and which might be rickettsiae that have not been identified properly on the basis of serologic or other reactions. Because of this difficulty, we have chosen not to consider the neorickettsiae in this review.