SALMONELLAE AND SHIGELLAE

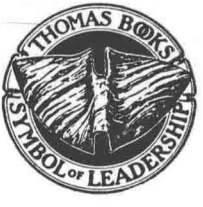
Laboratory Diagnosis Correlated With Clinical Manifestations and Epidemiology

BY

ALFRED J. WEIL, M. D.
Department of Bacteriology
The Bronx Hospital
New York, New York

and

IVAN SAPHRA, M. D.
Department of Bacteriology
The Beth Israel Hospital
New York, New York



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PREFACE

THE aim of this book is to make conveniently accessible the factual information needed for the handling of the laboratory aspects of salmonella and shigella infections. The description of methods will necessarily occupy a great part of these pages. But technical knowledge alone is not sufficient for purposeful laboratory work. For the proper approach, an understanding of the biology of these micro-organisms is needed. Thus it is attempted to supply the basic data concerning the biological position of salmonellae and shigellae as well as the rationale of our procedures. Furthermore, for the successful cooperation of the bacteriologist with the clinician and the epidemiologist, the former needs general information on the clinical and epidemiological aspects of the diseases caused by salmonellae and shigellae.

The emphasis will be on current viewpoints and methods. It is impossible, however, to disregard the past entirely. Our views as well as the very language we employ bear the marks of 2,000 years of medical observation and thinking. And it is similarly impossible to understand the present state of microbiology without consideration of the theoretical viewpoints of the two generations of bacteriologists whose work forms the basis on which the present generation is building.

It is hoped that enough references are provided to give access to the byways of our subject and its historical aspects. References have been selected with regard to this viewpoint rather than to priority.

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Alfred J. Weil
Ivan Saphra

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SALMONELLAE AND SHIGELLAE

CHAPTER I

INTRODUCTION

THE salmonellae and shigellae are members of a large and variegated group of Gram-negative, non-spore-forming rods commonly known as the family of *Enterobacteriaceae*. Their pathogenicity makes them of particular interest to human and veterinary medicine. The diseases caused by these micro-organisms, such as typhoid fever and dysentery, are, of course, much longer known than the bacteria themselves, though exact definition became possible only with the discovery of the causative agents. The bacteriology and immunology of shigellae and salmonellae, the epidemiology and the knowledge of clinical manifestations to which they are related have grown up together in mutual stimulation. The position of the one cannot be understood without relation to the others. This situation is reflected in the technical terms current in the field of enteric diseases, some of which go back to the time of hippocratic medicine. The gradual changes in their connotations reflect the developments of viewpoints.

History of Terms. Two terms in particular bear witness of this. The word typhoid fever is derived from the Greek originally designating a state of irrationality and coma. It eventually became associated with coma and fever, a syndrome which only modern medicine differentiated into clinical entities such as "status typhosus," meningitis, etc. Finally, the original term was restricted to the rickettsial disease "typhus," and the term typhoid fever ($\tau\upsilon\phi o\sigma$=typhus-like fever) is applied to the disease caused by the bacillus of Gaffky and Eberth. The Germans diverge from the English usage in that they created a special term for typhus (German: Fleckfieber=spotted fever) and reserve the word typhus for our typhoid fever. This explains why the German discoverers

of the microorganism named it "Typhus Bazillus," which they correctly latinized into *Bacillus typhi.* The English speaking world modified it—evidently with the intention of avoiding reference to typhus—to *B. typhosus.* (This is a linguistic monstrosity, as it means the stuporous bacillus.)

Dysentery is a Greek term meaning enteric disorder. In this age of etiological research it has been narrowed down to designate the infectious diarrhoeas.

After the typhoid bacillus had been discovered and named, it was found that related but not identical bacteria are also the causative agents of a clinically similar disease. Hence, these microörganisms acquired the designation paratyphoid bacilli (Greek παρα =by). Further investigation showed that paratyphoid baccilli form a group of related organisms rather than a uniform species. Within this group, individual entities became subsequently known by their bacteriological and serological properties; thus designations like paratyphoid A, B, and C originated.

The meaning of the term paratyphoid shifted gradually, because it became apparent that most of these organisms cause acute enteric infection in man and animals, but only rarely a typhoid-like disease. Other microörganisms, eventually recognized as belonging into this family, had in the meanwhile been described under independent names such as Gaertner's bacillus *(enteritidis)* or (when causing distinct animal diseases) as *B. pullorum* or *B. abortus equi.* The whole group eventually acquired a name by immortalizing a misconception. In 1885, E. D. Salmon and Th. Smith had described an organism of this paratyphoid group as the causative agent of hog cholera. The disease was subsequently recognized as due to a filterable virus, but the bacillus was stuck with the name *"Salmonella cholerae suis."* It is indeed a frequent secondary invader in hog cholera, but it is also most virulent for man, and could therefore claim the name *septicemiae hominis* with better right. As Salmon's description of the *cholerae suis* bacillus antedated all other paratyphoid observations, his name was eventually honored when a generic term was coined for

the paratyphoid group. Thus, the generic designation *Salmonella* came into being.

As to the typhoid bacillus, both the tradition of its particular virulence for man and its cultural peculiarities (which will be discussed in due time) caused the bacteriologists to keep it in a separate classification for a long time as Eberthella which caused no end of difficulties. Today, the genus Salmonella includes the typhoid bacillus as *S. typhi.*

Thus until recent years the confusing situation existed in which there was a disease (typhoid fever) caused by a group of closely related bacteria. The representative organism was placed in a genus by itself, whereas the others were placed in a genus comprising bacteria, some of high, others of low virulence to man, many of which became known to be pathogenic also for a variety of animals. An attempt to distinguish paratyphoid fever from typhoid fever clinically is as unsatisfactory as to employ a separate name for each type of meningitis.

The modern tendency is to designate the classical *clinical* entity as typhoid fever and range it together with other clinical manifestations due to *Salmonella* under the general heading of *salmonellosis.* Unfortunately, the classical English term enteric fever has not found favor in this country.

Principles of Salmonella Classification. The modern subdivision into groups and types is essentially based on antigenic properties. A widely recognized schema has been established through the work of a number of investigators which has become known as the Kauffmann-White schema (Kauffmann 1941-1951; Edwards *et al.* 1942, 1951). The types established with these techniques still carry names that reflect the historical development. Types are designated by three kinds of epithets: a few reflect particular pathogenicity for man—e.g., *S. typhi, S. paratyphi, S. enteriditis;* others, the pathogenic relation to animals, e.g., *S. cholerae suis, S. typhi murium.* It will be noted that most of these designations are of historical interest only because, as is the case with the types just named, we know by now that these types are by no means restricted to a particular

species or genus. *S. pullorum* (and *gallinarum*) is a lonely exception because this type is essentially a pathogen for fowls. The great majority of types is designated according to the locality where it was first isolated such as *S. montevideo*.

As to the bacteriological classification, it has now been generally accepted that all members of this group should be brought together as the genus *Salmonella* of the family *Enterobacteriaceae*. Disregarding a few borderline cases for the moment, the salmonellae form a reasonably homogeneous group of pathogens for man and/or animals, with a common pattern of bacteriological and serological characteristics.

Principles of Shigella Classification. The history of bacterial dysentery is in many respects a replica of that of enteric fever. In 1889, Shiga described a bacterium as the dysentery bacillus. The name *Bacillus dysenteriae* always has been properly restricted to this particular form. Within a few years, a whole array of related microörganisms were demonstrated as causative agents of dysenteric infection. In parallel with the paratyphoid bacilli, many of these organisms came to carry the name of paradysentery bacilli. The designations of the subforms varied greatly according to countries and schools. The confusion was certainly not lessened by the sometimes uncertain nature of the differential properties used for delimitation, by the complex serological make-up of the group, and by the difficulties encountered in the coordination of the serological differences and those established by existing cultural techniques.

Unfortunately the designations created during the years of painful search for Ariadne's thread through the maze survive in many textbooks and publications. A reasonable classification of types was eventually arrived at by the analysis of the somatic antigens: the type characterizing antigens could be demonstrated by absorptive analysis. And it could be shown that properly absorbed sera allow a speedy and reliable identification. As the type antigens dominate the immunological relations between host and microörganism, the establishment of the types has opened an approach to a rational in-

vestigation of these relations. In the meanwhile, it has become increasingly clear that the cultural differentiation is artificial. For instance, great importance for the classification of shigella was attributed to the ability of the organism to form acid from mannitol. We know now that strains which do and do not ferment mannitol occur within the same type and even within the same outbreak. For this and many other reasons, it would be the logical procedure to assign symbols to each serological type and dispense with the subclassification of shigellae by species. For the time being it is preferred to employ a compromise classification of shigellae which uses both species names for cultural and numerals for serological types. The obsolescence of cultural differentiation within the genus *Shigella* does not imply that cultural methods can be dispensed with. They remain of primary importance for the establishment of a microörganism as *Shigella* or *Salmonella.*

Why Typing? The question is sometimes asked: why do we invest so much time and work in differentiating types; in other words, why bother to fingerprint salmonellae and shigellae? Typing is done for clinical reasons in order to facilitate the diagnosis and prognosis, as will be seen in due course. And it is done for epidemiological reasons as an important tool in tracing and eliminating sources of infection, to provide vital information about the distribution in man and animals and the clinical significance of types. Such facts give the only reliable approach to problems of prevention.

Sources of Special References. In closing this introductory chapter, a few sources of more specialized references on many aspects of our subject may be listed: the chapter on enteric infection in Dowling's text on infectious diseases, the monographs on dysenteric diseases by Felsen and by Manson-Bahr, on Salmonella by Kauffmann (1941, and 1950) and the same author's book on *Enterobacteriaceae* (1951); the reviews on Salmonellae (Bornstein) and on dysentery (Weil, 1943, 1947) in the *Journal of Immunology*—on the typhoid bacillus in the *Archives of Pathology* (Weil, *et al.,* 1939), and salmonellosis by Seligmann and Saphra (1950).

CHAPTER II

THE BIOLOGICAL AND IMMUNOLOGICAL PROPERTIES OF SALMONELLA AND SHIGELLA

THE *Enterobacteriaceae* (Family: *Enterobacteriaceae* in *Bergey's Manuel,* in the 6th edition of which the genera *Salmonella* and *Shigella* are classed as a tribe, *Salmonelleae*), are characterized by the following properties: all *Enterobacteriaceae* are Gram-negative rods which do not form spores and which are not restricted in their oxygen requirements. Distinctions between the infinite variety of bacilli that fulfill these simple requirements become possible by determination of their cultural properties. In general these organisms are easily cultivated, a factor which greatly facilitates the investigation of their metabolic requirements and other biological characteristics.

General Properties of Salmonellae and Shigellae. The pathogenic *Enterobacteriaceae* have been sorted out from their commensal cousins by a slow process of trial and error. The following methods have proven themselves the most important tools for this differentiation.

Growth on agar: in general, Salmonellae and Shigellae grow less abundantly on solid media than saprophytic *Enterobacteriaceae*. None of them show quick spreading growth over the surface, i.e., the "swarming" which characterizes many strains of the genus *Proteus*. Agar cultures offer an excellent opportunity to test one property which is rarely mentioned in bacteriological textbooks, but which is of great help to those who have learned to pay attention to it: the odor. Our language does not contain terms to adequately describe odors, but our memory is an excellent repository for this kind of sensory perception, and it can be easily trained by