

ON THE CLASSIFICATION OF THE SHIGELLA TYPES

With Special Reference
to the Flexner Group

BY
STEN MADSEN



EJNAR MUNKSGAARDS FORLAG

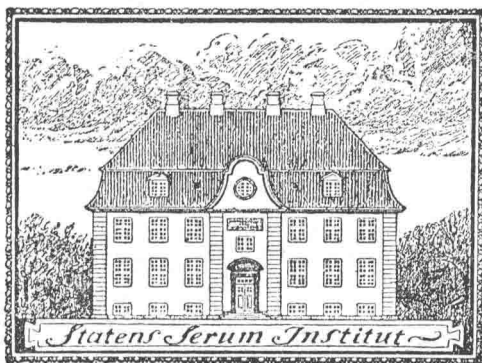
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København, den 3. oktober 1949.

J. ENGELBRETH-HOLM,
h. a. dec.

*Translated from the Danish
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Elisabeth Aagesen

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Preface.

The present work was carried out in the years 1947—49 at the State Serum Institute in Copenhagen. I am greatly obliged to the director of that Institute, Dr. *J. Ørskov* for the excellent working conditions and facilities placed at my disposal.

Dr. *F. Kauffmann*, chief of the International Salmonella Centre, has guided my studies of bacillary dysentery, and his extensive knowledge of enteric bacteriae has been of inestimable value to me during the performance of this work. I wish to thank him for his never-failing interest and stimulating criticism without which the present work would not have been possible.

I am also much indebted to Miss *Nini Petersen* for her technical advice and assistance; her skill and untiring efforts have been of the utmost value in the laboratory work.

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Furthermore I wish to thank the librarians of the Institute, Miss *Emma Wodschow* and Miss *Regitze Bussenius-Larsen* for their efficient help and encouragement.

Further thanks go to all the staff of the Institute who have been of assistance to me in one way or another.

Last, but not least, I should like to express my gratitude to the following who have furnished me with strains from various countries: *R. F. Bridges*, Oxford, England, *E. Boecker*, Berlin, Germany, *P. R. Carlquist*, Washington, U. S. A., *W. W. Ferguson*, Lansing, U. S. A., *A. E. Francis*, Beckenham, England, *M. Kristensen*, Copenhagen, Denmark, *National Collection of Type Cultures*, London, England, *A. J. Weil*, New York, U. S. A., and the late *K. M. Wheeler*, Connecticut, U. S. A.

Copenhagen, in October 1949.

Sten Madsen.

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Introduction.

A review of the comprehensive literature on dysentery shows plainly the difficulty of arriving at a proper classification of the dysentery group.

The constantly changing nomenclature, the frequent discoveries of new strains, and the difficulty of classifying them in the known systems have caused some confusion and disagreement within the literature on the subject.

Even though the recent investigations, based especially on the antigenic analysis, have contributed to greater stability in classification, the study of the dysentery group still gives rise to so many problems, that I willingly followed the suggestion of *F. Kauffmann* of taking up the entire question for revision.

The aim of the present work has thus been to contribute to an elucidation of the biochemistry and serology of the *Shigella* group, with special reference to the antigenic structure of the *Flexner* group, in which the greatest uncertainty prevails. Attention has here been focused particularly on the classification of strains "X" and "Y" and on the question of the justification of setting up the dual types, suggested among others by *Weil*.

For the routine work we tried to construct serviceable and easily applicable antigenic formulae and to prepare sera suitable for diagnostic purposes.

A number of *alkalescens* strains are included in the investigations but not designated as *Shigella* strains. According to *Kauffmann* (1949) these strains, together with the *dispar* group, are more closely related to the *Escherichia* group.

After a brief historical review the origin of each strain is indicated together with the nomenclature and classification used here. Next the procedure of the investigations is described. Then the biochemical and serological results are given, and finally these are discussed.

Chapter I.

HISTORICAL REVIEW

In 1898 *Shiga* (1898 a) reported that he had isolated a bacterium both from the feces and from the intestinal tract of 36 dysentery patients in Japan, a bacterium which agglutinated in fairly high dilutions in serum from these patients, but was not agglutinated by serum from normals. He described the bacillus, which he termed *Bacillus dysenteriae*, as a short, slowly motile rod, rounded at both ends. Morphologically it resembled the typhoid bacillus; it was non-stainable by Gram's method and non-sporing; it did not liquefy gelatine, did not coagulate milk, fermented glucose, and produced no indole. In addition *Shiga* (1898 b) found these bacilli to be toxic to guinea-pigs.

Shiga has since commonly been regarded as the discoverer of the dysentery bacillus.

Chantemesse and *Widal* had already described "le microbe de la dysenterie epidémique" in 1888. But they did not continue their investigations, which therefore were of no great importance to the dysentery research. The strain was, however, preserved, and in 1903 *Vaillard* and *Dopter* showed that the bacillus was really identical with that isolated by *Shiga*. In France *Chantemesse* and *Widal* are, however, still regarded as the proper discoverers of the dysentery bacillus (*Dopter* 1921).

Two years after *Shiga*'s publication *Flexner* (1900), and, at the same time, *Strong* and *Musgrave* (1900), claimed to have found — on the Philippines, in North America and in Puerto Rico — a bacillus corresponding exactly to that described by *Shiga*, which was also slowly motile. Moreover they stated that dysentery could be produced in man by oral administration of the bacillus. *Flexner*, as later demonstrated by *Martini* and *Lentz* (1902) and others, had isolated both *Shiga*'s bacillus and some distinct bacilli of the group which gradually came to bear his name.

Later that same year *Kruse* (1900) in Germany, isolated a bacterium from the faeces of dysentery patients, which in many respects resembled Shiga's bacillus, but which, nevertheless, *Kruse* did not regard as identical with the latter, partly because it was non-motile.

This question led to a discussion between *Shiga* and *Kruse*, a dispute which was not settled till a board declared the two bacteria to be non-motile and identical. *Shiga* was no doubt led astray by a very lively molecular movement. The bacterium was afterwards generally termed *Shiga-Kruse's bacillus*.

Already the following year *Kruse* (1901) published another paper in which he stated that in mental hospitals he had found a number of bacteria causing the so-called "Ruhr der Irren". The clinical picture was less violent and the course not quite so typical as that of ordinary dysentery. He named these bacilli pseudodysentery bacilli in contradistinction to the "genuine" dysentery bacilli. These *Bacilli pseudodysenteriae* differed serologically from the *Bacillus dysenteriae* (*Shiga-Kruse*), but resembled it morphologically.

The term *pseudodysentery* was replaced (*Collins* 1905) by that of *paradysentery*, a name since commonly used particularly by American writers. *Kruse* (1917), however, persisted in using his original name, having used that of *paradysentery* for some gas-producing and motile coliform strains.

In 1902 *Martini* and *Lentz* compared a great number of the dysentery bacilli then known, from Germany, Japan, America and the Philippines. They demonstrated *serologically* a marked difference between *Shiga-Kruse's bacillus* on one hand, and *Kruse's pseudodysentery bacillus* as well as *Flexner's* and *Strong's bacilli* on the other.

Thus they found two main groups:

Group I: *Shiga-Kruse's bacillus*.

Group II: "*Flexner*", "*Strong*", and *Kruse's pseudodysentery bacilli*.

Of these, group I constituted a separate entity, while group II comprised a number of serologically related bacilli.

Lentz (1902) continued the work on a biochemical basis. He stated that a fermentative differentiation corresponding to the serological one could be made on the basis of maltose, saccharose and in particular *mannitol* fermentations. But *Lentz* emphasised that a diagnosis based on fermentation could not compare with the serological diagnosis, "which within a few minutes can give absolutely exact results".

About the same time *Hiss* and *Russell* (1903), likewise on the basis of serological and biochemical investigations, made a classification agreeing

in the main with *Martini* and *Lentz*' results. In addition they described a bacillus "Y", which differed serologically from Shiga-Kruse's bacillus and fermentatively from Flexner's and Strong's bacilli. By agglutination, however, it could not be distinguished from Flexner's bacillus.

HISS AND RUSSELL'S TABLE

	Mannitol	Maltose	Saccharose
Group I: Shiga bacillus	0	0	0
Group II: "Y" (Hiss and Russell)	+	0	0
Group III: "Flexner"	+	+	+

Later *Hiss* (1904), on the basis of further serological and fermentative investigations, set up a table comprising four groups.

Group I: "Shiga-Kruse".

Group II: "Y".

Group III: "Strong".

Group IV: "Flexner".

Hiss furthermore pointed out the many overlapping agglutination reactions between groups II, III and IV. He was the first to suggest the theory of specific major or chief agglutinins in serum, removable only by absorption of serum by its own or another homologous strain. Besides the major agglutinins there exist various minor or partial agglutinins, common to all the strains and easily removable by absorption by heterologous strains.

Thus, *Hiss* touched on the question of antigenic structure, a question which later came to play a most important rôle.

Hiss' classification and terminology became extensively used, especially in Anglo-Saxon countries. Gradually it was realized, however, that the results of the fermentation tests varied too much to constitute a reliable basis for a classification.

Several different Y strains arose in the course of time, as this designation had been used indiscriminately for almost any strain which was not agglutinated by a Flexner or a Strong serum.

It was early shown, by *Conradi* (1903) among others, that Shiga-Kruse's bacillus excreted a very toxic substance, in contrast to the other dysentery bacilli, which were either non-toxic or only slightly toxic. A differentiation between *high-toxic* and *low-toxic* bacilli, therefore, seemed natural, a differentiation corresponding in some measure to the clinical picture.

The bacilli were now classified in a serologically well-defined, mannitol-negative, high-toxic group consisting of the Shiga-Kruse bacillus, and a

mannitol-positive, low-toxic group comprising a number of serologically related types. American writers often used the term "non-acid strains" for Shiga-Kruse's bacillus and "acid strains" for the other group. However, this classification had to be abandoned later (1917) after the incorporation in the dysentery group of Schmitz' low-toxic and mannitol-negative bacillus.

An important work on dysentery was *Kruse, Ritterhaus, Kemp, and Metz* (1907) publication based on investigations of about 50 "pseudodysentery strains". By agglutination tests and *Castellani's* absorption test these strains were classified in types with the letters A to F, of which A and D constituted the chief types. *Kruse et al.* emphasised, however, that they had hardly managed to include all pseudodysentery bacilli in this classification. (Subsequently type H was added, by *Hutt* (1913), and, after the discovery of Schmitz' bacillus, also type I (J)).

The work by *Kruse et al.*, who were the first to make extensive use of *serological* methods in the classification of the mannitol-fermenting dysentery bacilli, became very popular in Germany, but was practically ignored in English-speaking countries.

Moreover, *Kruse et al.* pointed out the unreliability of the results of fermentation investigations, especially as regards maltose, since bacilli which serologically constituted an entity could be seen to act in widely different ways on maltose. *Kruse et al.* do not seem to have realized how thoroughly old stock strains may change their fermentative properties, not least in the case of maltose.

In spite of *Kruse et al.*'s apparently distinct classification it proved difficult to place strains discovered later into their system. This was no doubt, in a great measure, due to the fact that different techniques were used for *Castellani's* absorption tests. Furthermore, the constantly changing nomenclature in the different countries contributed largely to the confusion and uncertainty.

No great progress was noted till *Sonne* in 1915 published his work in Denmark. He divided the Flexner group serologically in groups I and II. In addition he identified a group III, covering the bacillus now called *Sonne's* (or *Kruse-Sonne's*) *bacillus*. It constituted a serological entity, and was besides slow-lactose-fermenting.

This bacillus had, indeed, previously been discovered, among others by *Duval* (1904). *Castellani* found it in 1905 in Ceylon and called it *Bacillus Ceylonensis* A. *Kruse et al.* had likewise been working on it in 1907 and termed it *B. pseudodysenteriae*, type E. Hence it is often designated as "*Bacillus Kruse-Sonne E*" by German writers. Among French writers it is generally called *Duval's bacillus*.