BACTERIOLOGICAL,

CLINICAL AND EPIDEMIOLOGICAL STUDIES ON

EPIDEMIC INFANTILE DIARRHOEA

WITH SPECIAL REFERENCE TO

ESCHERICHIA COLI

(O 111: B 4 and O 55: B 5)

BY

ANNALISE DUPONT

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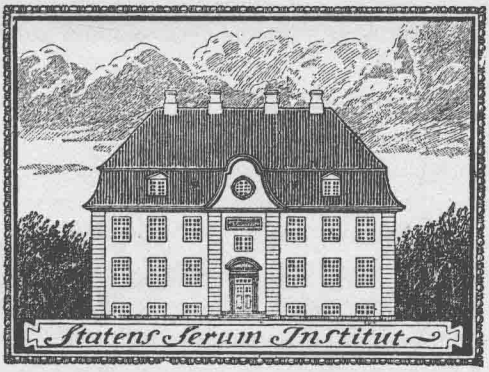

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Denne afhandling er af det lægevidenskabelige fakultet
ved Københavns Universitet antaget til offentlig
at forsvares for den medicinske doktorgrad.

København, den 19. april 1955.

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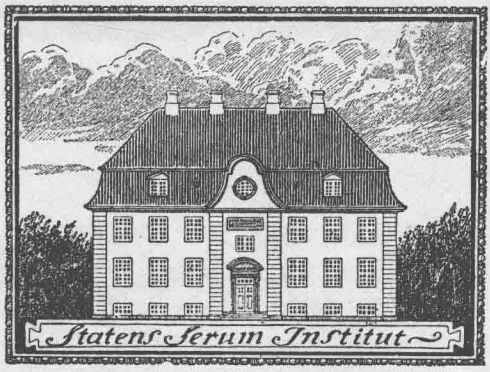

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PREFACE

The present work was carried out at the Statens Seruminstitut, Copenhagen, during my employment in the Diagnosis Department from 1950 to 1954.

I am greatly indebted to the Director of the Institute, *Jeppe Ørskov,* M. D., for the excellent working conditions offered me there.

My special thanks are due to *Martin Kristensen,* M. D., Chief of the Diagnosis Department, for his readiness to let me profit by his extensive knowledge and for his instructive criticism of my work.

I also wish to thank *Fritz Kauffmann,* M. D., Chief of the Pneumococcus Department and Head of the International Salmonella and Escherichia Research Centre for his never failing willingness to help and interest in my work, which I started in 1949 in his Department.

I beg Dr. *Svend Tulinius,* Chief of the Epidemiologic Department, to accept my thanks for having had occasion to profit by his epidemiological experience.

Professor *Preben Plum,* M. D., and Professor *Oluf Andersen,* M. D., have given me much valuable advice regarding clinical questions; and they have placed case records and specimens at my disposal, as have also the chief physicians of the paediatric, medical, and epidemic departments in Greater Copenhagen, Hjørring, Flensborg, Odense, and Usserød. I extend my thanks to all these chief physicians for their willingness to help.

Dr. *Biering-Sørensen,* Head of the organisation of Copenhagen Health Visitors kindly lent me the report of the epidemic in Odense in 1950. Further, I am grateful to him for his permission to let me use the case records of the health visitors and to let the health visitors collect specimens from normal infants. I wish to thank these 12 health visitors for the great extra work they undertook and carried out most conscientiously.

I am indebted to the physicians in charge and the heads of the infants' homes, day nurseries, maternity units, and kindergartens from which specimens were collected for the present investigation, as well as to the nurses of the hospital units concerned, who have all assisted med.

I express my gratitude to Mrs. *Kate Hansen,* laboratory assistant, for the great interest with which she assisted me in the daily laboratory work, and

to the staff of the Diagnosis Department for pleasant collaboration. Further, my thanks are due to the librarians of the Institute, Miss *Emma Wodschow* and Miss *Regitze Bussenius* for their helpfulness and kind assistance in the course of years.

I thank Miss *Aase Langhorn,* graduate in pharmacy, for her assistance in preparing nutrient media and reagents.

Finally, I wish to thank all my colleagues and the staff of the Statens Seruminstitut for pleasant collaboration.

The work was aided by a grant from *P. Carl Petersen's Fond,* to which I express my best thanks.

A specimen of the collection of case reports for 186 hospitalised children mentioned in the treatise will be available at the University Library, Department 2, Copenhagen, and the State Library, Aarhus, for 3 weeks prior to the day of public defence.

The translation is by *Elisabeth Aagesen,* cand. mag.

Hellerup, August 1955. *Annalise Dupont.*

CONTENTS

INTRODUCTION

The investigations here presented were commenced in 1949, inspired by some reports from England (*James, Kramer* and *Armitage* 1948) on the effect of antibacterial therapy in infantile diarrhoea, as well as by reports on the occurrence of special coli types in epidemics among infants (*Bray* 1945, *Bray* and *Beavan* 1948, *Taylor, Powell*, and *Wright* 1949).

The objects of the present study were: 1) a bacteriological, especially serological analysis of Danish and foreign strains belonging to the two *E. coli* groups: *E. coli* O 111: B 4 and *E. coli* O 55: B 5; 2) investigations into the incidence of these two *E. coli* groups; and 3) an attempt to throw light on the aetiological significance of these *E. coli* groups in infective infantile diarrhoea on the basis of Danish material.

CHAPTER I.

REVIEW OF THE LITERATURE ON ACUTE INFANTILE DIARRHOEA, WITH SPECIAL REFERENCE TO THE AETIOLOGICAL SIGNIFICANCE OF ESCHERICHIA COLI

Acute digestive disorders have always played an essential part among the diseases occurring in early infancy. In 1920 *C. E. Bloch* wrote that no other group seemed to play a more important part in paediatrics than diseases of the digestive apparatus of the young infant. There is no doubt that these diseases have at least previously predominated in paediatric units; and together with the chronic sequels they constitute the main subject of the paediatric text-books from the first 25 years of the present century. The interest shown in acute digestive disorders has varied considerably, having increased following outbreaks of particularly malignant cases. Such outbreaks may still occur, though the diseases can no longer be said to hold the same predominant position.

In 1789 the American physician *Rush* described the signs and symptoms of "cholera infantum" in young infants, and called attention to the relationship of the disease to hot weather.

The American writer *Dewees,* in his Paediatrics from 1825, mentioned cholera infantum as a disease peculiar to the U. S. A., where it is endemic. Mild forms do occur, it is true, in other countries, but they do not correspond to proper cholera infantum. He described the disease clinically and patho-anatomically.

Parrish (1826) mentioned the high mortality rate.

The first attempts to classify the gastro-intestinal diseases of infants according to symptomatology were made by the French paediatricians *Billard* (1837) and *Valleix* (1838). By that time these cases were regarded as manifestations of gastro-enteritis. *Barthez* & *Rilliet* (1853) described digestive disorders in children, and "catarrhe gastro-intestinal choleriforme" as a special form. They mentioned dentition, poor hygiene, and too early weaning as aetiological factors and stated that the disease occurred *exclusively* in late summer, being epidemic. *Trousseau* (1868) distinguished cholera infantum from Asiatic cholera. During the following years the patho-anatomical aspect of the disease predominated *(Baginsky* 1897).

Various diseases of the digestive apparatus are described in a Danish textbook of paediatrics from 1868 by *Levy*. One of these is cholera infantum, which "is more often epidemic than sporadic". Dentition is mentioned to be an aetiological factor.

Sevestre (1892) reported an epidemic of pulmonary infection associated with infectious enteritis in infants under two years of age.

About this time combined paediatric and bacteriological researches were started, which led to *Escherich's* description in 1886 of *Escherichia coli, B.lactis aerogenes,* and enterococci (the latter described in detail by *Thiercelin,* 1899); *Moro*'s and *Finkelstein*'s discovery of *B. acidophilus* in 1900; and *Tissier*'s discovery of *B. bifidus* in 1900.

Simultaneously the first reports appeared on minor epidemics of infantile diarrhoea in hospitals and nurseries.

Epstein (1881) and *Widerhofer* (1880) described institutional epidemics among young infants, including those breast-fed. In 1888 *Lesage* gave an account of a hospital epidemic. An infant suffering from diarrhoea with green stools was admitted among 20 other young infants, of whom eight developed green diarrhoea. A bacterium with a green pigment was cultured from the faeces of the affected children. When introduced into the small intestines of dogs and cats this bacterium gave diarrhoea in 18 out of 30 animals.

Rossi-Doria (1892) saw 20 infants in a Roman nursery affected with profuse diarrhoea and typhoidal symptoms, which he ascribed to *Escherichia coli,* present in large amounts in almost pure culture in faecal specimens as well as in the internal organs of 20 children examined postmortem. Coli bacteria were demonstrated by culture and microscopy of tissue sections, having the same arrangement as typhoid bacilli from typhoid patients.

As *Bact. coli* was found in faeces from both ill and healthy infants, the existence of so-called virulent coli bacteria was suggested on the basis of animal experiments, i.e. there was presumed to be a more virulent type in faeces from the ill than from healthy infants (*Macaigne* 1892, *Greene-Cumston* 1894, *Thiercelin* 1894). However, the results of these animal experiments have not since been confirmed. On the contrary, it soon appeared that coli bacteria pathogenic to animals could be found in faeces from both healthy and ill infants (*Escherich* 1898).

Attempts were therefore made to distinguish between the pathogenic coli strains and the saprophytic in a different way, a serological distinction being thereby aspired to. *Lesage,* in a brief report from 1897, mentioned a number of serological analyses (without describing the technique in detail). He summarized the results of his investigations as follows:

1) *Bact. coli* were agglutinated by serum from the same infant (50 cases: 40 positive and 10 negative).

2) The positive sera from these 40 infants agglutinated the coli bacteria from the 39 other infants with the same disease.

3) The positive reaction was of short duration, and the period difficult to find.

4) The reaction would cease if the disease developed into the chronic phase.

5) The reaction was absent in chronic cases.

6) A horse was immunized with a "good toxin secreted by the stated coli bacteria". This gave an antitoxic serum, in which other *Bact. coli* cultures from 113 infants suffering from the disease were agglutinated.

7) All these *Bact. coli* cultures derived from cases of infantile enteritis proved to belong to the same strain, since coli bacteria from normal infants were not agglutinated in serum from the ill, and serum from normal infants did not agglutinate pathogenic coli bacteria.

8) Typhoid serum agglutinated neither normal coli bacteria nor coli bacteria from cases of infantile enteritis.

9) The various strains of *Bact. coli* from adults (dysentery, diarrhoea, and other diseases) were not agglutinated by serum from affected infants, nor by antitoxic serum from an immunized horse.

10) It is important to study the various *Bact. coli* strains methodically.

11) Of the coli bacteria from cases of infantile enteritis agglutinated by antitoxic serum some coagulated milk, and some produced indole, while others did not.

Nobécourt (1899) has likewise employed serological methods. He immunized guinea-pigs and rabbits with living *Bact. coli* cultures and showed that serum from the animals specifically agglutinated the homologous cultures used. He concluded that the coli bacteria in cholerine can be characterized by means of animal serum. In addition, he carried out experiments similar to *Lesage's* (1897), but found that only six out of 20 human sera gave agglutination. *Escherich* (1898) found that the *Bact. coli* in the infant's faeces was agglutinable by its serum in only a few cases of infantile enteritis (which he called "colitis infectiosa").

Attention has not been focused exclusively on *Bact. coli* as a possible cause of infectious infantile diarrhoea. Several other micro-organisms are mentioned in this connection, especially streptococci (*Booker* 1897, *Escherich* 1899, 1900), *Shigella flexneri* (*Flexner* 1904), *Proteus* (*Metschnikoff* 1914), *Ps. pyocyaneus* (*Nobécourt* 1916). (*Escherich*'s "blaue Bacillose" is mentioned

in contemporary and subsequent reviews: *Marfan* 1899, *Monrad* 1900, *F. Bang* 1918, but is probably due to confusion with *Bact. acidophilus* and *Bact. bifidus,* first described in 1900; and "blaue bacil" simply means a Gram-positive bacterium).

In these papers the theory prevails that the aetiology is infectious, but others have been advanced, e.g. influence of the ground water temperature *(Ballard* 1881). Some writers *(Vaughan* 1890, *Spiegelberg* 1899) have shown that the intestinal mucosa of experimental animals is affected by toxic substances from milk, produced by micro-organisms (among which *Flügge's* proteolytic bacteria). Similar experiments were later carried out by *Czerny* and *Keller* (1906), who denied that the bacteria as such provoke the acute digestive disorders. In their opinion the digestive disorder is caused by the transformation of the milk by the non-pathogenic bacteria both before and after it has been ingested by the infant. They regarded the disease as due to acid poisoning and named it alimentary toxicosis (*Czerny* and *Keller* 1906).

The same idea has since been entertained by other workers, in particular *Finkelstein* (1910), who took infantile cholerine to be caused by toxic food constituents, thus attributing no aetiological importance to micro-organisms. Especially sugars were claimed to have a toxic effect on the intermediary metabolism.

These views regarding the toxic effects of the intermediary-metabolic products on the delicate infantile organism have given rise to valuable experiments and observations, particularly concerning the significance of acidosis for the clinical picture; but the theory itself has not yet been proved.

However, in the course of years, a number of the acute digestive disorders in infants have become recognized as being of infectious origin, even by the advocates of the alimentary theory. These are the diseases caused by the pathogenic intestinal bacteria of the *Salmonella* and *Shigella* groups.

Bahr (1910) as well as *Bahr* and *Thomsen* (1912) tried to utilize the experience gained by *C. O. Jensen* (1905) from diarrhoea in calves regarding the classification of *Bact. coli* for their examinations of *Bact. coli* in infantile cholerine. The material had been collected by *Ørum* (1910 a). It originated from cases in Copenhagen and comprised specimens of faeces, urine, and blood, as well as organ specimens withdrawn at autopsy.

Bahr (1910) and *Bahr* and *Thomsen* (1912) included in their analyses not only the *Bact. coli,* but also coliform bacteria, cocci, *Ps. pyocyaneus,* as well as typhoid and dysentery bacilli.

From their investigations they concluded:

In 117 cases of cholera infantum (62 alive and 55 dead) both faeces, blood, urine, bile and the internal organs as a rule contained bacteria of the coli-typhoid group: Coli A, coli B, Metacoli, and Pseudocoli. Typhoid bacilli