CHAPTER I

PLAN OF STUDY

Introductory Statement

It is a well recognized fact that not all members of the family Enterobacteriaceae are capable of inciting disease of the intestinal tract. Those which are generally accepted as agents of infective diarrhoea are the Shigellae, certain serotypes of the Escherichia coli genus, and the Salmonellae.

In a previous study (Bhat 1959) attention was focussed on the bacteriologic aetiology of childhood diarrhoea with particular reference to Shigellae, enteropathogenic E. coli (ECC), the Alkalasecens-Dispar group of organisms, and the Salmonellae. It was found that about 20 percent of the cases of diarrhoea studied were associated with these organisms. This left 80 percent with aetiology still to be defined (Bhat and Myers 1961; 1962).

In that study the major cause of infective diarrhoea in infants above one month of age appeared to be the Shigellae, 10-18 percent. In 5.5 percent of the cases ECC serotypes were found. Less than one percent was due to Salmonella infection. A study of short duration on new born nursery patients showed that ECC was the major cause of diarrhoea among the newborn with infection spreading rapidly. A high carrier rate was found then and later in groups affected by an ECC diarrhoeal outbreak (Bhat and Myers 1961; Bhat et al 1964). Shigellosis and Salmonellosis were not encountered among the newborn during those studies, but subsequently there were a few outbreaks of
Salmonella enteritidis in the nurseries and occasional sporadic cases of Shigellosis (Mach, unpublished data 1962, 1963). None of the Alkaliscens–Dispar group of organisms was isolated during that study, though search for them was made.

The observation that a large percentage of cases of childhood diarrhoeas remained aetologically undefined pointed to the necessity of further study.

Other groups in the family Enterobacteriaceae have been suspected often of producing diarrhoeal disease but there has not been enough evidence to incriminate them definitely as aetiological agents. In contrast to the pathogens mentioned above, which tend to spread in a community and which are found in sporadic cases of diarrhoea as well as in minor and major epidemics, these have shown little tendency to spread in a population. The organisms of as yet undetermined significance include those which previously had been classified vaguely as the "Paracolons" and members of the Proteus group.

The Paracolon group as first defined included a heterogeneous collection of unrelated bacteria whose only common characteristic was inability to ferment lactose, rapidly or otherwise. Such terminology was a reflection of the limitations of classification of such a large family as Enterobacteriaceae on the basis of a single biochemical property, namely the ability to ferment lactose. According to Edwards and Ewing (1955, 1962) the term "Paracolon" should now be considered only as a "designation of convenience to denote biochemically aberrant strains of the several groups and should not be interpreted as referring to any specific bacteriological group".
Each group of bacteria which ferments lactose promptly also contains atypical strains which may or may not ferment this sugar. Similarly, among strictly non-lactose fermenting groups, an occasional aberrant strain may arise with a lactose-fermenting characteristic. Hence, inability to ferment lactose, as in the former case or ability to ferment it, as in the latter case, should not be taken to exclude a culture from a group which it resembles in other properties. The identification of a culture must be based on a combination of tests for biochemical activities and not on a single biochemical property.

Some of the organisms which until recently have been considered as Paracolons are now well-defined members of the family Enterobacteriaceae. These are, in particular, the Arizona group, the Bethesda-Ballerup strains which have been placed with the lactose fermenting Citrobacter group previously known as Escherichia freundii and the Providence group. We have chosen to refer to the Citrobacter, including Bethesda-Ballerup, strains as CBB. The objective of the present study was to evaluate the role of these three groups of Paracolons and of certain Proteus species in diarrhoea in children up to the age of five years.

It has been found through the years that establishment of etiologic significance may not consist merely of identification of an organism in association with a case of clinical disease, as was attempted in our first study, though, classically, fulfillment of Koch's postulates rested on this as the first premise. The difficulty encountered in applying Koch's postulates in toto to the enteric group
of organisms, as pointed out by Ordway (1960), is for one thing that the clinical picture caused by these infections is extremely variable, and for another that production of disease in experimental animals has not been possible. Reiter (1956) remarked that the evidence of the pathogenicity of the Paracolobacterium group rested almost entirely on epidemiologic findings and that at least four biosero-types appeared to be pathogenic, including strains of the Arizona, Bethesda and Providence groups. In order to determine the pathogenic significance of a particular organism, it may become necessary to compare the incidence with which it is isolated from persons in health and in disease. If there is a significantly higher incidence of the organism in disease than in health, one might conclude that the organism is pathogenic. If the organism is found only in disease and not in health, it might be considered virulent. If an organism is isolated with the same frequency from persons both in health and disease one might have to infer that it is not pathogenic and that etiologic significance can not be ascribed to it. Yokutiel (1959) suggested that comparison of the bacteriological findings during an attack of infantile diarrhea with those during attack-free intervals in the same child as well as with those of other healthy children would be essential for drawing conclusions as to the pathogenic significance of the organisms found, particularly organisms of the E. coli, Parasolon and Proteus groups.

Another method of correlating the presence of an organism to occurrence of disease would be to demonstrate a significant serologic response in the host to that organism.
With these three approaches in mind, studies were planned, as described below, to determine the rates with which certain bacteria could be isolated from a series of diarrhoeal specimens; the incidences with which they could be detected in specimens from the same children during health and disease; and the frequency with which serologic response to homologous organisms could be demonstrated.

**Broad Delineation of Studies**

Plans were made to determine the incidence of diarrhoeal diseases which could be related etiologically to certain members of the family Enterobacteriaceae. Research grants from the Indian Council of Medical Research and from PL 480 funds, as approved by the ICMP, made it possible for collaborative studies to be undertaken. Clinicians, public health nurses and social service workers in one group were directed by Dr. Malati Jadhav*1, in another by Dr. Roger Feldman*2.

In addition as plans were formulated and as studies progressed the advice of an experienced statistician, Mr. P.S.S. Sundar Rao*3 was solicited. The bacteriologic studies were carried out continuously under the direct supervision of Dr. Prem Prabhoo Bhat*4, author of this thesis.

Specimens for the Diarrhoeal Disease Study were received through

*1 Malati Jadhav, Professor, Dep't of Paediatrics  
*2 Roger Feldman, Lecturer in Virology; Director, Enterovirus Research Project  
*3 P.S.S. Sundar Rao, Lecturer, Dep't of Biostatistics  
*4 Prem Prabhoo Bhat, Associate Professor, Dep't of Microbiology
Dr. Jadhav who was indirectly responsible for the accompanying forms and the data recorded on them such as name, age, sex, date, location or population group, case history, etc. Children were seen in their homes or in the hospital.

Specimens for the Longitudinal Study were received through Dr. Feldman who was, likewise, indirectly responsible for relevant data. Children recruited to this study visited the hospital as outpatients at regular intervals on specified days. They were examined and questioned by the clinician and rectal swabs were taken and designated as "routine" or "diarrhoecal" according to his findings.

Specimens for serologic examination were obtained by Dr. Ehat.

The criteria for classification of a case as one of diarrhoeal disease were those recommended in 1960 by the working group of the Indian Council of Medical Research for the Diarrhoeal Research Project. Thus, diarrhoea was defined as a condition characterised by the passage of three or more watery stools daily, either/or also containing blood and/or mucus.

The bacteriologic studies related to the nearly 5000 specimens which were subjected to a broad range of cultural procedures form the basis of the present report. Data were tabulated, percentages calculated and preliminary interpretations were made, after which Mr. Sundar Hao's advice was sought regarding the statistical significance of certain findings. Tests of significance were applied wherever such were essential and the method employed is indicated in the text.

For clarity of presentation, findings have been reported under the following five major headings.
I. Diarrhoeal Disease Study

(a) Association with the Shigella, including Shigella-like organisms; Interspathogenic E. coli (IEC), including the Alkalosensis-Disper (A-D) Group of Organisms*; and the Salmonellae. These are referred to in our study as "recognised pathogens".

(b) Association with Arizona; Citrobacter, including Bethesda-Ballerup (CBB); Proteus species; and Providence strains, in the absence of a recognised pathogen. These are referred to in our study as "suspect organisms".

II. Longitudinal Study

Association with the same bacteria as listed above. Determination of intestinal flora in disease and in periods of apparent health.

III. Paired Specimens Serology

IV. Biochemical and Serologic Classification of the Vellore Isolates designated as Suspect Organisms

V. Miscellaneous Studies

The material for the Diarrhoeal Disease Study, was obtained mainly from three groups of children; (1) those living in a rural area near the town of Vellore, (2) those living in a semi-urban area in Vellore and (3) those seen either in the Paediatric Outpatient Department or as inpatients of the Christian Medical College Hospital.

*Alkalosens-Disper organisms have been considered in the diagnostic section of the Microbiology Department of Christian Medical College Hospital, Vellore, as a group to be reported whenever they are isolated from specimens of diarrhoeal stools.
Vellore. In accord with the definition of the Indian Council of Medical Research, a "rural area" is one without facilities for drainage, a "semi-urban area" is one with open water drainage in contrast to an "urban area" which is one with facilities for closed water drainage. The first two groups of children were considered as "field groups". They were followed closely and specimens were obtained whenever they were suffering from diarrhoea.

Further details related to the population groups studied are presented later. Laboratory records were kept in such a way that it was possible to analyze the results under the following headings: a) Age of Children; b) Sex; c) Season of Illness; d) Sero- and Hiotypes of Bacteria Isolated; e) Mixed Infection.

Cases associated with mixed infection were not included with the others in the tabulations of results. Assessment of aetiological significance of an organism was considered to be more valid if it were the only one of concern to be detected in a diarrhoeal specimen. Specimens which yielded more than one recognized pathogen were not included in the tables of findings related to recognized pathogens. In the study of suspect organisms those which yielded doubtful or suspect organisms in association with recognized pathogens or with each other were not included in the tabulation of results related to them. Mixed infection specimens were, however, analyzed, and findings were tabulated separately.

For the Longitudinal Study, non-diarrhoeal specimens were obtained from rural and semi-urban children up to the age of five years who were swabbed monthly for this purpose, and diarrhoeal specimens were obtained
at any time the children had diarrhoea. In this section the results were analyzed as for the Diarrhoeal Disease Study but without reference to season.

Paired specimens of serum were obtained when possible from diarrhoeal children seen at the hospital either as outpatients or inpatients. This was undertaken only for a short time during the studies. Control specimens were obtained from children who were enrolled in another special study unrelated to diarrhoeal disease. Sera were tested by the indirect haemagglutination test.

In the section on classification of the Vellore isolates of the various groups of organisms included in the studies, the findings of other workers in the field of enteric bacteriology are cited for comparison, often in detail.

Miscellaneous studies included those pertinent to incidental findings. During the course of the studies, a new Salmonella serotype was isolated and a few strains of the rarely encountered Edwardsiella tarda and Chromobacterium typhiflavum. Brief literature review related to these organisms, biochemical and serologic characterization of Vellore isolates and a discussion of their significance in the aetiology of diarrhoea is included.

As indicated hereafter in a separate chapter the methodology for the studies was based largely on the recommendation of the Enterobacteriaceae Subcommittee Report of 1958 and on those of Edwards and Ewing (1962). Descriptions of special methods are included in the separate sections related to the various groups of organisms included in the study.
The main purpose of the studies was to evaluate the role of certain suspect organisms in comparison with that of the recognized pathogens. Review of the literature, as presented in the section on Literature Review, was orientated toward consideration of the role of the Pseudomonas and the Proteus species.

An evaluation of the media used in the isolation of Shigella, Salmonella and CUB is included in the Appendix which deals primarily with the media and reagents used in the study. A few case reports considered to be of general interest are presented also in the Appendix.