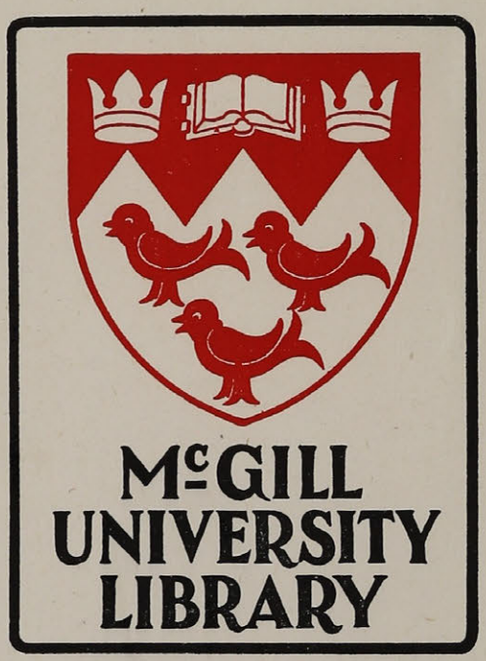

"ENTERITIS IN CHILDREN"

A THESIS

by

MARION CREELMAN SAVAGE

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INTRODUCTION

In the Children's Memorial Hospital in Montreal, as in all children's hospitals, children are brought in acutely ill with diarrhoea as the outstanding symptom. It is the responsibility of the laboratory to determine whether a state of infection exists, and the nature of such infection. The object of the work presented in this thesis was to explore the aetiology of enteritis in children in Montreal, especially in those instances from which no frank pathogen could be isolated.

During the year of study, no major outbreaks of intestinal disease occurred. One hundred and forty-three cases were investigated, and in all, including repeat specimens, a total of approximately two hundred specimens were examined. The number is admittedly small.

REVIEW OF LITERATURE

Enteritis is a disease so common in humans all over the world that it has received a great deal of attention in the past. The specific effects of enteritis on children, however, seem to have been overlooked by writers in the first three decades of this century. That it is, none the less, an important cause of disease and death in children has been pointed out in the last fifteen years, particularly in the Western Hemisphere.

Hormaeche (36) cites Bauza in saying that Uruguay has an infant mortality rate of 10%, and in 1935 28% of these infant deaths were due to gastro-intestinal disorders. In the United States in 1934, the State of Maryland, for example, 14.3% of deaths under one year of age were reported as due to dysentery (42). Weil says that this figure is typical of reports from all the United States (59). In Ontario (33), during the years 1928-1932, 3,812 children died from "Diarrhoea, enteritis and dysentery". This high mortality rate for dysentery remained stable over a period of from 1933 to 1940 while that of typhoid fever has steadily declined. The seriousness of this disease is emphasized by the fact that sanitary measures which have so markedly reduced deaths from typhoid have had no effect upon dysentery.

Nor do other countries fare any better than those of the Americas. Weil quotes Japanese, German and English figures to emphasize the extensive incidence of dysentery in those countries, with correspondingly high mortality rates. Weil, of course, makes it clear that, though the disease is undoubtedly a

serious one, such high death rates, especially among children, are due to the fact that only the most serious cases are admitted to the hospitals, and there is unquestionably a vast reservoir of cases both mild and moderately severe which recover clinically, and are never reported. The work done in the first third of this century was devoted largely to work on individual groups of organisms with special emphasis on adult epidemic disease and only scant attention to the isolated cases coming in to children's hospitals.

Most of the early literature on enteritis was devoted to the study of those pathogens which fall, in Bergey's latest classification (5), into the Family Enterobacteriaceae, and it is still true that the important epidemics of intestinal disease are due to these Gram-negative bacilli.

In any attempt to utilize Bergey's Manual to identify bacteria isolated in the laboratory, difficulties are experienced where species of Enterobacteriaceae are not sharply defined, but intergrade with one another to form an almost continuous series. Biochemical and serological proof of this is found in work on Escherichia, Aerobacter and Klebsiella (53) and a series from Shigella alkalescens to B. coli (56). Borman, et al. even go so far as to say that "The degree of interrelationship suggests that we are dealing with organisms having a common phylogeny but existing in a state of evolutionary flux (8)".

These same writers appreciate how misleading Bergey's taxonomic system, inconsistent as it is with this view, can be in its practical application. Pathogens isolated from patients'

stools, by it, can be dismissed as of no significance, and, conversely, harmless commensals mistaken for pathogenic organisms. Some of the differential criteria used in the past have been those of source, habitat, host specificity, colonial form and biochemical activity. These criteria alone, for the identification of enteric rods have now been proven inadequate. In recent years more and more time has been devoted to differentiation of these organisms on the basis of antigenic structure. The tendency, however, in the taxonomic field seems to have been simply to tack this vital information onto the description of an organism after it has already been pigeon-holed into a genus by the other, and often far less reliable, criteria.

Borman et al. (8) drew up a classification which seems much simpler and more flexible than that of Bergey, but one may question the division of Shigella-like and Salmonella-like organisms into two separate genera, when serological studies of Morgan for instance (46) show that the conjugated protein from the Shiga bacillus and that from the "O" fraction of the Typhoid bacillus are, as far as could be ascertained, chemically and immunologically indistinguishable.

However, for the sake of clarity in interpretation of the older literature, this review will retain the old names "Shigella" and "Salmonella" in the subsequent account of work done in the past on the association of these organisms with enteritis.

SHIGELLA IN INFANTILE ENTERITIS

As long ago as 1902 and 1904 Flexner and his co-workers found that 69% of 412 cases of infantile diarrhoea were due to organisms now classified as belonging to the genus Shigella. Other, more recent surveys, apart from epidemics, include that of Garcés in Chile (1931) (28) where 47% of 108 diarrhoea cases were due to Shigella; Cooper (1939) (17) in Cincinnati with 49% of 209 cases, and Hormaeche (1943) (36) in Montevideo, with 38% Shigellas of 668 cases admitted to children's hospitals. Several different frankly pathogenic species of Shigella have been recognized. Though serology is the "acid test", as a general rule they are biochemically characterized by the absence of or retardation of the ability to ferment lactose, lack of H_2S production, fermentation of glucose without visible gas, and, morphologically, by the lack of flagella.

In order that the serological methods employed in the experimental part of this thesis may be properly evaluated, an account of the human pathogens in the genus Shigella follows:

1. Shigella dysenteriae (The Shiga bacillus)

This organism was isolated first by Shiga in 1898 during a severe outbreak of dysentery in Japan. He thus established the etiological distinction between bacillary and amoebic dysentery. This organism is now found with frequency only in tropical countries, having almost disappeared even from Japan (55).

The characteristic which makes this organism outstanding in the group is its production of a powerful exotoxin.

It has a very marked effect upon the nervous system of rabbits and horses and less so in mice, guinea-pigs and rats. This toxin is thermolabile and antigenic. An extract produced at low temperature has been reported to have a M.L.D. of 1 microgram for the mouse (30).

The complete somatic antigen is also poisonous to some degree, namely in the order of 0.1 milligrams for the mouse. This quality,however, is shared with all members of the dysentery, Salmonella and colon-proteus group. Biochemically the Shiga bacillus is inert, the only positive result in the ordinary routine tests being acid production from glucose.

2. Shigella schmitzii (Hauduroy et al.)

This organism was isolated by Schmitz in 1917. It can readily be distinguished from the Shiga bacillus by its ability to produce indol from tryptophane, acid from rhamnose and the lack of the Shiga exotoxin.

2a. Shigella ambiguum (Andrewes) Weldin

This organism was described by Andrewes in 1918; similar in many respects to the Schmitz bacillus. Neter (52) feel that more complete study is required to decide whether these organisms form one single or two different species.

3. Shigella paradysenteriae (Flexner bacillus)

This organism was described first by Flexner in 1900 in the Phillipines and by Strong and Musgrave in Manila. In 1919, Andrewes and Inman (3) found that the majority of organisms which biochemically belong to this genus could be

classified into five "races" :- V, W, X, Y and Z. Each of these, they said, contained four major group antigens, V, W, X and Z in varying proportions, but with its own group antigen predominant: eg. the race W contained mostly W antigen, but smaller amounts of V, X and Z as well. The race Y, however, contained the four antigens in roughly equal proportions.

Boyd (10) from 1930 to 1940 made an extensive study of some four thousand strains belonging to this mannitol-fermenting group of dysentery bacilli. He classified about 66% of these as containing the V, W, X and Z antigens of Andrewes and Inman. The remaining 33% he found could be classified into nine additional types:- 103, P119, 88-Newcastle, 170, P288, D1, D19, P143 and P274. The real contribution Boyd made, however, was that he gave evidence that his types all had type-specific antigens, in addition to the cross-agglutinating group antigens which seven of them contained (namely V, W, X, Z, 103, P119 and 88-Newcastle).

Wheeler (61) in 1944, showed type-specific and group antigens in six of the seven Boyd types just named, but strains of X, as well as those of Andrewes Y seemed to have lost most of their type specificity through degradation, (as Boyd himself suggested previously). Wheeler shows that the group antigen is not one simple component shared alike by all types, as Boyd thought. It appears to be a much more complex aggregation of nine antigenic entities; some being broad components shared by four or more strains and other fractions shared by only two or three strains.

Wheeler also mentions, in addition to the group and type specific antigens, that there are also antigens common to Shigella, Salmonella, paracolon and coliform cultures. This supports the view, mentioned before, of Enterobacteriaceae as an

integrated series of types.

Weil, Black and Farsetta (60) carried Wheeler's work further to include a careful study of the antigenic structure of 136 Flexner strains. They found that there were fourteen main antigenic patterns in each of which one antigen predominated. By using suitable absorbed sera, they discovered that the secondary antigens existing in each of the fourteen patterns were different only quantitatively from the primary ones, thus returning to the principle first postulated by Andrewes and Inman. The primary (predominating) antigens agree well with the types of Boyd and Wheeler and are incorporated in a new Roman numeral classification similar to the Kaufmann-White schema for Salmonella.

Weil's Type	Race of Andrewes and Inman	Boyd's Type
I	V	"Flexner" I
II	W	"Flexner" II
III	Z	"Flexner" III
IV	---	"Flexner" IV (Type 103)
V	---	"Flexner" V (Type P119)
VI	---	"Flexner" VI (88-Newcastle group)
VII	X	-----
VIII	Y	-----
IX	---	Boyd I (Type 170)
X	---	Boyd II (Type P288)
XI	---	Boyd III (Type D1)
XII	---	Type D19
XIII	---	Type P143
XIV	---	Type P274

The great value of this recent work on the serology of Sh. paradysenteriae lies in the type specific absorbed sera which have thus been made available. Wheeler (61) and Weil (60) both describe methods for making such sera and the sources from which they obtained their strains.

With the inconsistencies of biochemical methods, such sera will prove the most valuable means of identification of this group of organisms for epidemiological study and control.

3a. Shigella sp. (Newcastle type)

This organism was first described in 1930 as the cause of an epidemic in Newcastle-on-Tyne (15). Antigenically, this organism is identical with Boyd's Type 88 Flexner strains, even though it does not ferment mannitol. The only other feature differentiating these organisms from known Flexner strains is that they sometimes produce small amounts of gas from dextrose and dulcitol when these sugars are dissolved in Lemco broth. This feature is not reliable, however, as both aerogenic and anaerogenic strains have been isolated from a single outbreak (32). Boyd, therefore, groups the Newcastle bacillus with his antigenically identical non-gas-forming Flexner strains into one group, Flexner VI. This group also includes the mannitol-fermenting, gas-producing Manchester bacillus. All available evidence seems to support this classification.

4. Shigella sonnei

It appears that Duval was the first to isolate this organism in 1904, but it remained for Sonne in Denmark (1915) to emphasize its frequent occurrence and suggest its pathogenicity.

Everyone agrees that this organism is very common in all parts of the world, but early workers believed that it was of only mild pathogenicity. This has latterly been disproved, however, with the work of Kobayashi et al. (39) who show that 40% of dysentery of the greatest severity in Japan is caused by the Sonne bacillus.

Biochemically, this organism is distinct from other dysentery pathogens in that it ferments lactose, albeit slowly (in from 1-20 days). Serology, however, still remains the only accurate way to identify the Sonnei group. Formerly it was regarded as an antigenically homogenous species, but in 1939 Glynn and Starkey (29) described two immunological types which they had identified. Weil, however, (59) feels that this work "needs further investigation in order to exclude mistaking an R variant for a true S strain". Wheeler also feels that the above types are identical on the basis of reciprocal absorptions. Rough and Smooth variants, however, are both antigenic, and typing antisera must contain antibodies against both to be of practical value.

5. Shigella alkalescens

This organism was described first in 1918 simply to differentiate it from Flexner-group species by its ability to produce acid from dulcitol, rhamnose and xylose. In 1938, however, Woolley and Sweet (64) described nine cases in which the organism was the only apparent cause of disease. Four of these were children with a diagnosis of acute pyelonephritis and who repeatedly showed catheter urine cultures

positive for this organism. In the four other children this organism was isolated from the stools, the diagnosis being enteric fever for two of them and dysentery for the other two. The ninth case was an adult nurse on ward duty who was suddenly stricken with diarrhoea and vomiting. Stuart in May 1945 also records an outbreak of epidemic gastroenteritis in Providence in which sixteen of twenty-eight ill infants' stools contained Sh. alkalescens.

SALMONELLA IN INFANTILE ENTERITIS

The organisms belonging to the genus Salmonella comprise the second large group of the Gram negative rods associated with enteritis. Up to the present, there are far too many different types of Salmonella described in the literature to give an account of each one separately. This review, therefore, will deal only with the general aspects of the group.

In 1929 White (63) pointed out that these organisms have, to begin with, one common quality:- "All the known species are pathogenic for man, animals or both." This pathogenesis is not confined simply to enteritis, for, in his comprehensive review Bornstein (9) cites many references showing a wide variety of conditions caused by members of the Salmonella genus. In contrast to the abundance of material on this subject, is the comparative dearth of references to be found on the organisms as they specifically affect children. Hormaeche (35) and his co-workers in Uruguay have provided a valuable series of papers on this point. They find that infants and children are highly susceptible to Salmonella infections, many of these, especially newborn children, taking severe septicaemic courses and even de-

veloping into meningitis. In Uruguay, the incidence of Salmonella infections in infants depends upon sanitation conditions, although several cases have been noted among children from homes with a high standard of living.

One of the few European references comes from Denmark where Kristensen, Bojlén et al. (40) have found that Salmonella infections are more frequent in children than in adults. This is probably referable to the fact that, apart from S. typhosa, the other species in small doses will infect children but not adults. This may be correlated with the marked difference in incidence in nurses and patients in institutional outbreaks in children's hospitals.

When the early work on this genus was done, biochemistry and pathogenicity were the two main criteria. With the publication of the Kauffman-White schema in 1934 (54) the antigenic formulae were established for those types already described on the basis of the older criteria. This scheme also brought organisms, eg. the typhoid bacillus, classed by Bergey's Manual under such genera as Eberthella, into the Salmonella genus, even though this organism usually fails to produce gas from dextrose. This process, however, of gradually classifying more and more biochemically non-Salmonella bacteria as belonging to the genus would soon render it impossible to regard the schema as a basis for the definition of a certain group of pathogenic bacteria. For instance, one of Bornstein's tables shows that a Salmonella antigen has even been found in Type 35 Pneumococci.

From a practical point of view, therefore, at the moment, Bornstein feels that it is advisable, despite aberrations,

to retain, for identification purposes, three characters in a definition of the Salmonella genus:- pathogenicity; serological relationships between its members and some of the biochemical characteristics of the type species Salmonella choleraesuis, such as non-fermentation of lactose and sucrose, production of acid and usually gas from dextrose and mannitol and negative indol production from tryptophane.

An understanding of the component antigens in the serological typing of Salmonella is essential for the best use of what typing sera are commercially available. Much valuable work has been done on these component antigens. Smith and Reagh first noted the difference between the floccular agglutination of the heat-labile flagellar H. antigen and the granular agglutination of the heat stable somatic O antigen.

But not only can a Salmonella vary between forms having both H and O forms with O alone; Arkwright in 1921 pointed out the smooth-rough variation manifested simply as a loss of the S. (smooth) antigen and the bringing to light of the R. (rough) antigen, or it may show up in a change of colony morphology as well - a change which gave this type of variation its original name. These R antigens, if allowed to confuse routine typings, will be responsible for considerable cross reactions and subsequent confusion in strain identification. The Salmonella can be degraded still further to lose the R. antigen, when two more antigens can be identified, Q and T.

Andrewes (2) in 1922 first showed that the flagella of Salmonella could also vary between two phases in the same culture. The antigens of these two phases occur in various types of Salmonella and the old names "specific","non-specific", "alpha", "beta",

etc. are now incorporated in the Kauffman-White Schema as phases 1 and 2. Unknown cultures may seem to exist only in one phase, but techniques have been devised, such as that of Bruner and Edwards (11) for discovering the second phase and even inducing more than two phases. These techniques materially assist the worker attempting to identify an unknown strain.

One other type of variation was shown following the work of Felix and Pitt in 1934 (25). They found that some of the strains of the typhoid bacillus failed to agglutinate in antityphoid "O" serum, yet were much more virulent for mice than agglutinable strains. They discovered that their agglutinable strains contained another somatic antigen, which they called "Vi", which appeared to alter their "O" agglutinating properties, and to have some relation to typhoid fever in humans, as the strains freshly isolated from patients and carriers almost always contained it. Such "Vi" strains are remarkably protective but must be carefully preserved to prevent loss of variation to the W type, as the strains deficient in "Vi" antigen are now described.

One more aid towards the final identification of the typhoid bacillus should be mentioned. Craigie (20) isolated bacteriophages which made strain differences of "Vi" typhoid bacilli easily demonstrable, thus adding another valuable weapon to the epidemiologist's battery in the war against this disease.

For the typing (serological identification) of Salmonella, commercially produced sera are now available. The genus is divided into groups A, B, C, D and so on according to the nature of the somatic antigens, and each group is then

further sub-divided into species on the basis of the flagellar antigens. Over a hundred species are currently recognized in this way.

OTHER ORGANISMS IN INFANTILE ENTERITIS

In addition to these known and recognized causes of enteritis, there are many accounts of bacteria whose possible pathogenicity, while not conceded, results in no little discussion in journals and textbooks. These organisms assume marked importance as one of the possible causes of summer diarrhoea in children, for very often they are the only potentially pathogenic organisms isolated from such patients, and sometimes are present in stool cultures in almost pure culture.

The genus Proteus has received a great deal of attention in this connection. This has been well reviewed by Topley and Wilson (58). Metchnikoff (44) in 1914 in Paris concluded that Proteus vulgaris was the specific cause of infantile diarrhoea and Morgan and Ledingham in London (1905-1909) found Proteus morgani in 43% of summer diarrhoea cases compared with an incidence of only 12% in normal children. Attempts to reproduce the disease in animals other than the chimpanzee were not successful, a criterion which has no particular value, and the theory lapsed in favour of more profitable study of Salmonella and Shigella.

During the last twenty-five years, few workers report such a high incidence as has been quoted above of Proteus organisms from cases of infantile diarrhoea. Widely differing reports on such cases from other countries have also tended to detract from the thesis that members of the Proteus genus cause

enteritis. In the last two years the need for a reinvestigation of their status has been felt, especially in the light of the better selective media available now. As an example of such work, in 1943 Neter and Farrar (49) in Buffalo described twelve cases of enteritis in infants. No agglutinins were demonstrable in the blood, but in three patients B. proteus was isolated, in seven B. morgani and in two both B. proteus and B. morgani. Two years later however, this same author (51) undertook a survey of lactose-negative organisms from stool cultures of normal infants. 34% had lactose-positive coliforms only, 40% had organisms of the Proteus genus and 22% had paracolon organisms, facts which in no way argued the essential pathogenicity of these bacteria.

These paracolon organisms are another group of this "Border-line" type. McClure (43) describes a series of outbreaks of epidemic diarrhoea in children of four Ontario hospitals. In the three institutions undertaking bacteriological examinations, no dysentery or Salmonella organisms were isolated either from stools or post mortem cultures. Haemolytic colon and paracolon organisms were isolated, the paracolon organisms being described as being identical with the colon, except that lactose is fermented in 2-4 days.

McClure produced toxins from these organisms which were heat stable when boiled, and produced vomiting and diarrhoea in cats on intraperitoneal injection. These toxins had no haemolytic titre for rabbit or sheep R.B.C. They produced symptoms in susceptible cats varying proportionately with the degree of severity of the epidemic from which they were isolated.

Anderson and Nelson (1) in Philadelphia also record

an outbreak of epidemic diarrhoea in the new born where the only organism isolated with regularity from the cases was a "para-aerogenes type of late-lactose-fermenting paracolon bacillus". Strains from this epidemic were included in a study of some 3,500 strains of "paracolon" bacteria made by Stuart et al. in 1943 (57). They classed all stable organisms under this heading which are isolated from man (rather than nature) but which differ from normal coliforms in their tardy fermentation of lactose, sometimes with little or no gas.

The careful antigenic studies of these workers not only shows a marked continuity between organisms of this group, but enabled the development of most illuminating comparisons of strains isolated from institutional enteritis outbreaks and from the food handlers in such institutions.

Neter and Clark (50) present data on thirty-nine patients with diarrhoeal disease, of which twenty-five carried paracolon organisms while the remaining fourteen, in addition to these organisms carried Salmonella, Shigella, B. proteus or B. morgani. Neter (51) in his most recent article (April 1945) sums up current opinion by saying:- "There is available at the present time suggestive evidence that paracolon and Proteus bacilli may be the incitants of diarrhoeal disease. This holds true particularly for epidemic outbreaks. In sporadic cases, however, it is not possible as yet to state, with any degree of certainty, whether the presence of these microorganisms is directly related to diarrhoeal disease."

VIRUSES IN ENTERITIS

One other possibility should be added, even though its proof is outside the scope of a routine bacteriology laboratory. This is the possibility of virus infection causing diarrhoea in children. Only one reference to this has been found, but it would seem a study worthy of more attention, despite the strong evidence that viruses, apart from lymphogranuloma, do not appear to attack the alimentary canal.

FOOD POISONING

No review of current literature on the subject of enteritis would be complete without some mention of food poisoning as one of its causes, particularly since this cause affects children so acutely. As Dolman (21) points out, wartime conditions have tended to bring about deterioration of the sanitation standards governing all phases of food production and consumption. Producers in Canada are faced with larger volumes of meat and dairy products than ever before. A great percentage of this type of perishable food is exported and thus commands a priority on limited cold storage facilities over that for domestic consumption. But not only is civilian food thus exposed to the threat of inadequate storage; often its producers, due to the labour shortage, must engage packers, dairymen and others without questioning their medical background.

Consumers, also, have been forced by war conditions to be less fastidious with regard to their food than heretofore. Shortage of domestic help often has resulted in the hiring of individuals in restaurants and in home kitchens who, unwittingly

often, are harbouring enteric pathogens. The standards regarding food for children are particularly in jeopardy due to wartime conditions. With parents working all day, the children often must be content with food improperly cooked and left over to be eaten on succeeding days without adequate refrigeration. Children, too, are particularly affected when safety standards in dairy products are threatened. Dolman (21) points out that there is still strong opposition to the pasteurization of milk. That unpasteurized milk and its byproducts, cheese and ice cream, may be a serious source of enteric disease is proven by the abundant literature tracing back to milk products cases of such infections as cholera, typhoid and paratyphoid fever, the dysenteries, Salmonella enteritis and Staphylococcus food poisoning.

There are two ways in which contaminated food can affect the human system. One is the "infection" type of food poisoning characterized by an incubation period generally between 8 and 36 hours and a variety of other symptoms following the multiplication within the body of pathogenic organisms contained in the food. The second is the "toxin" type, usually characterized by a shorter incubation period of 1/2 to 4 hours and by many of the same symptoms, but though they are sometimes more severe, the attacks are not as fatal and the recovery is more rapid than is the case with the infection type. Such an attack is due to the ingestion of food in which toxic substances have been formed as the result of bacterial proliferation.

According to Dolman (22) "The commonest, and in some respects, the least controllable form of food poisoning is that due to enterotoxin-producing Staphylococci". He carefully

distinguishes this enterotoxin from the alpha and beta toxins described by others by showing that it is remarkably heat stable, does not lyse rabbit red cells and gives severe gastro-intestinal disturbance upon intraperitoneal injection into kittens. Recovery from such an injection is usually prompt.

Repeated oral doses of enterotoxin apparently confer no immunity in the human, so the reservoir of potential victims is never depleted, although Dolman (23) shows that some measure of resistance can be developed by injections of formalinized enterotoxin filtrate.

The ubiquity of the Staphylococcus makes its contamination of food a very easy possibility and it is clear that the only reason this type of food poisoning is not even more common lies in the fact that not all strains are enterotoxigenic, even though they may produce alpha or beta toxin or both. Milk and its products are particularly susceptible, not only because of exogenous contamination, but because enterotoxigenic Staphylococci commonly cause a mastitis in cows and are sometimes found in the udders of healthy, uninfected animals.

Several authors feel that there is some ground for the belief in the production of a toxin by certain Salmonellas as in the case of Staphylococci. On this point, Bornstein in his comprehensive survey (9) says:- "Though it has been shown that intravenous injection of filtrates from young cultures of "food poisoning" organisms, in contrast to other Salmonellas, stimulates peristalsis in rabbits (24) a true Salmonella "toxin" that is responsible for the symptoms of the natural disease has not been demonstrated convincingly". This same author also points out (9) that children show a greater variety of manifesta-

tions to Salmonella infections than do adults, many of these being much more serious. It is imperative, therefore, that everything possible be done to prevent children from contracting such illness from food.

Organisms other than recognized pathogens have been reported as causing a food poisoning syndrome in children and adults. Cary, Dack and Meyers (13) isolated a "green-producing" Streptococcus from canned sausages which had caused nausea, diarrhoea and vomiting in 75 boys. Jordan and Burrows (38) and Davison, with Cary and Dack (14) also report outbreaks of food poisoning from which a similar organism was isolated. Most workers found that filtrates of such Streptococci did not give ill effects unless live cultures were swallowed as well, indicating that this type of poisoning may be due to infection with living organisms.

Wilson and Fulton, in wartime England, sent to Canada strains of B. coli and Proteus vulgaris both of which, associated with Streptococci, had been isolated from meat suspected of causing food poisoning. Dolman found that filtrates from these strains produced a definite vomiting reaction in kittens and cats, akin to that of Staphylococcus enterotoxin. Filtrates from other strains of Proteus and B. coli, however, also isolated from suspected foodstuffs failed to give this reaction in kittens, so Dolman undertook an extensive series of experiments with human volunteers. They first drank filtrates of suspected strains of P. vulgaris, B. coli and Streptococcus viridans, with no result. Nor were any results obtained when the above suspected organisms were inoculated into food. Dolman, therefore, concluded that their presence was the result of abundant oppor-

tunities for contamination between the time the food was prepared, eaten, incriminated and sent to the laboratory. Further, he concluded that, at the present time, no significant evidence proved that these organisms played any part in bacterial food poisoning due to preformed toxic metabolites.

The number of reported outbreaks of "toxin" type poisoning where the attempted isolation of frank pathogens failed, led Dolman to investigate the possibility of enterotoxigenic Staphylococci being overgrown by other bacteria in such foodstuffs. His experiments show that an initial contamination of food with Staphylococci, with consequent permanent intoxication, can give way after four days to the development of large numbers of a pure culture of Proteus vulgaris, even though this organism was introduced into the experimental food a day later than the other.

PERSONAL INVESTIGATIONS

MATERIALS

The following culture media were used during the course of this work:-

Peptone agar medium. 500 grams finely minced fresh beef heart, freed of fat, were added to a litre of distilled water, heated at 75° - 80°C for 1 1/2 hours and filtered through paper pulp in a Buchner funnel under slight suction. Sufficient agar to give a concentration of 1.5% was then melted in 1/10 of the volume of the meat infusion. The agar was either fibre (shreds of original bleached Gelidium spiriforme) or Difco granular. Then were added Proteose peptone (Difco) to

make 1% in the final volume, and salts to a final concentration of 0.25% NaCl, 0.02% KCl and 0.01% $CaCl_2$. The whole was added to the bulk of the meat infusion, heated at 60° - 80°C.

The medium was then set aside overnight to gel in order to adsorb the accessory growth factors. The next day it was melted at 100°C and the reaction adjusted to pH 8.5 using the Lovibond comparator type of colorimeter. The phosphates were precipitated by heating at 120°C in the autoclave for 20 minutes and removed from the medium by filtration through paper pulp. Finally, the reaction was adjusted to pH 7.2 with Normal HCl, and, after sterilization, the medium was ready for distribution.

Plain agar slopes. 5 cc volumes of melted medium were placed in 6" x 5/8" tubes, which were plugged, autoclaved at 120°C for 20 minutes, and slanted on a bench until cool.

Blood agar slopes. The medium above was sterilized, cooled to about 55°C, when 3% citrated human blood was added and sterile tubes filled with sterile precautions, slanted, cooled and incubated for sterility.

Ascitic agar slopes. The same procedure was followed as for blood, except that 5% ascitic fluid was added, the medium sterilly distributed in sterile screw-capped 6" x 3/4" tubes and 36 hours incubation was allowed as a check for sterility.

Semisolid agar. 0.3% agar was incorporated in the peptone agar broth base given above. The medium was placed in 10 cc volumes

in 6" x 3/4" tubes after which a 3 1/4" length of 6 mm glass tubing was inserted and the tubes plugged and sterilized.

Blood agar plates. To the peptone agar above, melted and cooled to approx. 55°C, was added 3% human citrated blood and the mixture poured in 20 cc volumes into sterile Petrie dishes (size: 100 x 20 mm) and incubated 24 hours for sterility.

MacConkey plates. Distilled water equal to half the required volume of medium, 1% lactose and 0.15% Difco bile salts were placed together in a flask, weighed, boiled 10 minutes in a water-bath, allowed to settle overnight, weighed again and lost water replaced and then filtered through Whatman #1 paper.

Then, to this was added a second mixture of distilled water equal to half the required volume of medium, 1.7% Bacto peptone (Difco) 0.3% Proteose peptone (Difco) and 0.5% NaCl. The pH was then adjusted to 7.6, the medium was heated for 15 minutes in a water-bath, compensated for evaporation and filtered through #1 Whatman paper. The pH was then brought down to 7.1-7.2 and 1.7% Difco or fibre agar added and melted in the autoclave (15 minutes at 120°C) followed by paper pulp filtration and the addition of 0.2% of a 1% neutral red solution. The medium was then autoclaved for sterilization and, after cooling to approximately 50°C, distributed in sterile Petrie plates.

Chandelier plates. For this medium the peptone agar given above was used, except that the agar concentration was 3%. To precipitate phosphates the pH was adjusted to 9.5, with a final pH of 7.0. To this base were added 1.0% lactose, and 10%

freshly prepared 3% aqueous solution of congo red. The medium was then sterilized, cooled and poured.

This medium prevents the spreading of Proteus, and grows Streptococci well.

S.S. agar plates. (for isolation of Salmonella and Shigella). 6% Difco SS agar was placed in a volume of distilled water equal to the required volume of medium. It was then heated to boiling in a water-bath until dissolved, cooled to 50°C and poured in 20 cc volumes into sterile Petrie plates.

This medium has been found to fade badly if left exposed at room temperature overnight. All plates, therefore, in this laboratory were stored in the refrigerator, not being removed until the time they were inoculated.

Tetrathionate broth. 4.6% Difco Tetrathionate broth base was mixed with a volume of distilled water equal to the amount of medium needed and distributed in 10 cc volumes in 6" x 3/4" tubes and autoclaved. Just before use, 0.2 cc is added of an iodine solution made by dissolving 30% iodine crystals and 25% potassium iodide in distilled water.

Peptone water base for sugars. To the required amount of distilled water was added 1% Proteose peptone, 0.25% NaCl, 0.02% KCl, 0.01% $CaCl_2$. The reaction was then adjusted to pH 7.0. After heating to 100°C the mixture was filtered, the pH readjusted to 7.0 and the medium autoclaved.

To the above medium 0.5% dry sugar was added and 0.1% of a 1.6% alcoholic solution of brom cresol purple. The medium was distributed in 2 1/2 cc volumes in 6" x 1/2" tubes, each

containing a fermentation tube of this shape and size:-

The tubes were plugged with wool, dyed a colour to correspond with the sugar, and autoclaved.

Tryptone water. for testing indol production.
The same formula was used as for peptone water, except that Bacto-Tryptone was substituted to give a reliable source of tryptophane from which the organisms can more consistently produce indol than if they must depend on the variable, and often inadequate, amounts of tryptophane found in ordinary peptone.

Kovac's reagent was used in testing for indol. This consists of 75 cc Amyl alcohol, 25 cc concentrated HCl and 5 grams of paradimethylaminobenzaldehyde. With the alcohol providing a better solvent than water for indol, and the HCl suitably altering the pH, this reagent seems to be a most delicate, reliable indicator of indol production,even after only 24 hours incubation of the tryptone water culture. The routine tests, however, were done by layering the reagent over the tryptone broth culture after 48 hours growth.

Nitrate broth. To the required volume of distilled water were added 1% Proteose peptone (Difco) and 0.2% potassium nitrate. The mixture was filtered, tubed, plugged and autoclaved.

Reagents for Nitrite test.

Solution A: 0.8% Sulphanilic acid dissolved in 5 Normal acetic acid.

Solution B: 0.5% Alpha-naphthylamine also in 5 Normal acetic acid.

Using a spot plate containing 1/2 cc of culture (at least 48 hours old) 1 drop each of Solution A and then B were added, when the development of a red colour indicated the presence of nitrites.

Litmus milk. The fat was first removed from the milk by centrifuging. Then 2% of a sterile 2% solution of azolitmin in water was added, the medium then being tubed in 6 cc volumes and autoclaved.

Plasma coagulase medium. 1% of a sterile 4% potassium oxalate solution in water was added to fresh rabbit or human blood. The blood was centrifuged and the plasma diluted 1:4 with 0.85% NaCl. It was then distributed in 0.5 cc quantities in 6" x 1/2" sterile tubes.

Gelatine tubes. 1.5% sheet gelatine was dissolved in the required amount of Peptone broth base as described above for Peptone agar. This was done at a temperature below 50°C, acidity being neutralized (pH 7.2) during solution. When the reaction remained stable at this pH for half an hour, the medium was placed in 6 cc volumes in 6" x 5/8" tubes, plugged, autoclaved at 120°C for 20 minutes and allowed to set, upright, at room temperature. Stab inoculations were made, and after incubating at 37°C, the cultures were placed in the ice-box before reading to solidify any unhydrolyzed gelatine.

Iron agar. (for testing H_2S production).

To the required volume of distilled water were added 2% Bactotryptone, 0.5% NaCl, and 1.5% agar shreds. The mixture was autoclaved at 120°C for 20 minutes to dissolve the agar and filtered through paper pulp, following which the pH was adjusted to 7.4. To the melted agar was then added 0.05% ferric ammonium citrate and 0.25% sodium thiosulphate. The medium was then tubed in 7 cc volumes, autoclaved and allowed to cool with the tubes upright, since they are used for stab inoculation. The

iron salt was used in preference to lead acetate as it is more readily precipitated by H_2S to form the black sulphide in the slightly alkaline medium necessary for the optimum growth of the pathogenic bacteria under investigation.

The sodium thiosulphate was added to give the bacteria the sulphur required for H_2S production in readily available form instead of relying on the sulphur compounds in the infusion broth, which are usually present only in inadequate amounts and often in a form unavailable for reduction to hydrogen sulphide.

This makes a medium which is most sensitive to the production of H_2S even showing a colour change around the inoculation stab as early as six hours after incubation.

METHODS

The methods used in this survey are described in detail below. Stool specimens were collected in a waterproof, sterile cardboard carton for transmission to the laboratory. Since the time elapsing between the passing of the stool and its inoculation onto the laboratory media was seldom more than three hours, it was not found necessary to treat the specimen with saline or other preservative.

First day In the laboratory the specimen was examined carefully for any signs of gross mucus or blood. Such portions, if present, were inoculated onto the edge of two plates of SS medium and one plate of blood agar. The inocula on one of the SS plates and on the blood plate were light while that on the second SS plate was heavier. A platinum wire spreader was used to spread the inoculum, first around the edge of the plate and then, lightly, across the centre. This resulted

in the isolated colonies growing in the centre, where they were easier to pick off than similar colonies on the edge of the plate. In addition to the three plates, a bean-sized portion of the stool was also placed into tetrathionate broth and shaken well to emulsify the inoculum. The media were then incubated at 37°C for from 18 to 24 hours.

Second day The blood plate was carefully examined for opaque haemolytic Staphylococcus colonies or tinted Pyocyaneus growth with its characteristic odour.

If Staphylococci were suspected, though overgrown with coliforms, the area was smeared and stained by Gram's stain and, on finding from positive cocci in clusters, growth from the area was subcultured onto Chandelier's medium. This effectively diminished the size of coliform colonies, and eliminated "spreading". Staphylococci showed up as discrete orange, opaque colonies. These were then isolated onto plain agar slopes and incubated overnight.

In the case of the Pseudomonas, production of a chloroform-soluble greenish-blue pigment was considered sufficient evidence for the organism being reported as Ps. pyocyanea. In any doubtful cases, the polar flagellum was stained with Cesares-Gil mordant, followed by impregnation with Holland's silver stain.

In examining the SS agar plates the feature which singled certain colonies out for further study was their inability to ferment lactose and thus take on a pink or red colour. Four lactose negative colonies were fished from each pair of primary SS plates and placed on agar slopes. If any differ-

entiation had showed up between H_2S producing and non-producing colonies, this was taken into account in the colonies chosen for fishing.

On the second day, also, the Tetrathionate broth was shaken well and a 3 mm loopful subcultured onto a MacConkey agar plate. All primary cultures, excepting the blood agar plate, were then reincubated for a further 24 hour period.

Third day The pure cultures of Staphylococci that were isolated from the blood agar plate were subcultured into oxalated plasma. If, in a water-bath at 37°C, the plasma was coagulated, the organism was reported as "Staphylococcus pyogenes" without any further test. If the plasma did not coagulate after a further 20 hours incubation, the organism was considered non-pathogenic.

The agar slope cultures of enteric Gram negative rods were subcultured onto lactose, dextrose, maltose, mannitol, sucrose and dulcitol peptone water sugars, as well as onto tryptone water and iron agar. Subcultures were also made of the isolated lactose negative colonies appearing on the first MacConkey plate subculture of the tetrathionate broth. If there were none, further subcultures were made of the original SS agar plate colonies, as these might well be colonies of dysentery bacilli which do not grow in Tetrathionate medium.

Fourth day The Tetrathionate broth was subcultured for a second time onto another MacConkey plate, and the pure cultures from the first subculture were placed on the primary sugars named.

The sugars from the SS agar subcultures were read and an effort made to classify the organisms, provisionally, into

genera. If the lactose sugar showed acid and gas on the first day, the organism was regarded as non-pathogenic. If the acid and gas were formed only on the second or third day, the organism was called a "late-lactose-fermenter" and also regarded as non-pathogenic.

If the lactose sugar showed acid only, the possibility of contamination was examined. In our experience, minute colonies of faecal Streptococci have, at 48 hours, been found growing underneath dysentery and other lactose negative colonies which were invisible at 24 hours. Therefore, as a routine measure, all strains producing acid only from lactose were replated to rule out the possibility that Streptococcus contamination was responsible for the anaerogenic fermentation of lactose. If, on replating, the strain still produced acid only from lactose in 24 hours, its motility was checked and if it was motile it was considered non-pathogenic.

If the lactose tube showed neither acid nor gas, the organism was regarded as a potential pathogen. The remaining metabolic tests were read and evaluated before any consideration of antigenic analysis.

If dextrose and all other sugars were negative, the organism was placed in the Alcaligenes genus, especially when the growth was viscous on solid media and a flaky pellicle formed in liquid media. If the organism showed poor growth at 37°C it was then subcultured at room temperature and if it grew well, it was termed a member of the Achromobacter genus.

Motile organisms of the dextrose acid and gas, mannite and dulcite negative group were classed in the Proteus genus, with two species distinguished: namely "Proteus morgani"

being indol positive and sucrose and H_2S negative, while "Proteus vulgaris" was H_2S positive and could be indol and sucrose positive or negative. Anaerogenic strains were still classed in this group with aerogenic ones, if they were mannitol negative and their motility could be proven, even with semi-solid agar if necessary.

Sucrose positive cultures almost invariably fermented lactose late, and sucrose negative, indol positive organisms were reported simply as belonging to the "paracolon" group.

Presumptive identification on the basis of metabolic activities was verified by agglutination tests. The agglutinating sera used were obtained from the Standards Laboratory, Oxford, England. Microscopic and macroscopic slide agglutination techniques were used, and confirmed by tube agglutination using halving dilutions of the serum.

"H" suspensions of motile organisms, and all suspensions of Shigella, were made either by incubating a tryptone water culture overnight at 37°C in the presence of 0.15% formalin, or, for rapid use, by adding 10% formalin to the same tryptone water culture. These methods are those recommended by the Oxford Laboratory.

"O" suspensions of motile bacteria were prepared by boiling tryptone water cultures for ten minutes.

All agglutination tests were incubated at 45°C in a water-bath. "H" agglutination was read in 24 hours in a viewing box, and "O" agglutination was read in 24 hours and again in 48 hours.

RESULTS

The stools of 148 patients were examined over a period of eleven months. From these patients a variety of organisms was isolated, often two or more from one patient. This point will be taken up more fully later.

Not counting repeat specimens, the numbers of the genera isolated from this group of cases were as follows:-

B. typhosa	3
Dysentery bacilli	8
Salmonella	5
Staph. pyogenes	16
Beta haemolytic Strep. Lancefield group A	1
B. pyocyaneus	7
P. vulgaris	46
P. morgani	40
B. paracolon	13
Achromobacter	5
Alkaligenes	5
Late lactose fermenter	3

In addition, twenty-five patients with diarrhoea showed none of the above organisms in their stool cultures.

An account will now be given of the work done in comparing the strains isolated in the first four groups listed above.

B. typhosa The three strains isolated were all biochemically and serologically typical. Biochemically, they fermented dextrose, maltose, and mannitol with the production of acid

but no gas. They had no action on lactose, sucrose or dulcitol. H_2S was rapidly produced. No indol was formed. The organisms were motile, but one strain not actively so. All agglutinated on slide test with "Vi" antiserum and agglutinated to titre in tubes with Typhosa "O" and "H" antiserum.

Two of the cases had positive blood cultures on admission and all had positive stool cultures for several days. The Widal reactions varied widely between one case, eleven days after admission, with a Typhoid "H" agglutination of over 1/5280 and another with a negative Widal three weeks after admission.

Dysentery bacilli There were eight cases of enteric disease from which dysentery bacilli were isolated. All the organisms appeared the same in colony morphology - growing in one to two mm. size colonies, translucent, mirror-like, with entire or slightly undulating margin, and a greyish colour when picked up on a loop.

All the strains promptly fermented dextrose and mannitol with acid but no visible gas. All were indol and H_2S negative and non-motile. All the strains which subsequently were serologically proven to be Shigella sonnei by tube agglutination fermented lactose in from one to two weeks. Since the exigencies of clinical practice necessitate as rapid an identification of pathogens as possible, it was found impracticable to wait until the lactose was fermented, so slide agglutinations were undertaken with the Polyvalent Flexner Nos. I and II and Sonnei serum as soon as the primary sugars had incubated for 18-24 hours. When the sera were first received from Oxford, little was known about the best technique for

using them. At first, they were used at full strength, one loopful of serum being mixed with a loopful of tryptone water culture. Also, in the beginning, only one serum was used, either Sonnei or Flexner according to the biochemical reactions displayed by the organism.

Prompt complete agglutination occurred with these single serum full strength tests, but the satisfaction at obtaining so clear-cut a distinction between the test and a simple saline control was short-lived, for further slide tests with the other Flexner and Sonnei sera on the same organism showed the same complete agglutination after fifteen minutes.

At this point it was discovered that normal rabbit serum would also produce complete agglutination of the particular organism under examination at that time (# 45-1415). Using this same organism, the sera were diluted 1:10 - as suggested in the Oxford notes on Salmonella typing (48), but with the same uniform non-specific agglutination. Older cultures agglutinated even more readily, nor did treatment with formalin effect any improvement. The organisms were then centrifuged down from the tryptone broth and resuspended in 0.6% NaCl. Tests with full strength sera still showed the normal rabbit agglutinin effect, but when the suspensions in diluted saline were mixed with diluted sera this disappeared.

There remained however, the problem of cross-agglutination. Since the constituent antibodies of the polyvalent Flexner sera have not been listed, the only course open, on obtaining specific agglutination with them, was that of doing slide agglutinations with the seven specific Flexner sera which were available. In studying the strain 45-1415 men-

tioned above, agglutination occurred with every one of these except Flexner V. Tube agglutinations were then set up with all those specific sera which were positive on slide test, as well as with the two polyvalent sera. This strain was completely agglutinated by the polyvalent Flexner No. I to a titre of 1:160, and showed partial agglutination at 1:320, that is, beyond the given titre of the serum. With the other sera, complete agglutination occurred to varying titres:- Newcastle and polyvalent Flexner No. II to 1:80, Flexner X, Z and 119 to 1:40 and Flexner W and 103 to 1:20. It seems inescapable that this organism is a dysentery bacillus of the Flexner group, but not agreeing with any of the described types.

In only one strain was a positive slide agglutination test limited to one only of the Flexner type sera (119), but this same organism failed to agglutinate with halving dilutions of 119 serum in tubes.

The cross agglutinating reactions within the Flexner group, as shown by the above strain 45-1415, are strongly reminiscent of the cross reacting "patterns" described by Weil et al. (60). The manufacture of absorbed sera on a commercial scale by Lederle Laboratories Inc., on the basis of Weil's fourteen types, would seem to be a most valuable contribution towards the speedy, definite identification of these organisms. Unfortunately, these sera, during wartime, could not be imported into Canada.

In continued work with the Oxford sera, it became apparent that this cross agglutination among the dysentery bacilli was not confined simply to the members of the Flexner group. In no case did any of the organisms isolated show any

cross reaction with Shiga or Schmitz serum. With Sonnei serum, however, the six other dysentery strains isolated from patients showed a graded series of cross agglutination reactions. All six of these strains were biochemically almost indistinguishable. All produced acid but no gas from dextrose, lactose (in one to two weeks), maltose, mannitol and arabinose. All failed to ferment sucrose, dulcitol, sorbitol, salicin, xylose or inositol in two weeks. All were H_2S and indol negative. Two failed to ferment rhamnose, while four produced acid, two in 24 hours and two after 4 - 10 days. This was the only biochemical distinction between the six strains, and it was not related in any way to the serological gradation.

By biochemical criteria, all six strains could be Shigella sonnei. One of them, however, showed complete agglutination to titre with polyvalent Flexner No. II serum, while the Sonnei serum agglutinated the organism to a titre of 1:80 only, which was little better than the other sera accomplished (Flexner polyvalent No. I to 1:20, Flexner V and 119 to 1:40).

The second organism of the six showed similar cross agglutination, though, this time, with the Newcastle serum showing up as the most powerful agglutinating agent. This serum completely agglutinated the organism to a titre of 1:320, even though no gas was produced and mannitol was fermented. Sonnei agglutination was also complete to titre, with Flexner polyvalent No. II complete to 1:80 and 103 to 1:20 and V partial only to 1:20.

(A note might be inserted here regarding the apparent inconsistency of the Newcastle serum agglutinating above its given titre. The Oxford serum titres are defined as "Not less

than 1:250", which allows for some sera being slightly higher than this, to offset possible deterioriation of titre over the six month period during which they are guaranteed.)

Two other organisms of the six showed marked slide agglutination with Flexner sera but no reaction with Sonnei serum. However, their biochemistry was so typical of Shigella sonnei, that tube agglutinations with this serum were done as well as with the sera of Flexner types giving positive slide agglutination.

In both cases, startlingly enough, the Sonnei serum completely agglutinated the organisms to titre, while the Flexner V, X and 119 sera showed only partial agglutination to 1:20 and 103 to 1:20 with one strain and 1:40 with the other.

In attempting to type dysentery bacilli with sera whose main characteristic seemed to be an extreme sensitiveness to homologous antigens on slide agglutination, it was not a little disconcerting to find these two organisms, ten months apart, failing to be agglutinated on slide, but promptly agglutinating in tubes.

The last two of these six dysentery bacilli were typical Sonnei strains, showing no reaction whatever with members of the Flexner group and complete tube agglutination to titre with Sonnei serum with positive slide agglutination as well.

Seven other strains of dysentery bacilli were also examined during the course of the year. Three were from adults - student nurses on ward duty who, within five days of each other were suddenly stricken with attacks of violent diarrhoea.

The biochemistry of two of the strains was identical with that of three strains isolated from children in the hospital during a period of sixteen days, five days after which the first of the nurses took ill. The serology, however, indicated that the nurses did not acquire their organism from those three dysentery cases, as all three strains from patients agglutinated more fully with Sonnei serum than did the nurses' organisms, and there were some differences in the cross agglutination reactions as well. However, both these nurses' strains and two of the patients' strains of that time did have in common an antigen which gave low-grade agglutination in 119 serum, while three of the four showed agglutination in 103, X and V sera, though not the same three in each case.

In this connection, Weil et al. (60) point out that such variation between the secondary antigens has been found to occur among strains from the same outbreak. They find that "strains from the same outbreak vary in this respect enough to make us cautious concerning "Tagging" by slight antigen differences, for instance for epidemiological purposes".

One important practical point was brought up by the examination of one of these two nurses' strains. This was the discovery that slide agglutination could occur with specific Flexner sera but not with the polyvalent Nos. I or II. Eight months later another strain isolated from a patient gave almost the same serological results, being negative in polyvalent Flexner Nos. I and II but slightly positive in Flexner 103, X and 119, corroborated by tube agglutinations. These two strains were also strongly positive for Sonnei serum. The only difference between them was that the one from the patient

fermented rhamnose late and agglutinated with Flexner V serum, while that from the nurse did not.

The third strain isolated from a student nurse showed the widest range of cross agglutination in the Flexner group found in this survey, very different from any strain isolated from patients. The biochemistry differed from the Sonnei strains by being consistently lactose negative and indol positive, suggesting the Flexner group. This was borne out by the fact that the Sonnei tube agglutination showed only partial agglutination to 1:20 while Flexner V serum agglutinated the organism to a titre of 1:320. The titres of the other sera in the group were as follows:-

Flexner Z, Newcastle, Polyvalent I and II	-	Complete to 1:80
Flexner X and 119	-	Complete to 1:40
Flexner W	-	Complete to 1:20
Flexner 103	-	Partial "P" to 1:20

Besides these three strains from adults, four other strains were studied from children. Two, one from the Royal Victoria Hospital, Montreal, and the other from the New York Babies Hospital, appeared to be serologically and biochemically almost identical, although no absorption experiments were performed.

Here, again, the slide agglutination test for polyvalent Flexner Nos. I and II were both negative, while those for Flexner X and 119 were positive as well as those for Sonnei. All of them were confirmed by tube agglutinations, with the Sonnei serum giving the strongest reaction, almost to titre, and the others in lesser degree.

The only difference between the two strains appeared to be that the one from New York showed some relation to Flexner 103.

The two last dysentery strains are of interest because of their negative results. One was isolated from a normal child during a routine examination. It agglutinated on slide test with polyvalent Flexner No. I and II, Sonnei and Newcastle sera but failed to give any reaction in tubes. The other strain was brought from the New York Babies Hospital. It was much less active biochemically than the Montreal strains, and would not agglutinate, on slide test, with any of the twelve dysentery sera available.

Such inagglutinable strains have been reported during the past year by Wheeler (62) and Weil et al. (60).

It might be mentioned in passing that the tests described above were performed on the freshly isolated organisms and were all repeated weeks or months later. The biochemical tests were kept for two weeks.

None of the patients with enteric disease due to dysentery bacilli died as a result of this infection. Neter (52) suggests that such a happy outcome could be due to the fact that no exotoxigenic strains were involved.

To sum up, though there may be some doubt about the validity of the name "Shigella sonnei" as applied to some of the strains isolated, all things considered, the general impression of the types of dysentery bacilli found in this Children's hospital is that Sonnei are the commonest, with occasional Flexner, but no Shiga strains. This ties in well with reports in the United States, such as (26).

Salmonella strains Six strains of Salmonella were isolated during the course of the year. Their identification was very much easier than that of the dysentery bacilli, as there was very little of the cross agglutination found with those organisms and no sensitivity to normal rabbit serum. The slide agglutination with the polyvalent serum in each case was prompt and complete, and confirmed by tube agglutination to the titre of the serum. Thus, the laboratory had no difficulty in rapidly reporting the organisms as members of the Salmonella genus. Species differentiation, however, was not as straightforward.

Biochemical reactions were remarkably uniform. All were lactose, sucrose and indol negative and produced acid and gas promptly from dextrose, maltose, mannitol, dulcitol, xylose and arabinose, with positive H_2S production.

Since variations in biochemistry have been recorded so often, all the seven available specific Salmonella sera were employed in slide agglutination tests. In four of the strains positive results were obtained with one of the seven sera only, while the other two strains showed positive results with only two of them. This speaks well for the high degree of specificity in these sera, especially in a genus where cross reactions are so common.

One strain agglutinated on slide with the Newport serum, but in tubes went to completion only to a titre of 1:20 and "P" agglutination only to 1:160, despite a serum titre of 1:250. The biochemical reactions were typical of the group of organisms of which the Newport variety forms a part.

The second strain biochemically fell within the same group, being also inositol negative. It agglutinated with

Enteritidis serum on slide, but this was not confirmed in tubes.

Of the other four species, two were inositol positive and two were negative. All showed positive slide agglutination with Aertryke "O" serum, corresponding to "Para B "O" antigen, but none of these went higher, in tube tests than complete agglutination to 1:80. It was doubted that these organisms could be Salmonella schottmuelleri as its specific antigen was not included in the polyvalent Salmonella serum, and these organisms all were agglutinated with this agent. This doubt was confirmed by the fact that only one of the four strains agglutinated with "Para B "H" antigen on slide and even this went to a complete titre of only 1:80 in tubes. The organisms, then, clearly, were not of the true Schottmuelleri variety, despite the fact that they agglutinated readily with Lederle "Anti-paratyphoid B serum" (no titre of "H" or "O" constitution given). This was borne out by a Widal done on one patient three weeks after admission. The reaction with known S. schottmuelleri "H" antigen was complete only to 1:10 while that with the "H" antigen from the patient's homologous organism was complete to 1:320. Dependence on the obviously non-specific Lederle serum would have led to the misnaming of this organism, and, no doubt, if widely used, to a marked increase in the number of Paratyphoid B. fever cases recorded.

It is of interest to note that one of these Salmonella strains was isolated from a middle ear infection; a reminder of the distinct possibility of extra-intestinal localization of infections caused by Salmonella in children. The Montevideo (36) group reports that 20% of 67 cultures of pus from the middle ear of children showed Salmonella.

As was the case with the dysentery agglutinations, all the tests were repeated on one day with simultaneously prepared antigens from fresh cultures. Reading of "O" antigens at 48 hours as well as 24 hours showed, more commonly than with dysentery, a moving along of complete and partial agglutination to the next dilution, i.e. at 24 hours the antigen may be completely agglutinated to 1:40 only, while at 48 hours, agglutination was complete to a dilution of 1:80.

Naming of the varieties was not attempted with the above results, the organism being reported simply as "A Salmonella showing the biochemical and some of the serological characters of the Paratyphoid B group".

Staphylococcus strains isolated Sixteen strains of Staph. pyogenes were isolated from patients' stools. All showed typical one to two mm. opaque colonies, with convex mirror-like surface, entire margin and strong beta haemolytic effect upon the human red blood cells in the medium. Fifteen strains showed typical pale orange-yellow colour, while one strain was white in colour.

All were plasma coagulase positive, as mentioned before, and were reported on that basis alone. For the more complete comparison of these strains, other media were tested with the following results:

Lactose and sucrose peptone water:-

All strains produced acid only.

Mannitol peptone water:-

All strains, except one, produced acid but no gas.

Salicin peptone water:-

Five strains produced acid, no gas.

Eleven strains were negative.

Nitrite production from Nitrate broths:-

Nine strains positive.

Seven strains negative.

Gelatine:-

Liquefied by only five strains after 10 days incubation.

These five strains became positive in 4-5 days.

Bergey's classification of the genus Staphylococcus makes no mention of the plasma coagulase test, so that a comparison of these strains with the biochemical characters described there for pathogenic Staphylococci would not seem to contribute a great deal.

It might be mentioned, however, that most workers in the past regarded positive lactose, mannitol, nitrite and gelatine tests as some indication of pathogenicity. At the present time, coagulation of plasma is considered a more reliable criterion (6) and it is interesting to note that of the sixteen strains here, all clotting plasma, seven failed to produce nitrites from nitrates and ten failed to liquefy gelatin. Perhaps these latter would have done so if they had been incubated longer, but no clinical laboratory can afford to wait even ten days before reporting such common organisms as Staphylococci.

Enterotoxigenesis, the most important criterion of the pathogenicity of Staphylococci for the human intestinal tract, was unfortunately impracticable, due to the shortage of kittens for this work. Nor were any serological methods available for the use of those who endeavoured to assay the significance of Staphylococci in stool cultures. Serological methods, although so valuable with the Gram negative rods, are, so far, not applicable to the Staphylococci.

The significance of Staphylococcus in stool cultures is difficult to evaluate. Dolman hinted that occasionally enterotoxin might be manufactured in the gut and give rise to vomiting and diarrhoea. It has been common experience that stool cultures rarely yielded Staphylococci, but the frequency in this series (16 out of 148) makes Dolman's suggestion an intriguing one. To incriminate the Staphylococcus, with certainty, however, would demand the demonstration of the absence of customary intestinal pathogens, and probably direct or indirect evidence of enterotoxin formation.

In only three of these sixteen cases were there intestinal symptoms in the absence of accepted intestinal pathogens. All three children were under six weeks of age, and were passing diarrhoeal stools containing mucus at the time of culture. One of them, the most acutely ill, showed Lancefield A haemolytic Streptococci in the stool on one occasion, another rare finding.

Two other cases were diagnosed clinically as dysentery and paratyphoid fever, and Shigella and Salmonella were isolated regularly, while the Staphylococci appeared only once.

Of the other eleven cases, the diagnosis ranged from such unrelated conditions as congenital heart disease to influenzal meningitis, and intestinal Staphylococci were apparently fortuitous. In six of these cases, nose, throat or mouth cultures showed Staphylococci, which might have resulted in the positive stool cultures through simple ingestion. However, this obvious explanation is probably wrong, for in another series, 12 throat cultures containing Staphylococci and 6 containing Lancefield A Streptococci were done within two days of subsequent stool cultures on the same patients, none of which showed either of these pathogens.

Excluding two children of 14 and 8 years of age, the average age for the other cases whose stools were found to contain Staphylococci was 5 1/2 months. This compares with an average age of patients with Salmonella of 2 years, with typhoid bacilli of 3 1/4 years and with dysentery bacilli an average of 5 1/4 years. At the present time, then, it seems wise not to attribute too much importance to the finding of Staphylococci in stool cultures. The extreme youthfulness of those who harboured these organisms is nevertheless noteworthy.

Seven patients yielded Pseudomonas pyocyaneus in stool culture. Six of the seven case histories gave no evidence whatever that this organism could be contributory to the cause of disease. In the seventh case "Pharyngitis and diarrhoea" was given as the clinical diagnosis. In addition to Ps. pyocyaneus, stool culture yielded, in the one specimen received, P. vulgaris and P. morgani.

These latter two organisms, it is clear from the figures on page 35, form the vast majority of the organisms iden-

tified from stools in this clinical laboratory. It is known, however, that they occur very frequently indeed in the stools of normal children. It is therefore extremely difficult to assay their significance when isolated from children who are ill, especially since repeat cultures following such isolations are not sent to the laboratory nearly as often as repeat cultures from patients, for instance, with typhoid bacilli.

However, an examination of the case records of children from whose stools the laboratory has isolated these organisms might give some indication of their significance in the large number of specimens containing them. Of the 46 patients whose stools contained P. vulgaris, 25 showed diagnoses completely unrelated to enteritis. Five of these were recorded as "Upper respiratory infection" and seven as "Otitis media", with the others ranging from three cases of "malnutrition" to "Syphilis" and "Poliomyelitis".

In thirteen of the cases from which P. vulgaris was isolated, a secondary diagnosis of "Dysentery" or "Intestinal intoxication" was mentioned, in addition to the primary diagnoses of "Pneumonia" "malnutrition", "Otitis media" etc.

The eight remaining cases were all diagnosed as "Dysentery", "diarrhoea", "Typhoid" or "Paratyphoid" fever. From four of these, frank pathogens and other organisms were isolated in addition to Proteus, but from three cases, P.vulgaris was the only organisms found, - the one other case having Proteus P. morgani as well. The average age for these last four cases was three months.

Forty patients' stools showed Proteus morgani. Twenty-one of these were associated with P. vulgaris; thirteen,

three and five strains respectively in the three categories described above according to the case record diagnoses.

Fifteen of the Proteus morgani cases had diagnoses irrelevant to enteric disease, with about the same incidence of "Otitis media", "Pneumonia" and "malnutrition" as was found with the similar group of P. vulgaris cases.

The remaining four cases were diagnosed as enteric conditions and blood and mucus found in their stools. A Salmonella was isolated from one and B. paracolon from a second case, in conjunction with Proteus morgani, the last two cases having the latter organism alone. All these last three cases were under three months of age.

The incidence of B. paracolon and of late-lactose-fermenting organisms will now be examined together, as most modern authors include all late-and non-lactose-fermenting organisms which ferment sucrose in the paracolon group. Of the sixteen organisms of this group isolated, ten of their cases showed unrelated diagnoses; with "Pneumonia", "Otitis media" etc. as common as before. Of the six remaining cases, two showed Salmonella and dysentery bacilli, as well as paracolon which would explain their enteritis.

Two of the remaining four cases had B. paracolon alone, and blood and mucus were seen in their stools. One other case showed B. paracolon, P. vulgaris and a late-lactose-fermenter after blood and mucus had been seen in the stool. The final case was that of a girl 14 years old, admitted acutely ill and showing colon ulcers with the sigmoidoscope; but the only suspicious organism found in her stools was a sucrose negative, indol negative aerogenic Gram negative rod fermenting

lactose after 6 days. This organism was isolated from only the first specimen.

None of the patients from whom Achromobacter or Alkaligenes were isolated showed any "Infection" type of enteric disturbance. Three children from the same family were admitted with symptoms of a "toxin" type of food poisoning. The mother and father were also taken ill, three hours after dinner, when all five shared some cooked meat. This laboratory, unfortunately, could not ascertain whether or not the meat was cultured, and the Montreal General Hospital did not isolate any pathogenic organisms from the parents. One child's culture showed Achromobacter, the second showed Pseudomonas, B. proteus and B. alkaligenes, while the third showed a Salmonella. The short incubation period, however, forces one to conclude that the Salmonella is more likely to have been present by coincidence, and that pre-formed toxins in the food were responsible for the acute symptoms. All three children recovered promptly as did also the parents.

In addition to these 152 cases from which known and "Border-line" pathogens were isolated, there were 25 children in whose stools none of the above listed organisms were found. Only three of these had case histories indicative of enteric disease. One case cleared up immediately on admission to hospital. One case had a negative proctoscopic examination, while the third showed reddened bleeding mucosa with considerable mucus and pus.

DISCUSSION OF RESULTS

The sixteen patients from whom frankly pathogenic Gram negative rods were isolated require little discussion. Clearly, any comment made should be centered upon the apparent discrepancy between the large number of cases of acute diarrhoea whose stools were cultured, and the small number of frankly pathogenic organisms isolated from them.

One answer to this may well lie in the fact that 68% of the patients whose cultures have been reported here are under two years of age. One of the main differences in the manifestations of disease between infants (0-2 years) and adults is the fact that, owing to the great instability of the infant's autonomic nervous system, focal disturbance may produce severe generalized systemic symptoms, while in adults, the main manifestations are focal, and the systemic manifestations, if present at all, are usually milder in character. It seems to be a common experience at a children's hospital that cases which are admitted with the clinical picture indistinguishable from that of an acute gastro-enteritis are found to have inconclusive stool cultures. At autopsy, as was the case with three of those cases listed above, no pathological lesions are found in the gastro-intestinal tract.

On physical examination, however, a focus of infection is found in the body such as pneumonia, upper respiratory infection or otitis media, and therefore the intestinal manifestations would have to be explained on the basis of a generalized toxaemia, rather than on the basis of a pathological state of the intestine itself.

This would seem to offer partial explanation as to why so many of the cultures were inconclusive, especially as the case records show just such evidence of focal infection in conjunction with acute diarrhoea.

There is, nonetheless, a small percentage of cases diagnosed as enteric infections from which each of the "Border-line" group of doubtful pathoogens has been isolated. This would seem to suggest the possibility of some pathogenic effect on the part of such organisms as the Proteus and paracolon group. It is to be noted that in this percentage, the children are, as a rule, very young and not in the best state of health, even without their enteric trouble. On this point Stuart et al (57) state that "case histories tend to show that colds, improper or inadequate diets or excessive fatigue may predispose an indiv-idual to attack by paracolon bacteria".

Possibly the same relation to inadequate diet may be postulated in the case of the Proteus group, for ten cases of malnutrition had mild to severe diarrhoea, with mucus and occasionally blood, from all of which this organism was isolated. In addition, the severe illnesses with which the majority of the cases described above are affected might conceivably lower their resistance to such organisms as those of the Proteus group and Staphylococci, even non-enterotoxigenic strains. Certainly, an association of the organisms listed above in seriously ill children is a finding very common in this laboratory. The dual incidence of **P.vulgaris** and P. morgani has already been noted, as well as the occurrence of these organisms with one or other of the frank pathogens, and with Achromobacter, Alkaligenes

B. paracolon and Pseudomonas. As association of these organisms, however, has been noted in normal stools, as well as in those of patients (35). At the Children's Memorial Hospital, of fifteen routine stools from normal children with no history of enteric disease, seven had no pathogens, five had Proteus vulgaris, three of which had P. morgani as well, while a fourth showed B. paracolon in addition to the other two organisms. The remaining three had B. paracolon alone, P. morgani alone and Achromobacter and Alkaligenes combined.

Two dysentery cases and one Salmonella case had Staphylococci associated with them, as well as P. morgani and P. vulgaris. One dysentery case was readmitted six weeks later and stools found to contain typhoid bacilli as well as the old dysentery strain. The last Widal done, fourteen days after the second admission, showed negative results for typhoid "O" and "H" antigens, but complete agglutination to 1:320 and ~~"P"~~ partial to 1:1280 with an antigen made from the patient's own dysentery strain.

On this point of mixed infections, Hormaeche (35) reports an incidence of 66 cases out of 3,592; including 10 cases of Shigella enteritis with positive blood agglutination, mixed with Salmonella organisms, 4 cases of Salmonella enteritis with positive Widal which carried Shigella, 28 cases with two types of Salmonella and even one case with five types. These astonishing results were probably due to the detailed examination of 40 colonies from each culture.

Such very conscientious work as this makes one feel that possibly the stools from enteritis cases from which only "Border-line" organisms are isolated really did contain frank

pathogens, but that these were masked by the greater numbers of the doubtful types. Cultural methods, then, must come under careful scrutiny.

In the case of coagulase-positive Staphylococci in this laboratory, the proportion of isolations from stools seem fairly high in comparison with other pathogens, simply by using blood agar plates. Chapman (16) is one of the few authors found who refer to this group of organisms as a possible cause of diarrhoea, but he makes no comment on the relative incidence of Staphylococci and pathogenic Gram negative rods. Chapman, in his work, isolated his strains of faecal Staphylococci with regularity by means of plating on Bacto phenol red mannitol agar and on Bacto brom-thymol-blue lactose agar. Probably the use of these two media in the routine clinical laboratory would increase the number of Staphylococci isolated from stools, but until some method is devised for testing their enterotoxigenicity more easily routinely than by kitten inoculation, such an increase in the number of isolations would seem to have little practical clinical value.

The use of SS medium for the isolation of dysentery bacilli seems to be endorsed by all who have compared it with the older types e.g. (31). Hynes' (37) modification of SS agar appears to give an even higher percentage of dysentery bacilli isolations from known cases, especially from patients in convalescence. Cooper et al (18) report that MacConkey's medium is slightly better for Sh. sonnei than SS agar, but, in our experience, the growth of so many B. coli strains on MacConkey's plates greatly hampers the isolation of single colonies of S. sonnei. Hormaeche (35) found that the use of

SS agar markedly reduced the number of cases of dysentery of unknown origin in Montevideo, especially among infants.

Tetrathionate broth, as an enrichment medium for Salmonella, has had consistently good reports. Hynes' work (37) showed that it is 80% effective in aiding isolation of both typhoid and Salmonella organisms from known cases. However, he points out that Proteus group organisms grow very freely in tetrathionate - as this laboratory has also found, and, because of this, he suggests, for Salmonella isolation, the use of Ruys' medium as well as tetrathionate for enrichment.

Plating of cultures in such media onto MacConkey agar seems to give almost as high a proportion of Salmonella isolations as any other, but Wilson and Blair's medium, Hynes found, picked up typhoid bacilli from an average of 50% more known typhoid cases than did MacConkey's medium.

It is consoling to see in the literature that other institutions do not find pathogenic organisms in all known dysentery cases. The Research Foundation of Cincinnati, Ohio, for instance (18), reports 74.5% positive cultures from such patients, while the Children's hospital in the same city report only 41% positive. Not as extensive cultural methods are used at the latter as at the former, and the latter use exclusive Selenite "F" enrichment, which is reported to be unsuitable for dysentery bacilli isolation. Halperin states (31) that, at the Children's Medical Center, Dallas, Texas, only 35% of cases of acute diarrhoea had positive cultures, and Cooper feels that a figure of approximately 43% is representative of most institutions in the United States.

One factor which may influence the percentage of negative cultures may be found to vary with the time during the disease at which the cultures are taken. In an examination of the case histories it was evident that, though acute diarrhoea was an outstanding symptom before admission, the child when in hospital often passed normal stools only. In addition, it was noted that the stool specimens of 17 patients with unexplained diarrhoea whose reports were inconclusive were not sent to the laboratory at a time when blood and/or mucus was present, but at times varying from one to five days after such physical signs of acute diarrhoea had disappeared.

The possibility must also be considered that, with negative cultures, the pathogenic organisms have died or been overgrown by the non-pathogens. However, Cruickshank and Gillespie (19) report that both Sonnei and Flexner organisms will survive in moist faeces left at room temperature (15-20°C) for up to four days. The relative numbers of Sonnei organisms decreased more rapidly under such treatment, than the Flexner organisms which persisted in apparently undiminished numbers.

In our laboratory, these organisms were viable in pure culture on ascitic agar slopes for as long as nine months at room temperature. Salmonella, typhoid and Staphylococcus cultures were also found to be alive for as long a period under similar conditions, though each strain was also lyophilized for permanency.

POSITIVE CULTURES FROM CONVALESCENTS. CARRIERS.

From the public health standpoint, it is of interest to note the relative length of time during which patients from whom pathogens were isolated retained these organisms.

With the three typhoid cases, the organisms disappeared in from five to fourteen days after the first isolation, and all subsequent cultures were negative.

With the sixteen cases from which Staphylococci were isolated, only two showed this organism in the second specimen. The Lancefield A Streptococcus was also isolated from only one specimen, subsequent cultures being negative. Pseudomonas, as well, appears to disappear rapidly, for the few cultures done on cases carrying this organism were also negative.

The dysentery bacilli seemed to be no more persistent. In all but one case, only the first culture showed the organisms; subsequent stools, when sent to the laboratory, having negative cultures. In the one exception, the patient from whom the organism was isolated was readmitted seven weeks later, and found to be carrying the same organism. Among convalescent carriers and healthy children alike, this harbouring of dysentery bacilli might be found to be more common if stool cultures were undertaken with greater frequency. In our laboratory, out of twelve children whose stools were examined as a routine measure for camp admission, one was found to be carrying an organism with the biochemical and some of the serological characters of a dysentery bacillus. This organism was still present in a specimen passed seventeen days after it was first isolated, but no further specimens were received from this carrier to con-

tinue the study. The embarrassing predicament in which the isolation of such an organism from a healthy child places its parents is self evident. However, that such cases are common among adults as well as among children is well recognized. Weil (59) points this out when he quotes that 2% of apparently healthy individuals in New York City are carriers of dysentery bacilli.

The convalescent temporary carrier is considered by Bojlen (7) to be the most important spreader of infection. He also estimated that 2.7% of dysentery in Denmark led to chronic dysentery. Felsen (27) reported an even higher figure of 8% for the United States. Thus it is clear that the reservoir from which children can be infected is very large indeed.

Sulphonamide treatment has been widely used in an effort to cut down the development of chronic dysentery and the carrier state. In a paper by Lyon et al (41) only three chronic infections resulted after treatment of 291 dysentery cases with sulphathiazole and sulphaguanidine. Contrary to this, however, is, for example, the report of Yannet et al. in the same year (1942) (65) concerning an outbreak of Sonnei dysentery where neither of these drugs prevented chronicity. In this connection Cruickshank (19) suggests that possibly Sonnei is more difficult to eradicate than Flexner.

The Montevideo survey shows a higher percentage of Salmonella carriers than of Shigella carriers there, but they feel that these figures are misleading as they do not feel that it has been proved that the methods for isolation of Shigella are as good as those available for the isolation of Salmonella.

During the course of this work it was found that the Salmonella organisms persisted far longer than those of any other pathogenic group, as a general rule. Salmonella was found to be present in stools in from at least two to seven weeks after primary isolation. The cultures were not continued, however, after three negatives had been reported, and, in two cases, the last culture done before discharge still showed Salmonella organisms. The repeat cultures on the stools of these patients left one with the uncomfortable feeling that sulphonamide dosage might be responsible for the final loss of the organism while the patient was still in hospital, but-as was the case with one patient - when this stopped on discharge the organism might have returned. Few specimens were ever sent to the laboratory to check on this point.

An effort was made to follow up the patients after discharge, but it was found that half the patients from whom frankly pathogenic organisms were isolated lived outside of the city, so that fresh specimens were unobtainable. Letters were written to the parents of those patients who lived in the environs of Montreal Island offering to collect and culture a stool specimen free of charge, if it was collected, refrigerated and a telephone call made to the laboratory. Only half of the parents answered the letter. None of the subsequent cultures showed any of the former pathogens. These follow-up cultures included stools of four patients who formerly had had Staphylococci, and one each of patients who had had Salmonella, Typhoid and dysentery bacilli.

There seems to be a singular loss of interest, by parents and physicians alike, in the possibility of pathogens

still being present once the patient has recovered from the symptoms of his illness. The hospital routine is that no patient should be discharged unless three negative stool cultures have been reported several days apart. It is often impossible, however, to continue to keep a child in hospital, even though his cultures are positive, when he is clinically completely cured and occupying a bed badly needed for acutely ill children.

It is only to be deplored, then, that parents are not sufficiently educated to appreciate the necessity of follow-up cultures on their children after they return home, not only in their own interest, but in the interest of their other children in the family and of the community in which they live.

One other point may be of interest, noticed during visits to the former patients' homes. Though most of the families appeared to be in the lower income brackets, there was a wide variation in the cleanliness of the homes, with one of the most persistent Salmonella infections coming from the neatest freshest house imaginable. On the whole, however, the homes visited did not present a very prepossessing appearance, with flies and dirt common features and, in one case, the most primitive of outside sanitary arrangements. Hormaeche (35) described the same set of conditions in Montevideo, about 3/5 the size of Montreal, where, with general sanitation standards being low, enteric disease is commonest among the low-income group of people, but is also found in the children who have had every advantage.

SUMMARY

This thesis presents an investigation into the aetiology of enteritis among children in the district surrounding Montreal. An effort was made to examine all possible causes of enteritis and treatment of the subject was not confined to one or other of the outstanding pathogenic groups alone.

The literature review, therefore, covered previous work in the fields of the dysentery bacilli, Salmonellas, Staphylococci and other organisms of "Border-line" pathogenicity. Materials and methods used were discussed briefly, with remarks giving some indication of the reasons for their selection.

The results comprised a study of all the organisms isolated from one hundred and forty-three cases. Sick children from whom the same organisms were isolated were grouped together and the various strains in each group compared both biochemically and serologically, where possible.

The significance in stool cultures of bacteria not normally regarded as intestinal pathogens is discussed. Mention is made of the carrier problem.

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