OCCURRENCE OF TYPHOID-PARATYPHOID BACILLI IN SEWAGE.

SOME FURTHER OBSERVATIONS.

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THE problem of the atypical mild or abortive case of enteric fever is one to which the attention of all concerned with the public health is being directed. Infections of the paratyphoid type may be extraordinarily protean in their manifestations, and it is a matter for speculation at the present time to what extent this disease is accountable for mild illness of short duration lacking all those grave features which have hitherto characterized classical enteric fever. Diagnosis of many cases ultimately recognized to be paratyphoid infection has been made only after repeated bacteriological or serological examinations, such assiduous search being prompted by a definite history of contact with a known case. Improved sanitation has led to a great diminution in the incidence of enteric fever of the severe type, and the danger exists of assuming that the disease is no longer a major factor to be reckoned with in public health work. Evidence is accumulating that such a sense of security is not altogether justified.

Scientific approach to the question is, however, difficult. The typhoid carrier rate in the general community affords one method of attack on the problem. This provides an index of the occurrence of the disease, since the danger of contact infection is always present, even when the conditions of sanitation approach the ideal. Among early attempts to arrive at an estimate of the incidence of carriers in the general population may be mentioned that of Minelli (1906),¹ who found one carrier among 250 persons examined in Strasbourg. Chapin (1916)² quotes the finding of organisms of the enteric group in the excreta of 3 of 986 healthy inhabitants of Washington, D.C. Welch and others (1925)³ carried out a systematic examination of 1,076 healthy dairy workers in an area where the incidence of enteric fever had been considerable during the years preceding the examination. In reporting the high carrier rate of 5.1 per cent. they stress the importance of the repeated examination of both urine and faeces. If results had been based on a single examination of faeces alone the incidence would have been only 1 per cent. Bromberg and Comaroff (1929),⁴ in a survey of 484 healthy persons in a district of Palestine which had been subject to frequent outbreaks of typhoid fever, found three carriers, giving an incidence of 0.64 per cent.

The figures show marked variation, which doubtless depends largely on the previous incidence of the disease and the thoroughness of the search. Even the lowest figure indicates that any city community in which enteric fever occurs must contain in the aggregate a very considerable number of carriers.

The work of Wilson (1928)⁵ in Belfast and Gray (1929)⁶ in Edinburgh suggested that examination of sewage might be a source of useful information regarding possible loci of enteric carriers. Wilson was able to demonstrate *B. typhosus* in the sewage of Belfast, and to arrive at a quantitative estimate of its numbers in the combined outfalls immediately before purification treatment commenced. Gray, in 1929, isolated *B. paratyphosus* B from the main sewer of one district of Edinburgh and proceeded to the examination of tributary sewers draining into this main. Positive results were obtained in three of seven such tributaries. It thus appeared that the method might be of value in localizing an infected district. In the absence of known cases in such districts the isolation of the organism from the sewage indicates a very active discharge either from carriers or undiagnosed cases, both of which are of considerable importance from the standpoint of public health.

The intention of the present work was to survey the main sewers of the city of Edinburgh in order to ascertain

how far the findings of Gray were generally applicable. By a study of the incidence of enteric infections in the various districts of the city during the five years prior to, and including, that during which the work was carried out, an attempt was made to ascertain whether any correlation existed between the localization of cases and the occurrence of specific organisms in the sewage. The examination of a considerable number of samples by different methods of selective culture provided an extended comparison of their sensitiveness, and it was thought that a standard method might be elaborated and suggested for routine use.

Methods.

One sample was obtained from each main sewer and cultures were made within three hours of collection of the specimen. Three methods were employed, as follows.

1. Direct plating on MacConkey's medium containing both lactose and saccharose (see Gray, 1929).

2. Ten tubes of brilliant green peptone water medium were inoculated each with a large loopful of sewage. The concentrations of brilliant green (Grübler) in these tubes ranged from 1 in 100,000 to 1 in 2,000,000. The peptone water used was standardized to pH 6.4. Incubation was for forty-eight hours at 37° C., when one to three loopfuls from each tube were spread on a plate of the lactose-saccharose medium referred to above, the amount of the inoculum depending on the growth visible in the peptone water tube.

3. Three plates of the glucose-iron-bismuth-sulphite brilliant green medium, as described by Wilson and Blair (1927),⁷ were heavily inoculated, a glass spreader being used to distribute three to five loopfuls of sewage over the surface of each plate. After incubation for forty-eight hours suspicious colonies were subcultured on to MacConkey plates. Pale colonies from the three sets of MacConkey plates were subcultured into tubes of peptone water and incubated for forty-eight hours. At the end of that time tubes showing pigment production indicative of *B. pyocyaneus* or allied types were rejected, and the indole test was carried out on the remainder, positive reactors being thus eliminated. The growth from those remaining was subcultured on a coagulated serum medium so that after incubation for forty-eight hours proteolytic organisms could be excluded. Biochemical reactions were then tested by fermentation of glucose, lactose, and mannite, and formation of H₂S in lead acetate glucose agar. In this way it was possible to select out possible strains of the enteric group from each sample with the use of the minimum of media. Identification was completed by biochemical reactions with a wide range of carbohydrates and the use of a high titre immune agglutinating serum.

Results.

In all, 58 samples of sewage were taken, each being from a different main sewer, so that the sewage of the whole city was sampled. Of these, 7 yielded organisms identified as *B. paratyphosus* B Schottmüller by direct agglutination and agglutinin-absorption tests. From 5 of these samples the organism was isolated only by the method of brilliant green enrichment; from 2 cases by Wilson and Blair's method. In no case was a positive result obtained by both methods. The direct plating of the sewage on MacConkey's medium gave negative results throughout.

In the case of the brilliant green enrichment method it was found that the most effective concentrations were 1 in 200,000 to 1 in 400,000. In those tubes to which smaller amounts of the dye had been added coliform bacilli of the *B. lactis aerogenes* type predominated, while higher concentration tended to inhibit growth of all organisms.

The results are perhaps not a true index of the selective properties of Wilson and Blair's medium, as it was found that batches made from the same constituents in apparently the same proportions showed great variation. In many cases no growth whatever appeared despite very heavy inoculation. With other batches good growth occurred, and in the two cases where positive results were obtained by this method colonies of *B. paratyphosus* B were numerous.

A further difficulty with this medium was that we were unable to determine with exactitude the typical colony appearances of the enteric organisms. Test plates inoculated respectively with pure cultures of *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B each yielded a variety of colony types; such variations were the cause of some confusion in dealing with the plates inoculated with sewage when subculture on to MacConkey plates of colonies presenting resemblances to these types showed a large number to be lactose fermenters.

Brilliant green enrichment with such a scheme as has been indicated for the further investigation of strains isolated appears to give the most consistent results, and it is suggested for use where a single routine method is required. A series of concentrations of the dye ranging from 1 in 125,000 to 1 in 500,000 has been found to be satisfactory. It must be noted, however, that in our work, as in that of Gray (1929), positive results were obtained by other methods when brilliant green enrichment failed. The use of Wilson and Blair's plating method in addition undoubtedly increases the possibility of isolating organisms of the enteric group. Our results suggest that the methods are to some extent complementary in that factors which militate against the success of one do not appear to operate when the other is used.

Discussion.

The areas drained by those sewers from which *B. paratyphosus* B was isolated could be divided into two groups. Four sewers yielding positive results were associated with adjoining districts comprising the whole port area of the city (District I). The remaining three drained part of the southern suburban area, the district being again well defined (District II).

By the courtesy of the medical officer of health for Edinburgh the incidence of cases of *B. paratyphosus* B infection in these districts was ascertained for the five-year period 1925-29 inclusive. The total number of cases notified in the city, excluding imported cases and those arising in institutions, was also available for the same period. Cases on diagnosis were, as a rule, removed at once to the City Hospital, so that recognized sufferers from the disease did not contribute to the discharge of organisms in sewage. The total number of cases arising in Districts I and II amounted to 46, while 103 cases had been notified during the same quinquennium for the whole city. Thus some 44.6 per cent. of the city cases occurred in these districts. It must, however, be noted that the population of these areas, as far as could be ascertained from available data, was approximately 30 per cent. of that of the whole city. It will thus be seen that, on a consideration of a prolonged period of time, little correlation is possible between the localization of enteric cases and the presence in the sewage of the specific organism in numbers sufficient to render isolation possible. This lack of correlation was further accentuated when the cases for the four years prior to that during which the examination was made were considered. During 1925-28 inclusive only 18.2 per cent. of the city cases originated in Districts I and II (8 out of 44 cases).

The figures for the year 1929, however, showed a more significant relationship to our findings. During this year 59 cases of *B. paratyphosus* B infection were traceable to infection within the city, and of these 38 appeared in Districts I and II. Thus approximately 30 per cent. of the city population provided 66.4 per cent. of the total number of cases. This would appear to show a marked relationship between the presence of paratyphoid bacilli in sewage and the topographical distribution of the disease.

Taking the finding in the case of each district separately further light was thrown on this subject. District I had an estimated population which was 19 per cent. of that of the whole city; 34 cases of *B. paratyphosus* B infection occurred in this district in 1929, or 57.9 per cent. of the total city cases. District II, on the other hand, representing 11 per cent. of the city population, provided only 6.7 per cent. of the total cases. Thus the two districts, from both of which specific organisms were being discharged in enormous numbers, gave widely divergent results in respect of case incidence.

The explanation of this may be in the difference between social conditions of the two population groups. District I represents a congested tenement area, while District II comprises for the most part a residential suburban area of modern construction. Probably the best index available for the expression of this factor is the population density. The most recent figures available are those of the annual report by the medical officer of health for the city for 1928. District I showed a mean density of approximately 48.8 persons per acre, subdivisions varying from 35.4 to 98.1, while that for District II was 5.8 persons per acre.

It seems probable that the finding of organisms of the enteric group in sewage indicates the presence of a potential or active source of infection, but that under conditions of life as found in an upper-class residential district the habits of the people are such that spread from such foci does not arise. In an overcrowded tenement area, however, the presence of a concentration of carriers, as indicated by the possibility of demonstrating the organism in sewage, is reflected in a high incidence of cases.

The sewer from which Gray isolated the organism in 1928 drained a district in which an outbreak of the disease had occurred in 1927. At the time of the present work (1929) this sewer yielded negative results. In the same way we were unable to demonstrate the specific organism in the sewage of other districts in which outbreaks of enteric fever had occurred during previous years. These findings would suggest that the permanent or chronic carriers which might be expected to result from such outbreaks are not sufficient to account for the massive discharge necessary to ensure isolation of the organism from a main sewer.

If any conclusion is possible from the present work it is that the presence of organisms of the enteric group in sewage indicates a concentration of missed cases, convalescents, and transient contact carriers in the general population. Such a circumstance may be found in association with, or immediately following, an outbreak of the disease. From a consideration of an upper-class suburban district it is suggested that such a focus may exist without any frank outbreak of the disease at the time of the examination or preceding it.

Summary.

1. A series of 57 specimens of sewage, which included samples from all main sewers of a city, has been examined with a view to the isolation of organisms of the enteric group.

2. By the use of three different methods of selective culture *B. paratyphosus* B Schottmüller was isolated from seven samples.

3. A simple routine method for isolating enteric organisms from sewage is indicated.

4. A comparison has been made between the incidence of *B. paratyphosus* B infection in areas drained by sewers giving positive results, and in the city as a whole.

We wish to acknowledge our indebtedness to Professor T. J. Mackie for the interest he has taken in this work; also to those of the sanitary staff of the city who have assisted in obtaining the necessary sewage samples.

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FOOD POISONING DUE TO EGGS.

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ONE habitually regards an egg as one of the safest and most wholesome forms of food. When the Three Musketeers had just escaped by a chance from death by the poisoned wine arranged for them by Lady Clarick, they sought safety in plain boiled eggs, and water drawn by Athos in person from the public fountain. But there is strong reason to believe that this innocence of the egg is, after all, only relative, and that, in fact, eggs are responsible for many cases of food poisoning which remain mysterious—that is, cases of severe or fatal gastro-enteritis due to salmonella infection in which none of the usually suspect articles can be incriminated. During the last three years there have been quite a number of instances of this kind, affecting both single individuals and groups of people. It has never been possible to bring absolute