there any albuminuria. In three of the cases of albuminuria there was no dyspnoea on walking, and in the other, the original very acute case, there was no way of telling, as he was in bed. It looks as if in some cases the chlorine or bromine damages the lung epithelium so severely that it does not allow absorption into the general circulation, while in others the gas passes through the lungs without affecting them permanently, but then sets up an acute nephritis.

One other case under my care, the result of gas poisoning, is difficult to explain. A well-built man of 24 was rendered unconscious by gas in April. He says that, when he came to, he was lying doubled up with his head between his knees, but he does not think that he was knocked over by shell concussion. Ever since he has suffered from severe attacks of pain in the upper chest, radiating down the left arm. These are relieved by amyl nitrite and trinitrin, and are true angina pectoris. His pulse is 80, the vessel of normal thickness and size, and tension is not high. On listening at the aortic cartilage there is a loud rough systolic murmur and a fair second sound—no diastolic murmur. The systolic murmur is loud along the course of the aorta to the left second space, and is heard in the carotids. The apex beat is in the fifth space in the vertical nipple line, the impulse is fair. There is a good first sound, a slight systolic murmur not conducted outwards, and a second sound; x rays show no enlargement of the aorta. There is no history of rheumatism or of syphilis, and the man was perfectly fit up to the day he was gassed. My impression is that he ruptured one of the cusps of the aortic valve in his struggle for breath.

THE ISOLATION OF TYPHOID AND PARATYPHOID BACILLI FROM FAECES,

WITH SPECIAL REFERENCE TO THE USE OF BRILLIANT GREEN AND TELLURIC ACID.

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AND

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THE methods for the identification of the various organisms belonging to the typhoid-coli group which cause epidemic disease by infection proceeding from the alimentary canal have now been well established.* As a preliminary to identification, however, it is necessary to isolate the suspicious organisms in pure culture from the infected subject. In the case of urine this is usually simple, provided that the patient is excreting organisms at the time of examination. When dealing with faeces it may be a matter of considerable difficulty owing to various coliform bacilli being present in great excess over the specific causal organisms. Under ordinary conditions these accompanying organisms tend to overgrow the specific types in culture media. In the early stages of the acute attack of such infections the method of blood culture is, of course, the most important diagnostic procedure, but in convalescent cases, or for the detection of carriers, the faeces and urine must be examined. (The Widal reaction is obviously of extremely limited value as an indication of infection in cases which have recently been inoculated with typhoid or paratyphoid cultures, singly or in combination.)

Unfortunately the argument does not apply that if the specific organisms are so scanty as to be readily missed, then such a case is relatively of only slight importance as a source of infection; on the contrary, it is now well known that the number of specific bacilli in the faeces and urine of a carrier may suddenly undergo marked fluctuations, and a case which is liable to be overlooked at the time of examination on account of the scanty number of organisms present may later on excrete the bacilli in great abundance.

Individuals who excrete these organisms are, of course, a very serious menace to a population unprotected by prophylactic inoculation; but their danger to an inoculated

community must not be underrated. It is to be remembered that immunity is only a relative state, and although the strongest proofs of the value of antityphoid immunization have now been afforded, there can be little doubt that a considerable proportion of individuals who have received the customary two doses of vaccine will still be susceptible to a sufficiently massive infection.

For these reasons it cannot be too strongly urged that every means should be adopted to ensure as thorough an examination as possible of the faeces of possible intestinal carriers. Not merely should the faeces be repeatedly examined,† but the most precise methods available for the detection of the specific organisms should be adopted. In cases where the specific organisms are scanty in proportion to the coliform bacilli, the customary method of spreading an emulsion of the faeces on the surface of a solid medium in Petri plates is obviously a somewhat imperfect procedure. In order to obtain a sufficient number of isolated colonies so as to include one of the specific organisms a very large area of medium may require to be inoculated. The media commonly employed, Conradi and Drigalski's, Endo's, and MacConkey's, do not have any effect in reducing the proportion of *B. coli* colonies; the results of Dreyer, Ainley Walker, and Gibson³ indicate rather that MacConkey's medium tends to suppress *B. typhosus*, and they therefore recommend the use of Endo's agar in preference.

A further defect of these media is that various coliform bacilli yield "pale" colonies resembling more or less closely those of the specific organisms, so that much time may be taken up in testing "likely" colonies which are scattered among others. Of course, in cases where typhoid or paratyphoid bacilli are numerous in the faeces any of such media will yield positive results. On the other hand, where the specific organisms form a relatively scanty proportion of the total number of viable bacilli present, the most hopeful means of isolation depends on the use of some agent which, while suppressing the others, will permit the typhoid and paratyphoid bacilli to proliferate. According to the results of Dreyer, Walker, and Gibson,³ ultra-violet light produces this effect, and they have recently described a most ingenious procedure whereby, after a plate has been inoculated with the emulsion of faeces, the contaminating organisms are killed off by graduated exposure to ultra-violet rays, whereas the typhoid bacilli survive and subsequently produce colonies when the plate is incubated.

Browning, Gilmore, and Mackie,¹ while investigating the antiseptic properties of a series of benzol dyes, found that "brilliant green" possessed a very marked differential action on organisms of the typhoid-coli group. Thus, in confirmation of Conradi, strains of *B. typhosus* were shown to be in general much more resistant to the action of this dye than were the common members of the coli group; *B. proteus* is also less resistant than *B. typhosus* as a rule; Gaertner's bacillus and paratyphoid bacilli are even more resistant than *B. typhosus* to brilliant green.†

The differential antiseptic power of brilliant green for the typhoid-coli group surpasses that of malachite green, and the former compound exhibits the property of permitting the growth of the specific typhoid-paratyphoid pathogenic organisms while inhibiting other coliform bacilli in a more marked degree than any other substance so far investigated. Gram-positive cocci, of course, are suppressed by it. Conradi and other workers had already employed brilliant green for the isolation of *B. typhosus*, but apparently without effecting any marked improvement on previous methods. Thus a solid medium was employed, and this is always disadvantageous if the specific organisms are scanty, since they will occur only where the plate is richly inoculated. Now rich inoculation tends to annul the antiseptic effect, and in consequence the contaminating coliform organisms are enabled to grow exactly where their presence will obscure the typhoid bacilli. In consequence, we recommended the use of brilliant green in fluid medium, a series of tubes of peptone water being employed, to which varying amounts of brilliant green were added; each was inoculated with an emulsion of the faeces, and after twenty-four hours' incubation at 37° C. subcultures were made on a solid medium

* In regard to the general problems of typhoid and paratyphoid carriers see Ledingham and Arkwright's *The Carrier Problem in Infectious Diseases*, London, 1912.

† Dysentery bacilli are not specially resistant to brilliant green, hence the method will not apply to the isolation of these organisms.

* For a comprehensive summary of the differential characters of these organisms see Henderson Smith, *BRITISH MEDICAL JOURNAL*, July 3rd, 1915.

containing an indicator. It was found that, in cases where only scanty colonies of *B. typhosus* developed on the plate inoculated directly from the faeces, practically pure growths of *B. typhosus* resulted in the subcultures from the brilliant green peptone water tubes; in a considerable number of instances typhoid bacilli were recovered by means of the fluid brilliant green medium, where the direct plate cultures failed altogether to show their presence. Although the common types of *B. coli* are more susceptible to brilliant green than are typhoid and paratyphoid bacilli, it was found that a particular group of coliform organisms was much more resistant; so far most of those brilliant green resisting types have been found to possess another property in common—namely, that they ferment inositol (*B. lactis aërogenes* is an example of this type). The inositol fermenters when present tend to overgrow typhoid bacilli in the brilliant green peptone water tubes. The striking fact was observed, however, that of all the colityphoid group the inositol fermenting types are the most susceptible to the antiseptic action of telluric acid (Browning, Mackie, and Smith²); hence by combining telluric acid with brilliant green it is possible to inhibit these organisms in addition to the ordinary forms of *B. coli*, while at the same time permitting typhoid, paratyphoid, and Gaertner bacilli to proliferate.*

We are indebted to several workers (Clarke, Smith, Stokes, Wood) who have tested the brilliant green method on an extensive scale; the original estimate of the utility of the procedure has been substantiated, and it has been shown that the employment of this method, in addition to direct plating, leads to a very material increase in the number of positive results. In the light of the experience so gained, several modifications have been suggested. As regards specimens of faeces which are to be examined for the specific typhoid-paratyphoid organisms by any method, two points are of great practical importance: (1) Cultures should be made from the motions as soon as possible after evacuation, preferably within several hours; faeces which have been allowed to stand for some time are unsuitable for the isolation of typhoid bacilli. (2) In cases where the faeces are normally solid—for example, in suspected carriers, it is advisable to administer purgatives and then examine the resulting fluid evacuations; there is a consensus of opinion that this procedure increases considerably the chances of successful isolation of the specific organisms.

Method for the Isolation of Typhoid, Paratyphoid, and Gaertner Bacilli from Faeces.

A fresh specimen of faeces, preferably fluid, is employed; plates of solid medium containing an indicator, such as Endo's preferably, or MacConkey's, are inoculated in the usual fashion, so as to secure a large number of isolated colonies. At the same time fluid medium containing brilliant green is inoculated.

The fluid medium consists of 2 per cent. peptone and 0.5 per cent. sodium chloride in distilled water, steamed in a Koch's sterilizer (for three-quarters of an hour) and then filtered through ordinary filter paper. The reaction should be corrected if necessary till it is only very faintly alkaline to litmus paper. The medium is then sterilized by steaming or in the autoclave; when large numbers of specimens of faeces have to be examined simultaneously time is saved by sterilizing the medium in bulk, otherwise it should be distributed in amounts of 10 c.cm. in test tubes and then sterilized. A stock 1 per cent. solution of brilliant green in distilled water is prepared; this keeps for many weeks or even months. Immediately before use a 1 in 10,000 dilution is freshly made up by adding 0.1 c.cm. of the stock solution to 9.9 c.cm. of distilled water, and then this dilution is added to the peptone water.

It was originally recommended that varying amounts of the dye should be added to the 10 c.cm. peptone water tubes, for each specimen of faeces a series of five concentrations being employed—namely, 0.1, 0.2, 0.35, 0.5, 0.7 c.cm. of 1 in 10,000 brilliant green. We believe that the employment of such a series is an important feature of the method since the conditions of growth of the specific bacilli depend on factors such as their numbers, the number and type of accompanying organisms, and the amount of organic matter present, none of which can be standardized.

When the number of specimens to be examined is considerable, however, the carrying out of the full procedure is expensive both in time and materials, hence a simplification consists in using for each specimen of faeces a single tube of fluid medium containing 0.5 c.cm. of brilliant green 1 in 10,000 to 10 c.cm. of peptone water. According to the experience of Clarke and Stokes, and also of Smith, this represents the average optimum quantity for the isolation of the specific organisms. When a large number of examinations have to be carried out at one time the mixture of brilliant green and peptone water may be made up in bulk and then distributed in sterile plugged test tubes (10 c.cm. need not then be accurately measured into each tube).

As regards the amount of faeces to be used, we originally employed a single loopful in each tube, in the case of very fluid faeces a large loop (up to $\frac{1}{4}$ in. diameter) being employed; solid faeces were emulsified by rubbing up with several volumes of sterile water. The tubes were then incubated for twenty to twenty-four hours, and subcultures were made on MacConkey's medium; but, in view of the results of Dreyer, Ainley Walker, and Gibson, Endo's medium would seem to be preferable. Three successive strokes are made from each tube; in this way one 4 in. plate will accommodate the subcultures made from three peptone water tubes. The plates are then incubated at 37° C. for eighteen to twenty-four hours, and the colonies examined, as in the case of direct plates.

Clarke and Stokes prefer the following procedure: The faeces are emulsified with several volumes of sterile water; the mixture is then allowed to sediment (half an hour), and then five or six large loopfuls of the supernatant fluid, free from grosser particles, are added to the tube of fluid medium. When such a rich inoculation is employed, subcultures are best made after nine hours' incubation at 37° C. When the specific organisms are found, they usually occur in abundance in one or more of the subcultures, thus saving much of the time which is taken up in testing a variety of "likely" colonies from the direct plates.

Tubes in which an abundant growth of organisms has occurred, as shown by marked turbidity and replacement of the original green colour of the medium by a yellowish discoloration, are not likely to yield *B. typhosus* in subculture. Where the series of doses of brilliant green is used the lower tubes may show this discoloration and the next higher ones are then the most likely to yield positive results. Should all the tubes appear clear after twenty-four hours and fail to yield growths in subculture, a positive result may yet be obtained after the fluid cultures have been incubated for a further period. When the single tube containing 0.5 c.cm. of brilliant green 1 in 10,000 is used the occurrence of discoloration indicates the following possibilities: (1) That corresponding to the amount of faeces used for inoculation the period of incubation ought to have been shorter—experience enables the amount of inoculation and the duration of incubation to be adjusted so as to secure the best results—or (2) that green resisting varieties of *B. coli* (usually inositol fermenters) are present. When these occur they tend to overgrow *B. typhosus*. The presence of inositol fermenters is probable when the subculture on solid medium shows a very abundant broad line of growth (suggesting in appearance the half melted creamy portion of the confection known as "chocolate cream").

With a view to suppressing the inositol fermenters telluric acid should be added to the brilliant green medium. The cultures in the green telluric acid mixture may be made along with the others, or, in order to save time, this may be omitted on the first examination. The examination of the first specimen of faeces from a case will probably show whether brilliant green resisting organisms are a disturbing factor; if they occur, then a subsequent examination of a fresh specimen of faeces should be made, using in addition medium containing 0.4 c.cm. of 1 in 1,000 telluric acid, along with varying amounts of brilliant green per 10 c.cm. of medium. Since the action of telluric acid when mixed with green is not precisely of the nature of a summation, it is advisable, in order to secure the optimum proportions in any given case, always to use a series of doses of brilliant green (not fewer than two amounts—namely, 0.25 and 0.5 c.cm. brilliant green 1 in 10,000), each along with 0.4 c.cm. telluric acid 1 in 1,000, per 10 c.cm. peptone

* Extended observations have shown that *B. paratyphosus* B is generally more resistant to telluric acid than *B. typhosus* (Smith).

water.* It was found by Browning, Mackie and Smith, in a case of enteric fever which they examined, that *B. typhosus* could be recovered from the faeces by means of telluric acid and brilliant green in combination, whereas direct plates as well as brilliant green by itself gave negative results.

The passage of typhoid and paratyphoid bacilli through media containing brilliant green or telluric acid has not been found to affect their fermentation or serological reactions. So far only incomplete series of results are available, but it appears that the employment of these procedures, in addition to direct plating, will increase the number of positive results by more than 25 per cent.

SUMMARY.

1. Specimens of faeces should be cultured within several hours after evacuation. In cases where faeces are normally solid it is advisable to give purgatives and examine the resulting fluid motions.

2. Make the usual smear cultures on plates of solid medium containing an indicator—for example, Endo's or MacConkey's. At the same time inoculate peptone water containing brilliant green; employ preferably a series of tubes for each specimen, but when time prevents this, use a concentration of 0.5 c.cm. of 1 in 10,000 brilliant green in 10 c.cm. of medium.

3. The direct plates and the brilliant green peptone water cultures are then incubated; if typical colonies are not found on the direct plates, or if "likely" colonies are scanty, make subcultures (successive strokes) from the green tubes on Endo's or MacConkey's medium. These are then incubated for twenty-four hours and examined in the usual way; should typhoid or paratyphoid bacilli be present in the subcultures from brilliant green medium they usually occur as an abundant growth of typical colonies, and it is possible to proceed at once to identify them by the usual fermentation and serum reactions.

4. In certain cases (where green resisting organisms are present) positive results may be obtained only when telluric acid is employed, in addition to green, in the medium. The cultures in the green telluric acid mixture may be made simultaneously with the others, or this medium may be reserved until the other procedures have failed. Thus, should direct plates fail to yield positive results, and the green medium be overgrown (inosite fermenters), then, in further examinations of the case, employ, in addition, peptone water medium containing a mixture of brilliant green with telluric acid.

REFERENCES.

- ¹ Browning, Gilmour, and Mackie, *Journ. of Path. and Bact.*, vol. xviii, 1913, p. 146, and *Journ. of Hygiene*, vol. xlii, 1913, p. 335. ² Browning, Mackie, and Smith, *Journ. of Path. and Bact.*, vol. xix, 1914, p. 127. ³ Dreyer, Ainley Walker, and Gibson, *Lancet*, March 27th, 1915.

HISTORICAL NOTE ON FARR'S THEORY OF THE EPIDEMIC.

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WHEN modern statistical methods are being applied with more or less success to the study of epidemics, it is, I think, interesting to recall the chief early work on this subject, especially since it has fallen into complete oblivion. The first attempt to describe epidemics quantitatively is due to Dr. Farr. No other person, so far as I can discover, had so early formed the idea that an epidemic was subject to definite laws, but his ideas were greatly in advance of his time, and his treatment of the subject was so episodic, that his discovery—I think that is the true description—has suffered almost complete neglect. His first publication was in the year 1840.¹ In that year he included in his report to the Registrar-General a short note on the course of epidemics, with special reference to

* Telluric acid can be obtained from W. Craven Ball, 45, Hop Exchange, Southwark Street, S.E. It should be kept as a 1 per cent. solution and sterilized by heat, otherwise it may become contaminated by moulds. The free acid keeps indefinitely in watery solution; it is therefore preferable to potassium tellurate, which was originally employed, but which is extremely unstable. Brilliant green may be obtained from various chemical dealers, or from Synthetic Chemicals, Ltd., Derwent Street, Derby. Graduated pipettes for measuring 1 c.cm. and fractions of a c.cm. can be obtained from most dealers in chemical glass apparatus, or from Messrs. Thomson, Skinner and Hamilton, 38, Sauchiehall Street, Glasgow.

the recent prevalence of small-pox. The numbers on which he based his conclusions were moderately large, more than 30,000 persons having died during the outbreak. He specially considered the decline of the epidemic, and fitted the figures to a curve calculated by a method described. Though he gives no equation of the form of the curve, it is quite obviously the normal curve of error. He finishes the article with a few remarks upon measles, typhus, etc., and then adds: "These exhibit the same regularity, but the laws which govern their course will be more conveniently discussed when the abstract of the observations has been extended over another year." He does not, however, in spite of frequent promises,² seem to have discussed the subject again for twenty-six years.

The hypothesis suggested, however, did not pass out of his mind. In fact, it seems rather to have become a part of his belief, for in 1866 he wrote a letter to the *Daily News*,³ a vivid and characteristic utterance in which he challenged the prevailing views regarding the progress of the cattle plague. This cattle plague had invaded England in the end of the year 1865, and caused very considerable damage. With the growth of the new year, week by week the number of cases steadily increased, till in the end of February Mr. Lowe,⁴ in a speech in the House of Commons, anticipated, in the absence of very determined measures, an epizootic of tremendous size. This statement Farr takes as the text of his letter. He definitely states his views regarding the progress of epidemics. In place, however, of assuming that the second difference of the logarithms is constant—a form which gives the normal curve—he calculates, in addition, the third difference from the observations, and uses this as well in his prediction. The curve obtained by this means is the same as that given in Case 2 of my note in the *BRITISH MEDICAL JOURNAL* of May 8th, 1915, though in his case the coefficient of the third power of the time is negative. Using this method, he prophesies an early maximum of the epidemic, with a subsequent decline—a prophecy which approximated with remarkable closeness to the actual facts.

The reception of this letter was not encouraging. The *Daily News* discussed the matter in a complimentary leader next day, yet no member of Parliament—though the cattle plague was being discussed nightly—seems to have thought it worthy of mention. The *Lancet* ignored the communication entirely. The *BRITISH MEDICAL JOURNAL*'s reception was distinctly unkind.⁵ It remarked:

Dr. Farr will not find a single historical fact to back his conclusion that in nine or ten months the disease may quietly die out—may run through its natural curve. Dr. Farr says again that the returns show that the weekly relative increase in the number of cattle which now fall with the disease is less than it was at first, and he attributes this to the view that the disease is running the usual course of epidemics. He quite forgets to take into account the fact that at the present time every one is satisfied as to the virulently contagious nature of the disease, and consequently takes measures to prevent it. When the plague first appeared, thanks to the stupid and puffed-up ignorance of the *Times*, the disease was generally set down as a local London cowshed nuisance, got, as "S. G. O." sagely suggested, by "Moonbeam" out of "Dungheap."

The letter was reprinted in the *Journal of Social Science* later in the same year (March 20th, 1866), as follows:

DR. FARR ON THE CATTLE PLAGUE.

March 30th, 1866.

My dear Sir,

I send you my letter to the *Daily News*. The epizootic has apparently attained its maximum, and is now going down, as the "law" led me to believe.

The thing was going up, and looked most alarming, when I wrote. I do not like to alter what I then wrote, lest it should be thought that the alteration is made to suit the facts.

I took the series containing the "back cases," as it was published in the second report of the Commissioners.

Before that, I had calculated a series from the weekly returns of cases reported in each week.

This series follows nearly the same course, but the numbers are about one-fourth less.

The Privy Council returns are all exceedingly imperfect; but in assuming that they are almost equally imperfect throughout, I do not think that I am very wrong.

Ever yours,

(Signed) W. FARR.