BRILLIANT GREEN AND TELLURIC ACID IN THE ISOLATION OF TYPHOID- PARATYPHOID BACILLI.

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OF the recent methods that have been advocated for the isolation of the enteric organisms (typhoid and paratyphoid bacilli) from the excreta of patients or carriers, probably that which has excited most interest and not a little controversy is the brilliant green method of Browning, Gilmour, and Mackie.¹ Support has been lent to it by the observations of Dreyer, Ainley Walker, and Gibson,² Stokes and Clarke,³ Robinson and Rettger,⁴ and others. Adverse opinions have mostly been expressed privately, though Robinson⁵ states definitely that it has no value. Teague and Clurman⁶ conclude that it must be regarded as a failure. In view of these latter opinions I venture to publish the results of a controlled test over a period of three weeks at the beginning of this year. The hospitals to which this laboratory is attached are the final hospital dépôts for soldiers convalescent from enteric and dysenteric diseases and obscure febrile conditions clinically resembling them. It is exceedingly important that no carriers should be allowed to rejoin their units in camp. Perhaps in the majority of cases the organisms are readily isolated from the faeces or urine of carriers; sometimes even they are so abundant and the condition of the bowel or its contents so abnormal that they appear in pure culture on bile-salt plates. Sometimes, again, we may fail to find evidence of their presence in known carriers, and it has been supposed that there are periods when the carrier passes no enteric bacilli. The probability, however, is that we only fail to find them because they are swamped by normal inhabitants of the intestines. At the time mentioned, the percentage of carriers found was below the average, for several months had in many cases elapsed since any illness had been complained of, or the history had been so obscure as to cast great doubts on the supposed nature of the previous illness. It seemed a good opportunity for testing Browning's method as against that adopted as a routine. Browning's method consists in the interposition of brilliant green peptone water as a means of suppressing much of the contamination before the suspected material is spread on solid media for the isolation of the bacteria. Various dye stuffs have been in use from time to time with the idea of cutting out the common bacteria of the colon—methyl-violet, malachite green, etc. Conradi was the first to employ brilliant green for the isolation of typhoid bacilli from stools. He used it along with picric acid in solid media, but as this dye develops its best bactericidal effects in media which are very faintly alkaline to litmus, Conradi obviously weakened much of its power by adding to it picric acid. Further, a mixture of organisms of various kinds with varying resistance, such as one gets in faeces, cannot be so well subjected to the uniform action of a chemical substance on solid as in liquid medium. If one may so express it, on the surface of a solid medium the organisms scarcely do more than wet their feet in the dye. In a liquid medium all the surfaces are equally prone to attack.

Browning, Gilmour, and Mackie,⁷ as the result of experiments with various dyes, concluded that brilliant green in very weak solution had a specially inhibitory effect on the colon bacillus as contrasted with typhoid and paratyphoid bacilli, whilst it had a powerfully bactericidal action on practically all other organisms. This fact I have already made use of in the treatment of septic wounds.⁸ It must be insisted on that in the isolation of enteric organisms the effect on colon bacilli is an inhibitory one mostly, and that if sufficient time is allowed, or if unsuitable concentrations of the dye are employed, or if there is too heavy an inoculation of our culture tubes, then the various adventitious bacteria may multiply to such an extent as to crowd out those of the typhoid-paratyphoid group. Again, all strains either of colon bacilli or enteric bacilli are not equally susceptible or resistant to the same concentration of the bactericidal substance; and, for this reason, Browning and Mackie advised the employment of five or six different concentrations of brilliant green in peptone water. This is a refinement which is practically impossible of

adoption as a routine measure in a laboratory dealing daily with a large number of specimens. From a careful test of 100 specially selected cases with five concentrations, I was not convinced that anything is to be gained by this; for in those cases in which enteric organisms were isolated by the brilliant green method where the direct plate failed, as also in those cases where direct plate and brilliant green method agreed, the typhoid and paratyphoid bacilli were caught by the two highest concentrations which the authors of the method advocate. Browning practically admits this, seeing that where he succeeded with his method when the direct plate method failed he generally obtained pure cultures in the highest concentration of brilliant green, and if he insisted on the longer method he would apparently do so only when a small number of cases was to be tested. Owing to the considerations already mentioned this is no doubt a useful precaution, but as a routine measure it is not convenient. Stokes and Clarke have already reported very favourable results with one concentration only, and my results agree with theirs.

In addition to brilliant green, Browning and Mackie used telluric acid in their medium because they found that certain organisms amongst others, giving the ordinary sugar reactions of the enteric group but differing from them in fermenting inositol, escaped the action of brilliant green alone, but were killed off by the addition of telluric acid.⁹ I have therefore always used both in conjunction. Even then we may get organisms surviving which we judge from all the tests we employ not to belong to the group, but the number of such is incomparably less than in the direct plate method. I have compared the results of Browning's method and the direct MacConkey plate, dealing with the same specimens, in over a thousand cases—a sufficiently practical test on which to pass judgement—and the conclusion can safely be drawn that, by Browning's method a larger number of carriers can be detected. It happens sometimes that the direct plate succeeds when the indirect method fails; and the hypercritical might suggest that when the indirect method succeeds and the direct method fails, such a result is only due to the increase in the usual number of plates spread—that is to say, if we spread three MacConkey plates directly instead of two MacConkey plates directly and another after the interposition of brilliant green, we should get the same results. From my results I should have no hesitation in preferring the brilliant green method alone if I were confined to a choice between that and the MacConkey plate, but the use of the two, where it is possible, would be nearer an ideal. Browning and his collaborators do not discard the ordinary method. By the use of brilliant green we cut out a great deal of unnecessary work by throwing out large numbers of "likely" colonies that would turn out in the long run to be quite useless for our purpose, and we suppress entirely, or restrain to moderate degrees, the growth of organisms normally found in faeces; above all, we succeed in obtaining pure growths or numerous easily identified colonies on plates of the organisms for which we seek.

In the method as given by its authors a loopful of liquid faeces is transferred directly to the brilliant green peptone water, incubated for twenty-four hours, and, from this, stroke cultures are made on MacConkey plates. When the faeces are not obtained in fluid form saline emulsions are made. Of course one can generally ensure that the stools are liquid by purging the patient. The difficulty when dealing with soldiers is that many will not swallow a pill, and we have to take the faeces as we get them. Consequently as a routine measure we emulsify the faeces in peptone broth by shaking up a roughly pea-sized portion with 5 c.cm. of bouillon. A drop of this is taken and spread on bile-salt agar plates by means of a glass spreader. I allowed these broth emulsions then to stand for a few hours in the incubator, and at the end of that time two loopfuls of the supernatant fluid were transferred to tubes of brilliant green peptone water with the dye in a concentration of 1 in 200,000 (0.5 c.cm. of 1 per cent. aqueous solution of brilliant green to 1 litre of peptone water), telluric acid, which does not affect the reaction of the medium, being added in a proportion of 1 to 25,000. These were incubated for eighteen hours, and from each three successive strokes were made on small MacConkey plates, using one plate for two specimens. I have convinced myself by control experiments that the best results are obtained by transferring some of the broth emulsion after incubation to the

brilliant green. By so doing we more frequently get pure cultures of the organisms of the group, and where they are not present we much more often succeed in obtaining sterile plates. Stokes and Clarke adopt pretty much the same technique. If we put the faeces direct into brilliant green peptone water it is exceptional to get pure cultures or negative plates. Frequently the medium is decolorized, and when it is decolorized we may be sure that colon bacilli are present in abundance. On the other hand, by the modification I have adopted decolorization of the medium is very uncommon; indeed, it is practically impossible to tell whether there is growth or not in brilliant green until after we have plated out.

When likely colonies are obtained, the same routine is adopted as in the case of the direct plates. They are transferred to lactose peptone water. If after forty-eight hours no fermentation results, the organism is tested on glucose, mannite, dulcitol, maltose and broth, and then submitted to the final test of agglutination by specific serums. The direct plates were examined by the whole laboratory staff—five in all—whereas the indirect method was carried on throughout by myself. That alone indicates the saving of work of which the method is capable.

Results of the Examination of 1,023 Specimens.

Result.	No. of Cases with Likely Colonies.	Lactose Fermenters.	Carried to other Sugars.	Necessary Agglutination Tests.	Actual Positives.
By direct plating	373	59	314	161	17
By brilliant green and telluric acid	120	39	81	45	21

In no single case did we obtain pure growths by direct plating; they were always mixed. By the brilliant green method there were pure growths of colourless colonies in 62 cases. In no single case by direct plating was the plate sterile, whereas 50 per cent. of plates by the indirect method showed no growth whatever. This means an enormous saving of time. When the growth on the indirect plate was mixed, the colourless colonies generally preponderated, and in all cases were easily detected. By the direct method the final test of agglutination had to be done on 161 cases, with a positive result, so far as typhoid-paratyphoid bacilli were concerned, in 17 cases, while, on the other hand, only 45 cases had to be carried to agglutination, with positive results in 21, in the case of the indirect method. By the direct method 6 cases of dysentery organisms were found in addition to these, whereas only one was discovered by the brilliant green method, for dysentery bacilli are apt to be killed off by brilliant green.

On referring to the specimen index we found that the 1,023 specimens were provided by 622 individuals. There were two cases of typhoid carriers in the lot. Typhoid bacilli were picked out on all of five occasions by the brilliant green method, and on four occasions by the direct plate. Paratyphoid A bacillus was picked out on the single occasion by both methods. Paratyphoid B bacilli were isolated on fifteen occasions by brilliant green and on twelve occasions by the direct plate; but this only gives one side of the picture, for it happened that no less than five paratyphoid B carriers were detected by brilliant green which were altogether missed by the direct method, whilst, on the other hand, two carriers were found by the latter method and missed by the former.

For the isolation of typhoid-paratyphoid organisms from urine I tried a slight modification of the method in 1,100 specimens. Equal quantities of urine and brilliant green peptone water (the dye being in a concentration of 1 in 100,000 in the latter) were mixed and incubated for twenty-four hours, and a loopful from each was spread on MacConkey plates. In only one case was an organism of the group isolated—a paratyphoid A. In almost all other cases the plates were sterile. The test was of no great value because it was uncontrolled, and no care had been taken to ensure the proper reaction of the composite medium. But I am enabled by the courtesy of my colleague, Dr. T. H. C. Benians, to give a short but striking experiment in favour of the value of the method. He took the urine of a carrier in which *B. paratyphosus* A and *B. coli* could be demonstrated by straight plates, and added portions of this urine to fifteen other urines in the

proportion of 1 to 10. To a parallel series he added brilliant green in a concentration of 1 in 250,000. After twenty-four hours' incubation plates were spread, with the result that by the direct method in only one case was the paratyphoid organism recovered, though *B. coli* were found in abundance on all plates, whilst by the interposition of brilliant green he found the paratyphoid organisms in nine cases in practically pure culture.

The method of Browning, Gilmour, and Mackie is therefore a useful method in detecting a number of carriers of typhoid-paratyphoid that are liable to be overlooked by the ordinary procedure. Both methods should be done together as a check on each other. Owing to the saving of time, if one method alone has to be used it should be the brilliant green.

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THE LOCAL TREATMENT OF BURNS ON A NAVAL HOSPITAL SHIP.

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THESE remarks refer to cases which have been under my care while afloat. They numbered 28, and were received (a) immediately after the *Natal* explosion, and (b) about forty-eight hours after the recent Jutland naval battle. The first application to the burn in every case was the Service picric acid dressing.

Out of the 28 cases, 15 were aseptic, and all these recovered. The remaining 13 cases were septic to a greater or lesser degree, and 5 of them succumbed, but one of these had other serious injuries. Four of the cases from H.M.S. *Natal* were virulently septic, and the two fatal cases developed erysipelas. I attributed this acute sepsis to the patients' immersion in water containing oil and sewage which had escaped from the sinking ship.

PLAN OF TREATMENT.

The treatment employed in my wards was as follows:

1. The blisters were punctured and the contents drained under strict antiseptic precautions, the surface being first sponged with carbolic acid solution, 1 in 20.
2. If it was a recent burn (for example, cases from H.M.S. *Natal*), I applied the Service picric acid dressing as the first dressing.
3. I smelt the dressings at my morning and evening duty rounds. Early sepsis in burns has an unmistakable odour, and the sense of smell can detect it early.
4. If there was no smell the first dressing was left on for two days, when it was removed in order to puncture any further blisters which might have formed. An ointment was applied, made of equal parts of boric ointment and vaseline. This was plentifully spread on strips of white "surgeons' lint," and was changed once daily. It is a good dressing, which does not tend to adhere, and so is not a painful procedure.
5. If the dressing had the septic smell the infected part was fomented with boric fomentations every four hours. The fomentation was four-ply, and the covering jaconet projected for $\frac{1}{2}$ in. beyond the margins of the focus in order to keep in the moisture. When the area cleared up—that is, when all sloughs, etc., had separated and a clean surface was left—the above-mentioned ointment was similarly applied once a day, according to the amount of pus which had been secreted from the raw surface.
6. If the burn was aseptic the loose skin of the blisters was not removed, but was left to act as a protection to the exposed deeper and painful layer of the skin.
7. If the burn was septic all loose skin was cut away; under these conditions the skin harbours harmful toxins which are readily absorbed to the detriment of the patient.