OBSERVATIONS ON THE CULTIVATION OF TYPHOID AND PARATYPHOID BACILLI FROM THE STOOLS WITH SPECIAL REFERENCE TO THE BRILLIANT GREEN ENRICHMENT METHOD.

By Captain J. W. McLEOD, R.A.M.C., 8th Mobile Laboratory.

The question of standardising bacteriological methods is at present under discussion and, although it is most undesirable that any one method should be forced on all bacteriologists, it is certainly reasonable that any method which has given excellent results in the hands of a number of independent workers should have a strong recommendation.

Browning, Gilmour, and McKie (1913) described a method for isolating Typhoid bacilli from the faeces by means of an incubation in dilute solutions of Brilliant Green prior to plating, and the value of the method has been investigated in many laboratories in the course of this war. Glynn and his collaborators (1917) summarised the published work dealing with this subject which had appeared up to the date of their report, and they expressed the general conclusion that "the available evidence indicates that the advantage of Brilliant Green, certainly of Browning's simplified technique, is not sufficiently established to justify its being recommended as an additional routine method in laboratories where enterics are examined, especially having regard to the slight extra labour and cost." In as much as the method has been, in my experience, one of the most valuable modifications of bacteriological technique which have been introduced and since it would seem to me to be a misfortune if the opinion expressed in the report quoted should deter any who are unfamiliar with the method from giving it a trial, I have not hesitated to publish the results which follow, although the conclusions may seem to some to be already well established.

The observations relate to the work of a Mobile Laboratory during the last four years. The opportunities for observing cases of infection of the Typhoid group have been the following: (i) a series of paratyphoid infections in the troops withdrawn from the Ypres sector to the area north of Albert in the summer of 1915; (ii) a small epidemic of typhoid amongst the civilians east of Doullens at the same period; (iii) a small epidemic of paratyphoid infection amongst the troops of a division which returned from Egypt in the early part of 1916; (iv) very occasional cases of typhoid or paratyphoid infections occurring amongst the troops or civilians in the area before and behind Cassell in 1917 and 1918; (v) a considerable outbreak of typhoid

https://doi.org/10.1017/S002217240000749X Published online by Cambridge University Press

infections amongst German civilians at Euskirchen in the end of 1918 and the beginning of 1919.

The observations made in the first two groups were mostly limited to blood culture, but in a considerable proportion of cases in the other groups a careful comparison between the value of direct plating and of Brilliant Green enrichment was made. In 1916 one tube only of Brilliant Green was used, a 1/250,000 dilution, and the method compared was that described by Ledingham and Arkwright (1912) with the modification that only one, not two or three plates were used for each specimen. In 1917, 1918 and 1919 1/250,000 and 1/500,000 dilutions of the dye were employed and the comparison was made with a literally direct method, *i.e.* the plate was inoculated with a small portion of faeces and spread immediately. The results of all investigations up to the end of 1918 in which the direct and Brilliant Green methods were compared are set down in Table I. These observations refer chiefly to paratyphoid infections and it is seen that out of the 16 results obtained 15 were

Table I.

Date	Name	Result: Brilliant Green	Result: Direct
30–4–16	Driver S.	Paratyphosus B, numerous colonies	Paratyphosus B, scanty colonies
30–4–16	Pte. C.	Paratyphosus B, numerous colonies	Paratyphosus B, scanty colonies
21–5–16	Pte. W.	Paratyphosus B, numerous colonies	Paratyphosus B, scanty colonies
30–5–16	Pte. Lambert (1)	Paratyphosus A, nearly pure culture	Negative
	,, (2)	Paratyphosus A, one colony	Negative
	,, (3)	Paratyphosus A, nearly pure culture	Negative
12–6–16	Pte. S.	Paratyphosus B, colonies	Paratyphosus B, colonies
5–7–16	Pte. A.	Paratyphosus B, numerous colonies	Paratyphosus B, scanty colonies
24–8–16	Pte. F. (1)	Paratyphosus B, colonies	Negative
	,, (2)	Paratyphosus B, colonies	Negative
*15–7–17	Pte. S.	Negative	*B. typhosus*
26–7–17	Pte. S.	Paratyphosus B	Negative
16–9–17	Pte. D.	Paratyphosus B, about 100 colonies	Paratyphosus B, about 20 colonies
29–11–17	Rflm. D.	Paratyphosus B	Negative
18–7–18	Civilian	Paratyphosus B, isolated	Negative
29–9–18	Civilian	*B. typhosus*	Negative

* A tube of Brilliant Green solution which had been standing for some time and become discoloured was used on this occasion.

positive by the Brilliant Green method and only 7 out of 16 were positive by the direct method.

It was the case of Lambert (Table I) in this group which brought out the value of the method in the most striking way. An investigation had been required of all possible carriers in this man's regiment on account of a rather severe outbreak of paratyphoid infection. After investigating without success the stools and urines of 60 other men, I obtained an almost pure culture of

https://doi.org/10.1017/S002217240000749X Published online by Cambridge University Press

Paratyphoid A from the Brilliant Green tube of this man's stool but nothing except *B. coli* and few colonies of coarse non-lactose fermenters from the direct plate. The examination was repeated twice with the results cited in Table I confirming the original finding.

The man's history was that he had had a sharp attack of diarrhoea in Egypt, and had suffered from irregularity of the bowels ever since. The regimental epidemic had been a mixed one, "A" and "B" infections occurring simultaneously, but the latter predominating. No more cases of "A" infection occurred after the detection of Lambert. A continued investigation for a "B" carrier was unsuccessful; the "B" infections gradually died out, however, and were possibly all due to case to case contact.

In view of the above result I find it impossible to accept the opinion expressed by Glynn and his collaborators, as quoted above. Extra trouble and cost would have been involved by omitting the use of the Brilliant Green method in this case, and at least one month's fruitless work would have had to be carried out and possibly a continued series of paratyphoid "A" infections would have occurred in the regiment.

The claim, however, originally made by Browning, Gilmour and McKie for the Brilliant Green method was not only that it facilitated the isolation of paratyphoid bacilli but that of typhoid bacilli. Most of those who from their practical experience of the method in this war have written in its favour, only recommend it, however, in respect of the isolation of the paratyphoid bacilli (Stokes and Clark, 1916; Fletcher, 1917).

Table II.

Name	Stool		Urine	
	Direct	Brilliant Green	Direct	Brilliant Green
Sophie U.	Abundant Paratyph. B	Abundant Paratyph. B	Abundant Paratyph. B	Abundant Paratyph. B
Gertde. M.	Negative	Scanty colonies *B. typhosus*	Negative	Negative
Christ. D.	Approx. 10 col. of *B. coli* to 1 of *B. typhosus*	Approx. 1 col. of *B. coli* to 4 of *B. typhosus*	Negative	Negative
Schwester A. ...	One or two col. *B. typhosus*, *B. coli* abundant	Approx. 5 col. *B. typhosus* to 1 col. *B. coli*	Negative	A few col. *B. typhosus*
Frau S.	Negative	A few col. *B. typhosus*	Negative	Negative
Jacob S.	Negative	Paratyph. B	Negative	Negative
Heinrich C.	Negative	*B. typhosus*	Negative	Negative
Helene P.	*B. typhosus*	Negative	—	—
Frau S. (2nd exp.) ...	Negative	*B. typhosus*	—	—
Frau K.	Negative	*B. typhosus*	—	—
Frau U.	*B. typhosus*	*B. typhosus*	—	—
Aug. U.	*B. typhosus*	*B. typhosus*	—	—
Frau K. (2nd exp.) ...	Negative	*B. typhosus*	—	—
Baxter, 1st spec. ...	Negative	*B. typhosus*	—	—
„ 2nd spec. ...	*B. typhosus* col. scanty	*B. typhosus* col. numerous	—	—

https://doi.org/10.1017/S002217240000749X Published online by Cambridge University Press

The occurrence of the civilian epidemic already mentioned in the early part of 1919 at Euskirchen, a small town in the British area of occupation west of the Rhine, afforded a good opportunity of retesting the value of the Brilliant Green enrichment method in cases of infection with *B. typhosus*.

The results obtained are given in Table II.

Excluding the two paratyphoid infections, there were 14 specimens derived from 11 different cases, 13 of stool and one of urine, in which a positive result was obtained. In 13 of these the result was positive by the Brilliant Green method, whereas in 6 only by the direct method. In my experience therefore the original claim for the efficacy of the method in isolating *B. typhosus* from the stool is fully vindicated.

Technique.

A few words about the technique appear relevant since it is probably the source of varying results amongst different workers. The dilutions of Brilliant Green in peptone water have been made by adding with a sterile graduated pipette the requisite quantities of $\frac{1}{5000}$ solution of Brilliant Green to sterile tubes of peptone water each containing 10 c.c. Solutions of Brilliant Green in peptone water were not autoclaved although I have no proof that this has any deleterious effect. The original technique was persisted in as it had given good results. The Brilliant Green tubes were always inoculated copiously, much more material being transplanted than could be adequately spread directly on several plates.

The peptone water used was capable of yielding a rapid and copious growth of *B. typhosus*, and suitable Brilliant Green was employed. These two factors are important. If peptone water is neutralised by a fixed addition of alkali and not titrated, it may easily happen that in frequent moving a laboratory will strike some water supply of an unusual grade of alkalinity or acidity, which if used to prepare peptone water according to formula will yield a product incapable of promoting a rapid growth of *B. typhosus*.

A number of bottles of "Brilliant Green" crystals have been issued through the Army Depots of Medical Supplies which have neither had the crystalline appearance nor the antiseptic properties of Brilliant Green. An old bottle of Grübler's preparation has been used throughout in these investigations, and it does not seem to me that any criticism of this method is pertinent unless the work is carried out with Grübler's Brilliant Green or with a specimen which has been proved equal to it in parallel experiment.

REFERENCES.

Browning, Gilmour and McKie (1913). *Journ. of Hygiene*, xiii. p. 335.

Fletcher (1917). *Journal Roy. Army Med. Corps*, xxix. pp. 544–549.

Glynn, Berridge, Foley, Price and Robinson (1917). *Report to Medical Research Committee*, No. 7, p. 5.

Ledingham and Arkwright (1912). *The Carrier Problem in Infectious Disease*, p. 104.

Stokes and Clark (1916). *Journal Roy. Army Med. Corps*, xxvi. pp. 466–467.

https://doi.org/10.1017/S002217240000749X Published online by Cambridge University Press