EPIDEMIOLOGY OF TYPHOID FEVER.

INCIDENCE OF TYPHOID CARRIERS IN A GENERAL POPULATION GROUP.

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Typhoid fever is one of the prevailing endemic diseases of Palestine. In 1925 and 1926 epidemic outbreaks of considerable magnitude occurred in various parts of the country (Kligler, 1927). As a part of a general programme of the study of the epidemiology of this disease, we have attempted to ascertain the incidence of typhoid carriers in a general population group. Owing to the large number of typhoid cases which occurred there in 1925 and 1926, Afule, a village of some 1200 inhabitants, was chosen as a desirable spot for such an investigation.

Although there is an extensive literature on the detection of typhoid carriers, methods of examination of specimens, etc., we found it necessary, before beginning the actual examinations, to perform a series of preliminary experiments, in order to determine the media and methods best suited for our purpose under the conditions in which we were working. The results of these experiments are of sufficient interest to warrant a brief summary before presenting the data of the examinations.

In our *preliminary experiments* we tried to determine three things:

(1) The best plating medium.

(2) The solution most suitable for shipment of stools.

(3) Rapid method for identification of suspected colonies.

Plating medium. We employed a constant quantity of fresh faeces, artificially infected with a known amount of a culture of *B. typhosus*. This mixture was emulsified in 15 c.c. of Brilliant-green-Bile[1], containing different concentrations (1 : 200, 1 : 400, and 1 : 800) of the dye. From these different concentrations platings were made on various media. The results of a typical experiment are shown in Table I.

These and other tests showed that artificially infected faeces plated after 24 hours' incubation in solutions of Brilliant-green-Bile gave equally satisfactory results, on all of the plating media tested. After 48 and 72 hours' incubation, however, there appeared a marked difference in results on different

[1] The Brilliant-green-Bile was prepared according to the method recommended by Havens (personal communication). Neutralised ox bile was sterilised and varying amounts of 5 per cent. Brilliant-green in *n*/1 HCl added. (5 grm. Brilliant-green were dissolved in 100 c.c. of HCl *n*/1 and evaporated to a brownish paste, then made up with distilled water to 100 c.c.)

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plating media. After 48 hours there was a preponderance of typhoid colonies on the brilliant-green plates, while after 72 hours these plates showed practically pure culture of typhoid bacilli, while on the others *B. coli* predominated.

Table I. *Results of plating stool suspensions of typhoid bacilli on different media.*

Dilution of Brilliant-green	Time interval of plating (hours)	Plating media: Brilliant-green (1–330,000) agar	Endo	Methylene-blue eosin
1–200	24	Mixed growth	Many *coli* and 6 *typhosus*	Many *coli* and *typhosus*
	48	Mixed growth	Mixed *typhosus* and *coli*	*Coli* and *typhosus*
	72	Pure strain of *typhosus*	More *coli* than in 48 hours	*Coli* only
1–400	24	Mixed growth *typhosus*	Pure *typhosus*	Pure culture of *typhosus*
	48	Mixed growth *typhosus*	Mixed growth	*Coli* only
	72	Pure strain of *typhosus*	More *coli*, occasional *typhosus* colonies	*Coli* only
1–800	24	Pure strain of *typhosus*	Pure *typhosus* colonies	Pure culture of *typhosus*
	48	Pure strain of *typhosus*	Mixed growth	More *coli*
	72	Pure strain of *typhosus*	Occasional *typhosus* colony	Occasional *typhosus* colonies. *Coli* only

A further comparison of Brilliant-green (1 : 330 T.) with MacConkey plates showed that both gave satisfactory results. Since the MacConkey medium is easily prepared in large quantities and is stable and especially since it is also

Table II. *Effect of various solutions on the survival of* B. typhosus *emulsified in stools.*

	Days 1		2		4		5		6		7		8	
Solution	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.
Brilliant-green-Bile* (1 : 200)	1+	0	0	1+	–	–	0	0	0	0	0	0	0	0
Brilliant-green-Glyc.† (1 : 1500)	1+		2+	4+	2+	2+	2+	2+	2+	1+	1+	1+	2+	3+

	Days 9		11		12		13		14		15	
	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.
Brilliant-green-Bile* (1 : 200)	0	0	0	0	0	0	0	0	0	0	0	0
Brilliant-green-Glyc.† (1 : 1500)	1+	2+	–	0	–	–	3+	0	–	–		

Mc. = MacConkey plates. Mc.G. = MacConkey + Brilliant-green.

The solutions were inoculated with 0·5 grm. faeces mixed with 500,000 typhoid bacilli. The same standard loop was used in plating from the emulsions. The number indicates the number of colonies of typhoid bacilli on the plates.

* *See* footnote, page 139.

† Brilliant-green-Glycerine, *i.e.* 30 per cent. glycerine in normal saline + different concentrations of Brilliant-green.

Table II *a*.

	Days 1		2		3		4		5		6	
Solution	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.
Brilliant-green-Glyc. (1 : 2000)	+	.	+	.	+	.	+	.	+	.	+	.
Brilliant-green-Glyc. (1 : 2500)	+	.	+	.	+	.	+	.	+	.	+	.
Brilliant-green-Bile (1 : 200)	+	.	+	.	+	.	–	.	–	.		

+ = Presence of typhoid colonies.

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possible to add Brilliant-green to this medium, we finally decided to use both plain and Brilliant-green-MacConkey-agar for all our platings.

Shipping fluid. In a study of this character, where the work cannot be carried out on the spot, the method of shipping of specimens is of primary importance, because upon it depends the survival of *B. typhosus* during transit. Consequently a considerable amount of time was devoted to the study of this aspect of the problem. Two different solutions for shipping specimens of faeces, one devised by Havens and his associates (1924), the other recommended by Wade, Kelly and Giblin (1925), were tested. As in the plating experiments given quantities of artificially infected faeces were inoculated into various concentrations of the two solutions. One of a duplicate set of bottles was kept in the laboratory, while the other was shipped to Afule and was returned to us immediately after it arrived there. Daily platings were made from these bottles on MacConkey and Brilliant-green-MacConkey plates. The results of two series of tests are shown in Tables II and II *a*. The difference between the two solutions is striking. It will be noted that the Brilliant-green-Bile is satisfactory for a period not extending over three days; in brilliant-green glycerine solution the number of colonies of *B. typhosus* remains the same for 10 to 15 days after the inoculation. It is apparent, therefore, that the latter solution is preferable where stools are to remain longer than three days in transit.

Having determined that for our purpose the brilliant-green glycerine solution was decidedly the more desirable, we attempted to define the optimum concentration of Brilliant-green. Different dilutions were inoculated with the same amount of faeces artificially infected with *B. typhosus* and kept at the laboratory. These mixtures were cultivated on the two kinds of MacConkey plates for various periods. Control specimens of artificially infected faeces were also shipped to Afule each month and on return plated as above. The results are shown in Tables III and IV.

Table III. *On survival of* B. typhosus *in various brilliant-green glycerine solutions.*

Laboratory experiment. Examinations after days.

Days	0		1		3		4		5		6		7	
Concentr. of Br.-gr.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.
1 : 1000	1	0	1	2	1	0	8	12	7	8	7	11	9	18
1 : 1500	1	0	1	.	2	.	15	25	10	8	20	24	40	35
1 : 2000	0	0	1	1	.	.	20	20	9	11	10	16	50	25
1 : 3000	0	0	3	2	3	10	.	24	30	29	33	36	28	60

Days	14		21		28		32		39		47	
Concentr. of Br.-gr.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.	Mc.	Mc.G.
1 : 1000	4	30	2	2	.	3	.	.	0	0	0	0
1 : 1500	5	11	8	6	.	5	2	0	0	0	0	0
1 : 1200	11	40	10	1	.	2	1	1	2	1	1	2
1 : 3000	35	7	17	31	.	11	4	2	1	1	1	0

Note: The number = the positive typhoid colonies on each plate.
Mc. = MacConkey. Mc.G. = MacConkey + Brilliant-green.

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Table IV. *Survival of* B. typhosus *in faeces shipped in various brilliant-green glycerine solutions.*

Con. of Br.-green No. 1	Ship.*	Rec.†	7. iv.	8. iv.	9. iv.	10. iv.	19. iv.	20. iv.	7. v.	24. v.	1. vi.	2. vi.	3. vi.
2000	30. iii.	7. iv.	+	+	+	+	+	+	+	+	+	+	+
2500	30. iii.	7. iv.	+	+	+	+	+	+	+	+	+	+	+

No. 2	Ship.	Rec.	27. vi.	28. vi.	29. vi.
2000	16. vi.	25. vi.	+	+	+
2500	16. vi.	25. vi.	+	+	+

No. 3	Ship.	Rec.	5. vii.	6. vii.	7. vii.	8. vii.
1000	30. vi.	5. vii.	+	+	+	+
1500	30. vi.	5. vii.	+	+	+	+
2000	30. vi.	5. vii.	+	+	+	+
2500	30. vi.	5. vii.	+	+	+	+

No. 4	Ship.	Rec.	31. vii.	1. viii.	2. viii.	3. viii.
1000	26. vii.	31. vii.	+	+	+	+
1500	26. vii.	31. vii.	+	+	+	+
2000	26. vii.	31. vii.	+	+	+	+
2500	26. vii.	31. vii.	+	+	+	+

No. 5	Ship.	Rec.	31. viii.	1. ix.	2. ix.	3. ix.	4. ix.	5. ix.
2000	24. viii.	31. viii.	+	+	+	+	+	+
2500	24. viii.	31. viii.	+	+	+	+	+	

Note: Brilliant-green-Glycerine inoculated 0·5 grm. faeces + drop of 24-hour broth culture of *B. typhosus* = approximately 5000 bacilli per c.c.

\+ = presence of typhoid colonies.

* Ship. = Shipment (with dates). † Rec. = Received (with dates).

Attention should be called to one other detail, which profoundly affected our first results: it was noted that the findings were sometimes quite variable. An examination of our materials focussed attention on the metal rod used in the shipping bottle. It was found that in some cases zinc rods had been used, and that the solution of the zinc affected the results according to the amount dissolved. The following protocol is illustrative.

Two sets of bottles with different concentrations of Brilliant-green-Glycerine were inoculated with 0·5 gm. of faeces containing 5,000,000 *B. typhosus* per grm. In one set a zinc loop and in the other a steel loop was inserted in the corks of the bottles. Plates made directly after the inoculation showed no marked difference between the two sets; however, those made after an incubation of 24 hours and more, showed a distinct inhibition in the set containing the zinc wire loop. The results are shown in Table V.

Attention is called to this observation in order to indicate the effect a minor detail might have on the results.

The procedure finally adopted was as follows:

Two-ounce, wide-mouth bottles were used. To each bottle 15 c.c. brilliant-green glycerine solution in concentration of 1 : 2000 or 1 : 2500 were added. The bottles were stoppered with corks into which were inserted steel rods with terminal loops of uniform size for collecting a more or less constant quantity

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Table V. *Effect of wire.*

Time of plating; days after the inoculation.

Wire	Concentr. of Br.-gr.	Direct	Days 1	2	4	5	7	9	14
Zinc	1000	18	2	0	0	0	0	.	.
Zinc	1500	17	6	0	0	0	0	.	.
Zinc	2000	18	1	0	0	0	0	.	.
Zinc	2500	50	+	2	1	0	0	0	0
Zinc	3000	11	0	0	0	0	0	.	.
Steel	2000	34	1	6	21	.	0	+ +	50
Steel	2500	+	1	5	+ +	+	+	13	42

Note: The numbers under days (1–14) indicate the number of typhoid colonies per plate. A standard loop was used for all platings. The flasks were inoculated with 0·5 grm. faeces containing 5,000,000 *B. typhosus* per grm. The bottles containing the steel rods were still positive after 45 days.

. = No plating that day. +, + + = Colonies, too many to count.

of stool. These bottles were sent out in a wooden box divided into squares for 24 specimen bottles. An extra supply of boxes and bottles was also sent in order to insure a reserve supply on hand.

Arrangements were made with the local physician and sanitary inspector[1] to collect specimens from all of the inhabitants and mail them regularly to our laboratory within a day or so after collection. Specimens received at the laboratory were immediately plated out with a standard loop on brilliant-green MacConkey and plain MacConkey plates. These platings were repeated daily on three successive days.

After 24 hours' incubation at 37° C. the seeded plates were examined, and suspicious colonies fished into Kligler's lead acetate triple sugar medium (Kligler, 1916) which enables one to make a presumptive differentiation of *typhoid, paratyphoid A* and *B*, and *dysentery bacilli* in the one tube. This medium consists of standard nutrient agar containing 1 per cent. lactose, 0·1 per cent. glucose, 1 per cent. saccharose, and 1 per cent. Andrade indicator. The triple sugar medium is prepared first, distributed in measured quantities in flasks, and autoclaved. After removal from the autoclave the flasks are cooled to 60° C. and 0·025 per cent. lead acetate solution added from a sterile 0·5 per cent. solution of the basic salt. The medium is thoroughly mixed and distributed into test tubes with precautions to avoid contamination.

Bailey and Lacy (1927) suggested a modification in the technique, consisting principally in mixing all the ingredients and autoclaving. We have found, however, that the above method gives more constant results, in that we avoid too much autoclaving which is liable to break up the sugars, and particularly because, despite all precautions, autoclaving with the lead acetate in the medium results often in flocculation, spoiling large batches of media.

On receiving a characteristic suspicious growth on Kligler's lead acetate triple sugar, agglutination tests were made with specific anti-sera, and at the same time semi-solid media containing various sugars were inoculated. Thus complete identification was possible in about 48 hours after plating.

[1] We are indebted to Dr Temkin for his co-operation which made this work possible.

Results of the tests. During 1927 we examined 869 specimens, of which six examinations were positive for *B. typhosus*. The specimens were taken from 484 inhabitants, of whom three proved to be typhoid carriers. The bacilli isolated from faeces agglutinated to the full titre 1–12,000 with specific serum. Both smooth and rough colonies were differentiated. Where the number was sufficiently large, the ratio of rough to smooth colonies was approximately 10 : 1.

The results are recorded in Tables VI *a* and VI *b*. It appears that despite the warm climate and the epidemic of 1926, the percentage of carriers was not high—only 0·62 per cent. All of the carriers were women; one aged 40, the

Table VI *a*. *Incidence of carriers in Afule in* 1927.

	No. of persons examined	No. positive	No. of times	Total No. of examinations	No. positive
	164	0	1	164	2
	258	1	2	516	3
	59	2	3	177	1
	3	0	4	12	0
Total	484	3		869	6

Table VI *b*. *Monthly examinations; carriers found.*

Month	No. of specimens examined	Number positive
January	—	—
February	44	0
March	113	0
April	169	1
May	123	2
June	182	2
July	143	0
August	63	0
September	32	1
Total	869	6

second 52, and the third 9. The first gave a history of typhoid 30 years ago; in her neighbourhood five cases of typhoid occurred in 1925, but among her own family there were no cases. The second was sick with typhoid in 1926 and has since remained a carrier. The third gave a negative history for typhoid, but both her brother and sister were ill during the 1926 epidemic. One of the carriers was a milk dealer and thus probably had a large share in the epidemic.

Not only is the number of carriers no larger than that reported in populations living in temperate regions, but there appears to be no difference in the seasonal prevalence of carriers. The figures are too small for broad deductions, but the indications are that neither of these factors is of primary importance in the evolution of the epidemic. The origin of the epidemic was undoubtedly due to the presence of the carriers, but the rapid spread was apparently caused by the introduction of a new susceptible population living under poor sanitary conditions.

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