ON THE VALUE OF THE PETROLEUM-ETHER METHOD FOR THE ISOLATION OF B. TYPHOSUS FROM FECES

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The use of petroleum-ether in the isolation of typhoid bacilli from feces was suggested by Bierast (1914). He added a finger's breadth of petroleum to a fecal suspension in broth and shook the mixture at intervals. After allowing this to stand in a cool place for sixteen hours, he plated the sediment or some of the broth from the bottom of the container. He reported that 2 out of 23 specimens were positive only by this method. The work was interrupted because he developed typhoid fever. Jaffe (1915) had favorable results with 24 specimens, as follows:

NUMBER	DIRECT PLATING	AFTER SHAKING, ETC.
5	—	—
5	+	+
0	+	—
4	—	+

He calls attention to the danger of the method and reports a case of typhoid fever due to its use.

Hall (1915) working with the method found that the activity of the different fractions obtained by the distillation of crude petroleum varied inversely with the boiling point. He found that the fraction with a boiling point about 40°C. gave the best results. This fraction would contain a large proportion of pentane (C_5H_{12}) and for convenience we shall refer to it as such.

Hall modified the Bierast procedure as follows. To a moderately heavy suspension of feces in broth is added one-half its

volume of pentane. Close the bottle tightly with a rubber stopper and shake for one-half hour in a shaking machine. The container is then allowed to stand for one and one-half to two hours at room temperature, the bottom fluid or sediment removed with a pipette and plated. His results may be summarized as follows:

SPECIMENS EXAMINED	DIRECT PLATING	PENTANE SHAKING
69	Positive, 13 Positive, 1 Negative, 7 Negative, 48	Positive, 13 Negative, 1 Positive, 7 Negative, 48

Thus, while one specimen positive on direct plating was negative after shaking, 7, or 33 per cent of the total positives, were positive only after shaking.

These results were so favorable that we investigated the method. Unless otherwise stated, we have followed the method of Hall.

Our first attempts were made with stools from typhoid carriers and with greatly diluted broth cultures of recently isolated typhoid strains. The shaking was for one-half hour, and after shaking, the emulsions were allowed to stand for two hours before plating on the Endo medium. The results may be roughly illustrated by the following examples:

MATERIAL	DIRECT PLATING	AFTER SHAKING
Stool suspension	Positive—5 per cent typhoid	1 typhoid colony
Stool suspension	Positive 4,000 total colonies; 75 per cent typhoid	100 total colonies; 90 per cent typhoid
Stool suspension	Positive 5,000 total colonies; 5 per cent typhoid	200 total colonies; 1 per cent typhoid

Broth suspensions of several cultures (pure) yielding by direct plating from 400 to 5000 colonies per plate, after shaking, and then plating an equivalent amount of material, gave no colonies.

While, with the one stool cited above, there was a relative increase in the proportion of typhoid to total flora, it would

obviously be dangerous to expose stools less rich in typhoid to this marked absolute decrease in typhoid colonies. We next tried shortening the time of shaking, and found that in no case did the proportion of typhoid increase on shaking longer than ten minutes, whereas the total number recovered frequently took an alarming drop, and in some cases none was recovered after thirty minutes. It was also determined at this time, that more typhoid bacilli were viable after one than after two hours' standing. The results with this modified technique—that is, time of shaking reduced to ten minutes, and of standing to one hour—may be summarized briefly as follows.

TOTAL EXAMINED	DIRECT PLATING	AFTER SHAKING
12	Positive, 10 Positive, 1 Negative, 1	Positive, 10 Negative, 1 Positive, 1

These stools were quite rich in typhoid. Of those in which bacilli were found both before and after shaking, the percentage of typhoid to total flora was increased in three and decreased in seven cases. In 3 of the 7, less than 10 per cent of the number of colonies on the direct plates developed after shaking. In the case of the 3 specimens showing relative increase, 2 of the 3 specimens showed loss of typhoid bacilli, in 1 case to 3 per cent of the original number. The stool which gave positive results only on direct plating had 75 per cent of typhoid (total colonies 900) and yielded 150 non-typhoid colonies after shaking. That in which bacilli were found only after shaking had 6000 colonies on two badly diffused direct plates, which were reduced on shaking to 400, of which 20 were typhoid. In other words, the tendency, even with the reduction of exposure, is to a rapid reduction of the number of typhoid bacilli with which a relative increase compared with the accompanying flora is not necessarily associated.

While these experiments were under way, we conducted a number of tests with pure cultures of recently isolated strains of typhoid, designed to develop a technique more favorable than

that which we were employing. First, we determined that an emulsion of typhoid in broth was not more susceptible to the action of pentane than a similar emulsion in either a normal stool suspension, or in the same suspension sterilized by steaming for one-half hour. Thus:

Summary of reactions of several strains: shaken ten minutes, stood one hour

<i>Direct plating</i>	<i>After shaking</i>
In broth 2500 colonies.....	0
In stool suspension 2500 colonies, 95 per cent typhoid.....	0
In sterilized stool suspension 2500.....	0

On attempting to find the approximate percentage of typhoid bacilli which would survive shaking alone, we found wide variations between individual strains. With some, 250 colonies per drop were completely lost on shaking; with others, the numerical reduction varied from 40 to 98 per cent. With this reduction in numbers, there was frequently a concomitant reduction in the size of the colonies developing after shaking. It was thought that the physical effect of shaking might be responsible, but on shaking without pentane (with equivalent saline dilution) practically no reduction occurred, whereas, in this case, 5000 colonies per loop were killed by pentane. It was thought that the bacilli might be contained within the supernatant pentane rather than in the subjacent broth, but culture from the pentane in two cases where the broth yielded from 3 to 25 colonies per drop, showed it to be sterile.

We then investigated more accurately the effect of standing after shaking. The results with several strains were:

DIRECT PLATING	IMMEDIATELY AFTER SHAKING	STOOD 15 MINUTES	STOOD 30 MINUTES	STOOD 45 MINUTES	STOOD 1 HOUR
5,000*	3,000	3,000	2,000	2,000	1,800
3,000	600	160	65	27	25
1,400	400	21	12	12	3

* Strain (in other tests) shows unusual resistance to pentane.

In a final series of stool examinations, small amounts of carrier stool were added to normal stools so as to give mixtures poor in

typhoid. Obviously, it is only with this class of material that the method, if of value, will aid in diagnosis. In order to determine more accurately the initial number of typhoid bacilli, we incorporated the use of the brilliant green medium described by Krumwiede, Pratt and McWilliams (1916), which has proved extremely successful in the isolation of typhoid bacilli from feces. As preliminary tests had shown that in stools as well as in broth cultures, the typhoid bacilli were more or less rapidly lost on standing after the shaking was over, we plated immediately after shaking ten minutes with pentane, and obtained the following results.

TOTAL EXAMINED	DIRECT ENDO	DIRECT GREEN-DYE	ENDO AFTER SHAKING
50	Positive, 21 Negative, 7 Negative, 9 Negative, 13	Positive, 21 Positive, 7 Positive, 9 Negative, 13	Positive, 21 Positive, 7 Negative, 9 Negative, 13
Total positives	21	37	28
Percentage positive.....	42.0 per cent	74.0 per cent	56.0 per cent
Percentage positive compared with total number positive.....	56.7 per cent	100.0 per cent	75.6 per cent

These results indicate that the method with very short exposure has some value as compared with the direct plating on a non-restraining medium (Endo) alone. The general tendency to reduction in the number of typhoid bacilli, not necessarily associated with a markedly greater decrease in the fecal flora, was again observed in this series. On the whole, the data presented indicate that the method is not a reliable one for the relative enrichment of typhoid bacilli in stools. Compared with direct plating on a differential medium (brilliant green agar) the method is evidently inferior.

A report by Schuscha (1916) recently available only in an abstract indicates that his results with the method have not been more satisfactory than with direct plating.

We might add that the method of pentane shaking is unsuited to routine laboratory work. The pentane itself is highly inflam-

mable, and the containers are protected from leaking during agitation in the machine only with difficulty. Two workers abroad have developed typhoid fever while using the method and we feel that there is a large element of danger in its use because of the tendency for the contents of the bottles to blow out when opened, due to the highly volatile pentane.

CONCLUSIONS

The petroleum-ether method for the detection of *B. typhosus* has no advantage over direct plating on a medium which restrains the growth of the associated fecal types. With short periods of exposure, it may be successful where direct plating on Endo alone fails. There is a strong tendency to reduction of the number of *B. typhosus*, not necessarily associated with a greater reduction of the accompanying fecal bacteria. The danger of infection to those using the method, however, is sufficiently serious to warrant its condemnation, especially as it offers no advantage over direct plating on differential restraining media.

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