A STUDY OF THE CHICK-MARTIN TEST FOR DISINFECTANTS

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Of the merits which may be demanded of a test for disinfectants two of the chief are capacity to yield uniform results, and that of affording a true estimate of efficiency under conditions of actual use. The simpler methods, such as the Rideal-Walker, of obtaining a phenol coefficient possess the first of these merits, and when all the conditions of the test are rigidly controlled furnish results which for what they are worth have a high degree of accuracy. But in one important respect at least they fail to reflect the conditions of practical use and hence to indicate the results to be expected under such conditions. The only ingredients in the test mixture employed in the Rideal-Walker method apart from the disinfectant itself and the distilled water with which it is diluted are those of the broth culture, of which 0·1 c.c. furnishes the inoculum. In practice a disinfectant may have to contend with a variety of other material, and the combination of its active constituents with this material may prevent the total destruction of bacteria unless the disinfectant is present in sufficient excess. A test of practical efficiency must therefore introduce such material, and it should as far as possible be appropriate to the purposes for which the disinfectant is to be used. Although these uses are many and varied, they fall clearly into two classes, which may conveniently be termed surgical and excremental: in the former are included all the uses of disinfectants about the human body, in the latter their use for destroying bacteria outside the body, which often either are contained or originate in excremental material. Just as blood or serum are in many ways appropriate as added material, or "organic matter," in a test of the former class of disinfectant, so are faeces, and to a limited extent urine, appropriate to the latter, though other materials less deliberately imitative have been proposed, such as gelatin, starch, and milk.

At the time of its publication the test proposed by Chick and Martin (1908), now known by their names, was an important advance on any recognised method then in use, and the work of these authors was of great service in establishing the principle that an adequate amount of appropriate organic matter should be incorporated in the test mixtures in order that the results obtained might thereby gain in practical significance. They had in mind disinfectants of what has been referred to as the excremental type, and after experimenting with a number of other materials chose human faeces as organic matter. Since the proportion of solid matter in faeces varies, a

necessary preliminary to the use of a constant quantity was drying, and the essential feature of the test is the presence in each tube employed of a weighed amount of dried faeces. The effect of this addition is to reduce the activity of all disinfectants considerably, and in as far as that activity persists it is undoubtedly a measure of efficiency under some of the severest conditions of use. This is its advantage, but the test is not free from objections. The preparation of dried faeces, which involves grinding and passing through a fine sieve, is unpleasant and theoretically even unsafe owing to aerial diffusion of the powder. The accurate weighing of a small quantity of dried faeces for addition to each tube is a laborious process, and might puzzle anyone not familiar with the test; it is, in fact, rendered necessary by the peculiarly unstable nature of the suspension formed, which cannot therefore be prepared in bulk and distributed in smaller quantities. Even when prepared in the individual tubes in which it is to be used, the suspension has the disadvantage that numerous particles tend to adhere to the side of the tube above the fluid level.

Except in as far as they affect the accuracy of results given by the test, these drawbacks are of secondary importance and might be accepted. A more serious criticism, however, has been that the test gives irregular results. It must be required of a test which is adopted officially and on which important commercial interests may depend that its results shall be consistent. These, according to some workers, they are not.

What would appear to be an obvious source of variation in results is the composition of the organic matter itself. Human faeces is by no means a product of constant composition; it may contain an almost unlimited variety of food residues, varying quantities of bacteria, and varying quantities of digestive secretions and of mucus. Its reaction may under normal conditions be either acid, neutral or alkaline. Nevertheless, according to Chick and Martin's original work, tests with different specimens of faeces did yield almost concordant results. Another possible source of discrepancies, especially between the results obtained by different workers, is to be found in minor variations of technique, such that instructions cannot exactly define them. Any test sensitive to such influences cannot be satisfactory. There is evidence in the experiments about to be described that such factors as these can influence the results obtained. These experiments, in which the process of disinfection under the conditions of the Chick-Martin test has been studied somewhat more intimately than in the routine performance of the test itself, were undertaken in order to identify the factors concerned in producing variable results.

EXPERIMENTAL STUDY

The properties of the faecal suspension

At the outset about 80 g. of powdered faeces were prepared in the manner prescribed for the test, and this material was used throughout. These observations have therefore no bearing on the possible effect of variation in the composition of the faeces used. The instructions for the test are that 2·5 c.c.

of distilled water shall be added to 0·15 g. dried faeces in tubes, which are then autoclaved. Such suspensions had the following characters. The supernatant fluid, which was clear and dark brown in colour, was distinctly acid to litmus, and contained 0·98 g. of material per 100 c.c. in solution. The deposit, which consisted mainly of particles large enough to be seen individually by the naked eye, reformed rapidly after shaking the tube. When such a suspension in a graduated tube was allowed to stand for 30 min. (the period of the test), and then subsequently centrifuged to constant volume, the bulk of the deposit was only further reduced by about 15 per cent. during centrifuging: the deposit formed during a test is therefore about 85 per cent. solids. The particles forming this deposit were enumerated in a counting chamber and measured, under different conditions, with somewhat surprising results. The first counts were performed after the lightest shaking which would bring the deposit into suspension, the second after 30 sec. further gentle shaking, the third after 5 min. vigorous shaking in a corked tube with beads. Two types of particle were counted separately, those of the size of bacteria ("small" particles) and those of larger size, up to and including those visible to the naked eye ("large" particles).

	Particles per c.mm.	
	Large	Small
After slight agitation (to mix)	4,360	12,000
After 30 sec. shaking	13,600	81,200
After 5 min. shaking with beads	696,000	8,120,000

The average diameter of the "large" particles before shaking was 47·3 microns, after 5 min. shaking 8·4 microns. It is clear from these observations that shaking rapidly disintegrates the particles of dried faeces, and in as far as the extent of this disintegration affects the result of a test it constitutes a variable factor which cannot accurately be controlled, since it is likely to be affected not only by the extent to which the tubes of suspension are shaken, but by the vigour with which the dried faeces have previously been ground, and the precautions taken during sieving to retain the finer particles, which tend to remain suspended in the air, and are carried away by the slightest draught. That the degree of this disintegration does affect the result of a test is shown in the following experiments.

Counts of surviving bacteria in test mixtures

Complete disinfection is a crude criterion of the progress of events in a test mixture: more exact indications can be obtained by enumerating the surviving bacteria, either at different time periods, or after a fixed period in different dilutions of the disinfectant. Such enumeration was obtained as follows.

Method. Autoclaved suspensions of dried faeces (6 per cent.) were prepared as for the Chick-Martin test and mixed with an equal volume of dilutions of a disinfectant. The disinfectant used throughout was a "white fluid" of coal-tar origin which gives a Rideal-Walker coefficient of 18–20 and a Chick-Martin coefficient of about 2·5. The tubes were placed in a water-bath at 20° C. After

a sufficient interval 0·1 c.c. of a 24-hour broth culture of *B. typhosus* was added: the culture used, except where otherwise stated, was the "Lister" (Rawling) strain. After a specified time, 0·5 c.c. of the contents of the tube was diluted with 49·5 c.c. of sterile distilled water in a flask, and from this dilution a further 1 in 100 dilution was made in a second flask, each being well shaken. From each of these dilutions 1 c.c. was added to a melted tube of beef heart extract peptone agar kept at approximately 46° C. which was then poured as a shake plate culture; additional thin layers of agar were employed both below and above the inoculated layer to obviate spreading growth on either surface. The colonies in these cultures were counted after 2 days' incubation at 37° C. How this method can be used to illustrate the progress of disinfection is shown in the following experiment.

Counts at different time intervals. Two tubes each containing 0·3 g. dried faeces and 5 c.c. distilled water were autoclaved. One of these was then corked and vigorously shaken with beads. To each was added 5 c.c. of the same dilution of the disinfectant (actually a dilution of 0·308 per cent., a critical dilution for this disinfectant in the Chick-Martin test), 0·1 c.c. of culture was added, and dilutions and plate cultures were made as described, at different intervals of time. The contents of all the tubes were lightly shaken to mix them, both on the addition of their ingredients, and before removing samples by pipette. The colony counts obtained are given in Table I.

Table I. *Numbers of surviving* B. typhosus *at different time intervals in mixtures of faecal suspension and disinfectant.*

	Unshaken tube		Shaken tube	
Time	0·01 c.c.	0·0001 c.c.	0·01 c.c.	0·0001 c.c.
Zero	+ +	56	+ +	119
5 min.	109	1	+ +	64
15 ,,	15	0	+ +	23
30 ,,	6	0	1024	14
60 ,,	1	0	515	9

+ + = an uncountable number of colonies.

Of this experiment it should first be said that the "zero" cultures (from dilutions made as expeditiously as possible directly after the addition of culture to the tubes) are not a satisfactory control, since it is clear both from the discrepancy between the counts from the two tubes and from subsequent control counts (dilutions being made in distilled water) that some of the bacilli added have already been killed. This, however, does not invalidate the conclusions to which the results lead, which are these. Disinfection in the Chick-Martin mixture, at least by a class of disinfectant to which the method is commonly applied, is a slowly progressive process, a small proportion of the bacteria surviving long after the majority are killed. This obtains for critical dilutions for any disinfectant, and whether, as seems possible, the survival of the few most resistant bacteria is unduly protracted for a test employing an arbitrary and comparatively short period of exposure, could only be ascertained by further comparative experiment. What is quite clear, however, is that

previous disintegration of the faecal particles by shaking greatly retards the process of disinfection; the number of survivors at 15 minutes and after is at every stage more than a hundred times greater in the shaken suspension than in the unshaken. The degree of subdivision of the faecal particles must therefore materially affect the result of a Chick-Martin test, and it has already been pointed out that this may vary with the method of grinding and sieving, and been shown that it may also be affected even by light shaking. It is possible also that some disintegration occurs during storage of prepared tubes.

In an experiment identical with this the deposit formed was not disturbed before removing material for culture, and separate cultures were made from the supernatant fluid and deposit. The former were completely sterile, the latter were not. The survival of bacteria in both portions of the mixture was therefore studied separately in detail.

Separate counts from supernatant fluid and deposit. Twelve tubes each containing 0·15 g. dried faeces and 2·5 c.c. distilled water were autoclaved; six of these were corked and shaken with beads. Three dilutions of phenol and three of the disinfectant were added in 2·5 c.c. amounts to pairs of these tubes (shaken and not shaken). Thirty minutes after the addition of 0·1 c.c. culture, dilutions and cultures were made; those from the unshaken tube being made first from its supernatant fluid, then from the contents of the tube after shaking up the deposit. The quantities of disinfectant stated in the table are those contained in the 2·5 c.c. of disinfectant dilution added as described in the original technique for the Chick-Martin test. The results of this experiment are set out in Table II.

Table II. *Numbers of surviving* B. typhosus *after* 30 *min. in mixtures of faecal suspension and different dilutions of disinfectant.*

Contents of tubes	Colony counts: 0·01 c.c.	0·0001 c.c.
0·1 c.c. culture in 5 c.c. water	—	361
Tubes containing:		
Phenol 5 %:		
0·66 c.c.: Supernatant, unshaken tube	+ +	71
Deposit, unshaken tube	+ +	70
Shaken tube	+ +	95
0·77 c.c.: Supernatant, unshaken tube	1104	8
Deposit, unshaken tube	1656	11
Shaken tube	+ +	18
0·92 c.c.: Supernatant, unshaken tube	61	—
Deposit, unshaken tube	180	—
Shaken tube	516	—
A "white fluid" 2 %:		
0·66 c.c.: Supernatant, unshaken tube	0	0
Deposit, unshaken tube	299	5
Shaken tube.	+ +	320
0·77 c.c.: Supernatant, unshaken tube	0	0
Deposit, unshaken tube	92	0
Shaken tube	+ +	65
0·92 c.c.: Supernatant, unshaken tube	0	—
Deposit, unshaken tube	0	—
Shaken tube	170	—

+ + = an uncountable number of colonies. Where no figure appears no cultures were made.

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The results of this experiment, which are typical of those also obtained in a number of others, clearly indicate two things; that in at least two critical tubes in a Chick-Martin test the surviving bacteria are contained exclusively in the deposit, and that the subdivision of faecal particles produced by shaking greatly increases the number of survivors. Whether or no a positive culture is obtained from any of several tubes will depend in the first place on the degree of subdivision of the particles of faeces, a factor which has already been shown to be incapable of exact control, and in the second place on how the loopful for culture is removed, the controlling factor here being the inclusion in it of some of the deposit. This apparently minor detail of technique is not covered by the instructions for the test, and could be a prolific source of error.

These considerations apply chiefly to the tubes containing the disinfectant tested; it will be observed that in the phenol tubes the differences in the number of surviving bacteria are much less considerable. This is almost certainly referable to the fact that the phenol is in solution and the disinfectant in suspension.

Further experiments were carried out in order to determine whether similar effects would be obtained with different types of disinfectant and with a different strain of *B. typhosus*. It may be said at once that all such experiments gave results having the same significance; two will be described.

An experiment with a "black fluid." An experiment identical with the foregoing was carried out with a "black fluid" (Rideal-Walker coefficient 18–20). The results are given in Table III, expressed as numbers of surviving bacteria per c.c. in the test mixture. By cultures from dilutions of a mixture of 0·1 c.c. broth culture with 5 c.c. of water it was determined that the living bacterial content of each tube at the outset of the experiment should have been 5,130,000 per c.c. It appears from the results that the black fluid behaves in the same way as the white fluid previously tested, completely sterilising the supernatant fluid in all the tubes but the first of four, leaving a small proportion of survivors in the deposit of all tubes but the last, and a much larger proportion of survivors in all the tubes, including the last, when the suspension of faeces had been disintegrated by previous shaking.

An experiment using the "S" strain of B. typhosus. The Lister (Rawling) strain of *B. typhosus* is sometimes objected to for disinfectant tests because in most laboratories it is now rough. The condition of the culture used in the foregoing experiments is as follows: its colonies on agar are rough, and it is non-motile, but cultures in broth are free from even microscopic clumps, a wet film showing only single bacilli. The only rational objection to the use of a rough culture, namely that it may form clumps of varying size within which bacilli may escape the action of a disinfectant, does not therefore apply. Nevertheless, an experiment was done with *B. typhosus* "S," the strain commonly used for the Chick-Martin test, which is smooth and motile, in order to determine whether this difference in the characters of the culture will affect its behaviour in a mixture of disinfectant and a suspension of faeces. That it will not is

shown by the results of an experiment given in Table IV; *B. typhosus* "S" was exposed to four dilutions of the disinfectant (the white fluid previously used), *B. typhosus* Lister to two of them for comparison. The only difference shown is that the former strain is rather more resistant; the distinction between shaken and unshaken tubes, and between the supernatant fluid and deposit in the latter, are the same. The actual counts in the case of the Lister culture correspond very closely with those obtained in the experiment detailed in Table II. It is possible that the control count of the "S" culture was in error, since two of the figures in the last column represent an excess over the calculated inoculum.

Table III. *Numbers of surviving* B. typhosus *per c.c. after* 30 *min. at* 20° C. *in suspensions of dried faeces + different dilutions of a black fluid.*

Original inoculum = content of 5,130,000 per c.c.

Amount of 2 % disinfectant in tube c.c.	Number of surviving bacteria per c.c.		
	Unshaken suspension		
	Supernatant fluid	Deposit	Shaken suspension
0·48	100	160,000	5,090,000
0·55	0	140,000	940,000
0·66	0	2,400	200,000
0·77	0	0	2,500

Table IV. *Numbers of surviving* B. typhosus *per c.c. after* 30 *min. at* 20° C. *in suspensions of dried faeces + different dilutions of disinfectant (white fluid).*

Original inoculum of *B. typhosus* "S" = content of 3,240,000 per c.c.; that for *B. typhosus* Lister not determined.

Amount of 2 % disinfectant in tube c.c.	Number of surviving bacteria per c.c.		
	Unshaken suspension		
	Supernatant fluid	Deposit	Shaken suspension
B. typhosus "S"			
0·55	1,030,000	1,870,000	5,120,000
0·66	178,400	280,000	6,020,000
0·77	0	112,800	3,920,000
0·92	0	1,400	6,700
B. typhosus Lister			
0·66	0	35,300	3,450,000
0·77	0	3,400	510,000

Discussion

The Chick-Martin test is designed to imitate the conditions under which disinfectants are used, but it begins by converting highly appropriate organic matter into a type of material unknown outside the laboratory. The pulverisation and complete desiccation of faeces produces a material which is non-miscible with water in the cold, and autoclaving the mixture results in a suspension which has peculiar and unsatisfactory properties. It rapidly forms a compact deposit, in which small numbers of bacteria survive long after those in the supernatant fluid have been destroyed. Within fairly wide limits the result of a test depends, in fact, on the progress of events in this deposit, on the

fate of bacteria in small interstices between solid particles of faeces. The number of these survivors may demonstrably be so small that whether a positive culture is obtained may depend largely on how a loopful is removed, and in part on pure chance. The effect of this condition is illustrated in the frequent occurrence of "irregular tubes" in the resulting cultures. The experiments which have been described also show that the capacity of an emulsified disinfectant to destroy bacteria in this type of faecal suspension depends to a large extent on the degree of subdivision of the particles in the suspension, an uncontrolled factor liable to variation from several causes.

It is clear that these considerations, apart from variations in the composition of the faeces, a factor which in spite of Chick and Martin's original work cannot confidently be held entirely blameless, would account for discordant results in the performance of this test. There are certain steps which, without changing its character, would probably reduce its liability to error. The chief of these would be to restore the faecal suspension to a natural condition by mechanical shaking, continued for such period as might be necessary for complete disintegration of the particles into their original constituents. The reaction of the suspension would also be controlled. Further than this, since in the present test the period of contact between the organic matter and the disinfectant before the addition of culture is unspecified, and the degree of adsorption of one by the other may therefore possibly vary, this factor should be brought under control. Rather than introduce another arbitrary time period, it would be preferable to make a change which at the same time brings the test closer to practical conditions, by mixing culture and faecal suspension beforehand and adding the disinfectant to this mixture. Finally, a closer spacing of dilutions would make for greater accuracy in results, unless there were still some inherent property in the test which rendered such accuracy unattainable.

The alternative is to employ another form of organic matter, and if a substitute could be found which served the purpose equally well, and obviated the unpleasant process of preparing dried faeces, workers in this field would welcome it. The following observations are offered on this point.

A possible substitute for faeces

In considering this question it was necessary to determine which elements in a faecal suspension play the chief part in reducing the activity of disinfectants, and experiments with this object were carried out which need not be described in detail. When the fluid and solids of an autoclaved suspension were separated, the latter being resuspended in water, and tests were done with both, it was found that the fluid portion was without effect in any of the dilutions of disinfectant used, whereas the action of the solids was almost unaffected by the substitution of water for the original fluid. The solid particles in the suspension are therefore chiefly responsible for its action. In order to determine what type of particle mainly exerts this effect suspensions of fresh faeces (in which

the ratio of moist to dry weight was subsequently determined) were filtered through glass wool, yielding a fluid in which almost all the particles were of the size of bacteria; tests with this material showed that its effect was little impaired by the removal of its coarser ingredients. It therefore appears that of the solid particles the smallest are the most active, and experiments already described illustrating the effects of mechanical disintegration point to the same conclusion. These particles consist largely of bacteria. According to Strasburger (1902) a variable proportion, averaging about one third, of the dried weight of faeces, is accounted for by bacteria. A determination by Strasburger's method (repeated differential centrifugation) with a fresh specimen of the faeces used in these experiments indicated that the proportion of bacteria in the total dried weight was 53 per cent. Since not only a large proportion but the most active element in a suspension of faeces consists of bacteria, it would seem feasible to use a preparation of bacteria alone as organic matter in a test for disinfectants. A suspension of bacteria would have the advantage that many of its properties (particle size, chemical composition, etc.) would naturally be constant, and others could be accurately controlled. But to furnish a quantity of organic matter of the order used in the Chick-Martin test large volumes of culture would be necessary.

A possible solution to this difficulty presents itself in the form of commercial yeast. When preliminary tests had shown that a suspension of this material does in fact reduce the activity of disinfectants to about the same extent as a suspension of faeces of similar concentration, yeast was obtained from ten different retail sources, and its properties were studied.

Table V. *Characters of* 1 *in* 5 (*moist weight*) *suspensions of ten samples of yeast.*

Sample	Dried weight %	Volume of solids %	Solids in solution %	Cells per c.mm.	Reaction (*p*H)	Disinfectant coefficient given
1	29·9	15	1·86	2,740,000	6·4	2·5
2	30·1	14	1·64	2,835,000	6·2	2·2
3	32·7	15	1·6	4,400,000	6·2	2·5
4	29·1	14	1·9	2,840,000	6·2	2·5
5	28·1	13	1·9	1,940,000	6·1	2·2
6	29·6	15	1·8	3,320,000	6·3	2·2
7	31·0	15	1·74	3,280,000	6·3	2·2
8	27·5	14	1·8	2,970,000	6·3	2·2
9	28·5	13	1·4	2,500,000	6·0	2·2
10	28·7	14	1·9	2,670,000	6·1	2·2

Properties of yeast suspensions

A weighed amount of each fresh moist yeast was emulsified in distilled water and made up to such a volume that 1 g. of moist yeast was contained in 5 c.c. This suspension was autoclaved; its reaction was then determined, and it was brought to neutrality by the addition of the required amount of $N/1$ NaOH. Meanwhile a weighed amount of the original yeast was dried to constant weight at 55° C. and reweighed. The ratio of moist to dry weight was

thus found to vary singularly little. The suspensions were studied as follows. A measured amount was centrifuged to constant volume; this varied from 13 to 15 per cent. of the total volume. A measured amount of the supernatant fluid obtainable after centrifuging was evaporated to dryness and found to contain from 1·4 to 1·9 per cent. of material in solution. The yeast cells in the suspension were counted, and found to vary from 1,940,000 to 4,400,000 per c.mm. Finally a disinfectant test was carried out by the Chick-Martin technique using each of these suspensions as organic matter, and a set of tubes containing 6 per cent. dried faeces as a control. The coefficient obtained with the latter was 3·0; with all of the yeast suspensions it was either 2·5 or 2·2. These data are given in detail in Table V.

Yeast suspensions prepared in this way therefore vary somewhat in their properties, although in certain of these (notably particle size and number and presumably chemical composition) they vary much less than suspensions of faeces. It should be possible to reduce the degree of these variations either by requiring that the yeast used shall conform to certain standards or by obtaining it from a specified source. The objection to making a volumetric suspension in accordance with moist weight could be met by a subsequent determination of the dry weight percentage and adjustment of the volume of suspension accordingly. In spite, however, of such variations in character as these suspensions exhibit, their effect in reducing the activity of the disinfectant is almost constant; a difference of only one tube in the Chick-Martin range covers the whole extent of the differences between them. As regards the degree of this effect, it is somewhat more pronounced than that of a roughly equal amount of dried faeces (the concentration of the latter having been the usual 6 per cent.; that of the dry yeast can be calculated from the first column in Table V to have varied from 5·62 to 6·54 per cent.). Should a test employing yeast as organic matter be found satisfactory, it will probably be advisable to reduce the concentration of the suspension to less than that used in these preliminary experiments.

There is therefore a *prima facie* case for considering the suitability of yeast for this purpose. Further investigations are in progress.

SUMMARY OF CONCLUSIONS

1. A study of the suspension of dried faeces used in the Chick-Martin test, and of the process of disinfection in this test has shown that:

(*a*) The suspension of faeces is unstable, and rapidly forms a compact deposit.

(*b*) Surviving bacteria in critical dilutions of the disinfectant are contained exclusively in this deposit.

(*c*) Uncontrolled factors affect the degree of subdivision of the particles of faeces.

(*d*) The degree of subdivision of these particles materially affects the activity of a disinfectant in their presence.

2. It is suggested that these facts among others may account for inconstant results which the test is said to give.

3. A suspension of yeast is proposed as a possible alternative form of organic matter in such a test.

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