Observations

(ON

THE VALUE OF SOME OF THE METHODS USED FOR TESTING DISINFECTANTS.

By E. KLEIN, M.D., F.R.S.,

Lecturer on Advanced Bacteriology, St. Bartholomew's Hospital
Medical School.

THE usual method of testing and comparing disinfectants consists in exposing for a given time cultures or emulsions of a particular microbe to dilutions of the disinfectant. This, no doubt, is of value in comparing with one another various disinfectants or their dilutions, and although it affords a sufficiently reliable index of the efficacy and power of a given disinfectant in a definite dilution and for a definite time exposure, it cannot, for reasons to be mentioned presently, supply an absolute guide for the application of the disinfectant in actual practice. In practical life a disinfectant is applied by a surgeon, by the sanitarian, or by the nurse, not to an artificial culture or to an emulsion of an artificial culture, but to materials, such as excretions, secretions, morbid products, and morbid tissues, derived directly or indirectly from the human or animal bodies. The microbes to be acted upon by the disinfectant are embedded in, surrounded by, and mixed with various materials. This condition cannot obviously be compared with that of a microbe in a broth culture, or with a watery or salt emulsion of the growth taken from an artificial culture. The surgeon who wishes to disinfect a wound and to keep it antiseptic, has to apply the disinfectant to tissues and not to a watery emulsion; the nurse who is supposed to disinfect a typhoid stool with a given disinfectant is not acting on a watery or saline emulsion of a culture of the typhoid bacillus, but on materials of a highly-complex composition, and altogether of a different nature from the laboratory culture.

An illustration is sufficient to prove the difference between the one kind of disinfection and the other. *Staphylococcus pyogenes aureus* (of a recent and active culture) is justly considered one of the hardest non-sporing microbes, at any rate as far as chemical disinfectants are concerned, and for this reason bacteriologists are in the habit of using this microbe in their laboratory tests.

Now, the *staphylococcus aureus* taken from a culture (broth or agar) and placed into a watery solution or distributed in a disinfectant, is by simple agitation in most cases readily emulsified; the fluid viewed under the microscope shows the microbes almost uniformly distributed as single cocci or as diplococci or as very small groups. Compare with this a microscopic specimen of the contents of an abscess or of the secretion of a wound or ulcer. Here you find, besides crowds of various tissue elements, leucocytes, debris, tissue fibres, fatty granule-cells, etc., large and small clumps of cocci and diplococci not only in the fluid medium, but within the protoplasm of leucocytes, within debris and within masses of fibres.

The disinfectant which is to act on the microbe so surrounded and lodged has a task to fulfil quite different from what it has when it is added to a broth culture or other uniform emulsion of the microbe. The same, *mutatis mutandis*, applies to diphtherial membrane, to sputum, to the typhoid stool, and other animal excretions. In the following experiments I wish to show by direct observation that in a number of tests which I have made in this direction, marked differences exist in the action of a certain disinfectant, with which I have been well acquainted for some years, to wit, izal, as applied to microbes, such as *staphylococcus aureus*, *B. coli*, *B. typhosus*, in different surroundings; in one case the microbes were taken from artificial cultures, and in the other were in their natural habitat. The izal used was the fluid sold as ordinary izal in the ordinary pharmacy.

METHOD.

1. In the case of pus of acute abscess, the pus and blood, immediately after collecting them from the abscess in sterile test tube, were diluted with sterile distilled water, 1 part of pus being added to 49 parts of water. From this dilution a control culture was made on agar, one platinum loopful of the mixture for one agar surface culture. The result of this control culture indicated the amount of *staphylococcus* present, and allowed the number of colonies in the control tube to be

compared with those that appeared in the culture tubes after medication. To the above dilution of the pus was added a definite amount of izal direct as sold, and then well shaken up. The proportion of izal to the volume of diluted pus represented the strength of izal actually used in the experiment.

2. In the case of urine a definite amount of izal was added, well shaken, then after being kept for the required time, the necessary cultures were made on agar and in broth.

3. In the case of the typhoid stool only typical fluid (pea-soup) stools were used; of these a definite quantity was added to a definite quantity of sterile water. This was well shaken up; to it was added a definite amount of izal as in the other cases.

Also in the last two instances control cultures on agar with one platinum loop were made from the diluted materials previous to the addition of the izal, so as to be able to compare the actual number of microbes of the coli-typhoid type before and after exposure to the disinfectant.

After the addition of izal cultures were made at the desired periods from the medicated fluids on agar surface, and in broth, each culture tube receiving three platinum loops of the medicated material; these tubes were then incubated at 37°C. Inspection and notification were made after the tubes had been incubated for 3 or 4 days, for by this time all growth, if any, had become complete.

EXPERIMENTS.

First Series.—Pus of acute abscess.

Experiment 1.—Of pus and blood of an acute abscess of the female breast, 1 c.cm. was added to 49 c.cm. of sterile distilled water (1 in 50).

One platinum loop yielded about 100 colonies of *staphylococcus aureus*.

Added to diluted pus 0.1 c.cm. of izal well-shaken up. This made, therefore, 1 izal in 500.

Cultures were made with three platinum loops for each tube after 5, 15, and 30 minutes with this result:—

a. After 5 minutes: agar tube has 12 colonies of *staphylococcus aureus*. Broth tube uniformly turbid.

This broth-tube was subcultured, with the result that it proved a pure culture of *staphylococcus aureus*.

b. After 15 minutes: on agar two colonies of *staphylococcus aureus*. Broth-tube uniformly turbid.

Subculture proved this broth-tube to be a pure culture of *staphylococcus aureus*.

c. After 30 minutes: on agar two colonies of *staphylococcus aureus*. Broth-tube greatly retarded growth, only faintly turbid after 3 days' incubation.

Considering that a single loop of the diluted pus yielded before the addition of the izal about 100 colonies of *staphylococcus aureus*, whereas three loops (same loop as used in control) yielded after 5 minutes' exposure to the izal only 12 colonies, the process of disinfection must be considered already after this short space of time of a fair degree; this is well shown in experiment c, after 30 minutes' exposure, when three loops yielded only 2 colonies; that is to say, of 300 microbes only two had survived. The broth tube bears witness to this, since the broth showed greatly retarded and scanty growth.

The result of this Experiment 1 is, then, that izal 1 in 500 may for practical purposes be considered a fair, though not complete, disinfectant for pus of acute abscess (*staphylococcus aureus*) after 30 minutes' exposure.

Experiment 2.—The same pus as above was used; 1 c.cm. of the pus was added to 39 c.cm. of sterile distilled water (1 in 40), then 0.1 c.cm. of the izal was added (1 in 400), well-shaken, and cultures were made as above after 5, 15, and 30 minutes on agar and in broth, using three platinum loops for each tube.

The result was this:—

a. After 5 minutes' exposure: on agar 15 colonies of *staphylococcus aureus*. Broth uniformly turbid.

Subculture of this proved pure culture of *staphylococcus aureus*.

b. After 15 minutes: both tubes free of growth.

c. After 30 minutes: both tubes free of growth.

From this it follows that izal 1 in 400 completely disinfects in 15 minutes; in 5 minutes already the number of living microbes being greatly reduced (300 to 15).

Experiment 3.—Pus of an acute submental abscess (mixed with blood) was obtained; 1 c.cm. of the pus was diluted with 39 c.cm. of sterile distilled water (1 in 40).

With one loop of this dilution made control agar culture. In this about 12 dozen colonies came up; these were partly colonies of streptococci, partly colonies of *staphylococcus aureus* and albus.

0.1 c.cm. of izal was added to the diluted pus (1 in 400); after well-shaking it and keeping it for 5, 15, and 30 minutes, cultures were made on agar and in broth, using for each tube three loops. Incubated at 37°C. for 4 days.

The result was this:—

a. After 5 minutes: on agar 15 colonies of mixture of staphylococcus aureus and albus. No colonies of streptococci. Broth slightly turbid.

Subculture of the broth showed mixture of staphylococcus aureus and albus, but the great majority were those of staphylococcus aureus. No colonies of streptococci.

b. After 15 minutes: both tubes were free of growth.

c. After 30 minutes: both tubes were free of growth.

From this experiment it follows that izal 1 in 400 disinfects completely streptococcus in 5 minutes; that it reduces living staphylococcus aureus and albus in 5 minutes to a considerable degree (from 432 to 15) and that it completely disinfects them in 15 minutes.

The following table summarises the preceding results as regards the capability or incapability of izal to disinfect the microbes of pus of acute abscess.

TABLE I.

Sample.	Nature of Dilution.	Time of Exposure.		
		5 minutes.	15 minutes.	30 minutes.
Pus No. 1	1 izal in 500	+ reduced	+ reduced	+ greatly reduced
" " 1	1 izal in 400	+ reduced	—	—
" " 2	1 izal in 400	+ reduced	—	—

Positive sign means growth, negative sign means absence of growth after medication.

Experiment 4.—This experiment is a control to the preceding experiments with staphylococcus aureus, and serves to compare the effect of the disinfectant when used on a culture of the microbe in watery emulsion.

Of a 48-hours' agar active culture of staphylococcus aureus added to sterile distilled water, sufficient to form slight but distinct turbidity.

One loop of emulsion brought forth on agar an almost confluent mass of staphylococcus aureus.

a. To 50 c.cm. of the staphylococcus emulsion added 0.1 c.cm. izal, 1 in 500.

b. To 60 c.cm. of the staphylococcus emulsion added 0.1 c.cm. izal, 1 in 600.

c. To 75 c.cm. of the staphylococcus emulsion added 0.1 c.cm. izal, 1 in 750.

After 5 minutes' exposure made with three loops one agar and one broth culture, incubation at 37° C., with result as follows:—

a. 1 in 500, 5 minutes' exposure. No growth in either tube.

b. 1 in 600, 5 minutes' exposure. No growth in either tube.

c. 1 in 750, 5 minutes' exposure. Agar shows 18 colonies.

Broth uniformly turbid with staphylococcus aureus.

Table II summarizes the preceding results.

TABLE II.

Sample.	Nature of Dilution.	Time of Exposure, 5 minutes.
Watery emulsion of culture of staphylococcus aureus	1 in 500	—
" " "	1 in 600	—
" " "	1 in 750	+ greatly reduced

From this it follows that for staphylococcus aureus of culture izal 1 in 600 is a complete disinfectant in 5 minutes. This result is in so far interesting as it shows that staphylococcus aureus of culture is disinfected quicker and with greater dilutions of izal than when it is acted upon in its natural habitat, that is, pus. It will be remembered that for pus izal 1 in 400, fifteen minutes' exposure, was the limit for completely disinfecting staphylococcus aureus, whereas for disinfection of this microbe in watery emulsion 1 in 600 izal for 5 minutes' exposure was sufficient.

This result emphasizes the importance of testing the disinfecting power of any given substance on the pus microbes while in their natural media, for only thus is an absolute index for practical guidance acquired.

Second Series.—Typhoid Stools.

Experiment 5.—Typical (pea-soup) typhoid stool (1) of a case of typhoid fever, 22nd day of illness.

This stool was tested in high dilutions by means of Drigalski-Conradi plates, and was found to contain about 14 millions of B. coli, about same number or a little more of streptococci, and 3 millions of the B. typhosus per 1 c.cm. of original stool.

The disinfection experiment was made in the following manner:—

1 c.cm. of the stool was added to 49 c.cm. of sterile distilled water (1 in 50); this was well-shaken up till a fair and uniform emulsion was obtained. Control agar culture with one loop showed innumerable colonies of above microbes. To it was then added 0.1 c.cm. of the izal and well-shaken up; this made, then, a dilution of 1 izal to 500.

Cultures were made after 15 minutes, after 30 minutes, and after 1 hour exposure, three loops of the medicated fluid being added to each agar and broth tube.

The result was this:—

a. After 15 minutes: on agar one colony of streptococci. Broth slightly turbid.

Subculture of this broth showed colonies of streptococci only.

b. After 30 minutes: on agar no colonies. Broth very slightly turbid.

Subculture of this broth showed colonies of streptococci only.

c. After 1 hour: on agar no growth. Broth very slightly turbid. Streptococci.

From this experiment it follows that the microbes of the coli-typhoid type were completely disinfected in 15 minutes by izal 1 in 500, but the streptococci, although disinfected for agar in 30 minutes, were not quite disinfected for broth though very largely reduced in number in 1 hour.

Experiment 6.—The same stool (2) as in Experiment 5 was treated with izal 1 in 600, after exactly the same manner as described above.

Result:—

a. After 15 minutes: agar free of growth. Broth slightly turbid; B. coli.

b. After 30 minutes: both tubes free of growth.

c. After 1 hour: both tubes free of growth.

Experiment 7.—The same stool (3) treated with izal 1 in 750.

Result:—

a. After 15 minutes: agar showed five colonies (B. coli). Broth uniformly turbid (B. coli).

b. After 30 minutes: agar shows three colonies (B. coli). Broth very slightly turbid (B. coli).

c. After 1 hour: agar no growth. Broth no growth.

The result of these two experiments, 6 and 7, is then:—

Izal 1 in 600 completely disinfected the microbes of the coli-typhoid group in 30 minutes, but not in 15 minutes. Izal 1 in 750 completely disinfected these microbes in 1 hour, but not in 30 minutes.

Experiment 8.—Typical (pea-soup) typhoid stool (4) of a case, 19th day of illness, was treated in exactly the same manner as the stool in Experiment 5, namely 1 in 500, with this result:—

a. After 15 minutes: no growth on agar or in broth.

b. After 30 minutes: no growth on agar or in broth.

c. After 1 hour: no growth on agar or in broth.

In this case, then, izal 1 in 500 caused in 15 minutes complete disinfection of the microbes of the coli-typhoid type as well as of the streptococci. (Control experiments made of this stool in high dilution by means of Drigalski and Conradi plates showed: 16 millions B. coli, 2 millions B. typhosus, and about 10 millions streptococci per 1 c.cm.)

Experiment 9.—The same stool (5) as used in Experiment 8 was treated with izal 1 in 600.

Result:—

a. After 15 minutes: agar, no growth. Broth, no growth.

b. After 30 minutes: agar, no growth. Broth, no growth.

c. After 1 hour: agar, no growth. Broth, no growth.

From this it follows that izal as 1 in 600 completely disinfected in 15 minutes.

Experiment 10.—The same stool (6) as in Experiments 8 and 9, izal 1 in 750.

Result:—

a. After 15 minutes: agar shows two colonies (B. coli). Broth uniformly turbid (B. coli).

b. After 30 minutes: agar, no growth. Broth uniformly turbid (B. coli).

c. After 1 hour: both cultures free of any growth.

From this it follows that izal as 1 in 750 completely disinfected in one hour, but not in 30 minutes.

Experiment 11.—Semi-solid stool (7) of a case of typhoid, 10th day of illness. Added 1 gram to 50 c.cm. of sterile distilled water. (From this dilution, 1 in 50, made with one loop one agar culture, with the result that after incubation the agar surface was covered with innumerable colonies, in many places confluent). To the above dilution, 1 in 50, added 0.1 c.cm. izal, that is 1 in 500. Cultures from the medicated fluid were made after 15 and 30 minutes and after one hour, using for each culture tube three loops.

Result:—

a. After 15 minutes: agar shows one colony of sporing B. mesentericus. Broth tube turbid due to cocci and streptococci; no bacilli.

b. After 30 minutes: both tubes free of growth.

c. After an hour: both tubes free of growth.

In this experiment, then, izal 1 in 500 disinfected the microbes of the coli-typhoid group in 15 minutes, though not those of cocci.

Experiment 12.—The same stool (8) was used as above, izal 1 in 600.

Result:—

a. After 15 minutes: agar free of growth. Broth free of growth.

b. After 30 minutes : agar has one colony (*B. coli*)? Broth free of growth.

c. After 1 hour : both tubes free of growth.

From this it follows that izal 1 in 600 completely disinfected the microbes of the coli-typhoid group in 1 hour, but already in 30 minutes the reduction of all microbes was enormous, since only the agar tubes showed one single colony, but it was not quite clear whether *B. coli* or not. For practical purposes 30 minutes would therefore suffice.

Experiment 13.—The same stool (9) was used as above. Izal 1 in 750.

Result:—

a. After 15 minutes : agar shows large number of coli-like colonies, Broth clear.

b. After 30 minutes : both tubes without growth.

c. After 1 hour : agar shows one colony of sporing *B. mesentericus*.

The following Table III summarizes the results of all experiments made with typhoid stools in respect of the microbes of the coli-typhoid group:—

TABLE III.

Sample.	Nature of Dilution.	Time of Exposure.		
		15 minutes.	30 minutes.	1 hour.
Typhoid stool (1)	1 in 500	—	—	—
" " (2)	1 in 600	+ reduced	—	—
" " (3)	1 in 750	+ reduced	+ reduced	—
" " (4)	1 in 500	—	—	—
" " (5)	1 in 600	—	—	—
" " (6)	1 in 750	+ reduced	+ reduced	—
" " (7)	1 in 500	—	?	—
" " (8)	1 in 600	—	—	—
" " (9)	1 in 750	+	—	—

Third Series.—Urine.

Experiment 14.—Turbid urine of a case of typhoid (end of second week). An agar control culture was made with one loop of the urine; it showed after incubation 36 colonies of various kinds of microbes, chiefly belonging to the coli-typhoid group. To 70 c.cm. of the urine (practically the whole of the urine obtainable) 0.1 c.cm. izal was added; this represents, therefore, 1 in 700.

Cultures were made, using three loops for each culture tube, after 5, 15, and 30 minutes, with this result:—

a. After 5 minutes : agar, no growth. Broth turbid.

b. After 15 minutes : agar, no growth. Broth, no growth.

c. After 30 minutes : agar, no growth. Broth, no growth.

From this experiment it appears that 15 minutes was sufficient for izal 1 in 700 to cause complete disinfection of this urine.

Experiment 15.—Normal urine was filtered and then sterilized by heating it for 10 minutes at 70° C. To the sterile clear urine added of a recent culture of bacillus typhosus sufficient to produce slight turbidity. One platinum loop of this was transferred to the surface of gelatine. It brought forth a very large number of colonies of the *B. typhosus*.

a. To 50 c.cm. of the typhoid urine was added 0.1 c.cm. of izal, this being equal to izal 1 in 500.

b. To 60 c.cm. of the typhoid urine was added 0.1 c.cm. of izal, this being equal to izal 1 in 600.

c. To 75 c.cm. of the typhoid urine was added 0.1 c.cm. of izal, this being equal to izal 1 in 750.

After 5 minutes' exposure, cultures were made on agar and broth, each tube receiving three platinum loops. Incubation 37° C.

The result was as follows:—

a. 1 in 500, 5 minutes' exposure. No growth in either tube.

b. 1 in 600, 5 minutes' exposure. No growth in either tube.

c. 1 in 750, 5 minutes' exposure. Good growth in both tubes, proved to be pure *B. typhosus*.

It follows from this that 1 in 600 for 5 minutes is the limit of izal disinfection for *B. typhosus* distributed in urine.

Experiment 16.—For control and comparison of the above experiments sterile distilled water was substituted for the stools and urine. In other respects all conditions remained the same, namely, of a recent culture of *B. typhosus* (same as used above) sufficient growth was added to the water so as to produce slight, though distinct, turbidity.

A single platinum loop of the watery emulsion yielded a very large number of colonies of *B. typhosus*.

a. To 50 c.cm. of the typhoid water added 0.1 c.cm. izal, 1 in 500.

b. To 60 c.cm. of the typhoid water added 0.1 c.cm. izal, 1 in 600.

c. To 75 c.cm. of the typhoid water added 0.1 c.cm. izal, 1 in 750.

After 5 minutes' exposure made cultures on agar and in broth with 3 loops. Incubation at 37° C. with the following result:—

a. 1 in 500, 5 minutes' exposure. No growth in either tube.

b. 1 in 600, 5 minutes' exposure. No growth in either tube.

c. 1 in 750, 5 minutes' exposure. No growth on agar, slight turbidity of broth due to *B. typhosus*.

This, then, agrees with Experiment 15 as to complete disinfection of *B. typhosus* by izal 1 in 600 in 5 minutes, but as

regards izal 1 in 750, the typhoid watery emulsion appears to be more amenable to disinfection than the typhoid urine, for while in the latter good growth appeared in the broth tube, in the former the broth only showed retarded and diminished growth of the *B. typhosus*. That the slight turbidity of this broth was really due to *B. typhosus* was proved not only by subcultures but by the positive agglutination test, the bacilli becoming completely agglutinated by typhoid serum (of a typhoid protected rabbit) 1 in 200 in 5 minutes.

Comparing these experiments of watery emulsion of *B. typhosus* with those of the typhoid stool the difference becomes obvious; for while izal 1 in 600 completely disinfected the watery emulsion in 5 minutes, and even as 1 in 750 greatly reduced the number of bacilli in 5 minutes (no growth on agar, slight turbidity in broth), in the typhoid stool (2), izal 1 in 600 did not disinfect in 15 minutes, though it succeeded in doing so in 30 minutes; in another case (stool 9) izal 1 in 750 had no effect in 15 minutes, though it caused complete disinfection in 30 minutes.

EXPERIMENTS ON THE DISINFECTION OF BACILLUS TYPHOSUS WITH SANITAS, FORMALIN, AND CARBOLIC ACID.

By DAVID SOMMERVILLE, B.A., M.D., M.R.C.P.Lond., D.P.H.Camb.

(From the State Medicine Laboratories, King's College, London.)

In this age of numberless disinfectants it is impossible for the busy medical practitioner to ascertain the true value of many products claimed to be reliable germicides, and his faith in particular preparations is not reassured when he is informed, and rightly informed, that their chemical purity and strength may be everything to be desired, whilst as destroyers of bacteria they may be quite worthless.

In the present state of our knowledge of the manner of action of disinfectants—an action combining physical as well as chemical factors—the best method of determining the efficiency of a given disinfectant seems to be a bacteriological one, in which an appeal is made direct to its powers of germicidal work (quantitative as well as qualitative) on a given micro-organism.

The following investigations were undertaken to discover the relative bactericidal values of "sanitas," "formalin," and carbolic acid, when applied to cultures of *B. typhosus*, and in line with a suggestion thrown out in a critique of a previous paper which appeared in the BRITISH MEDICAL JOURNAL of April 9th, 1904, more detailed information respecting the tests and the life-history of the bacillus used is now given.

Throughout this work, which was executed according to the method suggested by Rideal and Ainley Walker, extreme care was taken that, as far as possible, uniformity in the following conditions should obtain, so that the results in all three cases should be strictly comparable. The conditions are: Time of contact of disinfectant with microbe; age of culture of microbe used; constitution and reaction of medium upon which microbe is grown; vital resistance of species of microbe used; quantity of disinfectant added to culture; temperature of medication; temperature and time of incubation.

A virulent culture of *B. typhosus*, recently isolated from a typhoid spleen, was procured, and sown in broth prepared from "Lemco," after a formula constantly used in these laboratories. This broth, it may be noted, is rendered slightly acid to phenolphthalein (but neutral to litmus), the acidity being equivalent to 15 c.cm. normal NaOH per litre. One loopful of a 24 hours' culture was daily taken from a test-tube containing 10 c.cm. culture, and with this a second 10 c.cm. broth tube was inoculated. The latter in turn at the end of 24 hours yielded its quantum of seed for a third, and so on, throughout the period of experimentation. During this period no variations in the biological phenomena of the organism, as determined by motility, number of flagella, and Durham-Gruber reaction, could be detected.

A sample of phenol standardized by Br. supplied definite quantities of pure carbolic acid, and a 1 per cent. solution was found a convenient dilution with which to work.

A series of 30 test-tubes containing 5 c.cm. each of the bouillon was duly sterilized and placed in racks in six groups of 5 each.

The sample of sanitas to be tested was put in competition with 1 per cent. pure phenol, and was diluted in varying degrees with sterilized distilled water. (All vessels, flasks, pipettes,