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,

TABLE I.—*B. Typhosus*, 24 Hours' Broth Culture at 37° C. (Room Temperature, 15 to 18° C.)

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Sanitas	1 in 1	—	—	—	—	—	—	48 hours	37° C.
"	1 in 2	×	×	×	—	—	—	"	"
"	1 in 3	×	×	×	×	—	—	"	"
"	1 in 4	×	×	×	×	×	—	"	"
Phenol	1 in 100	×	×	×	—	—	—	"	"

Carbolic Acid Coefficient, $\frac{1}{100} = 0.02$.TABLE II.—*B. Typhosus*, 24 Hours' Broth Culture at 37° C. (Room Temperature, 15 to 18° C.)

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Formalin (40 per cent.) ...	1 in 10	—	—	—	—	—	—	48 hours.	37° C.
"	1 in 20	—	—	—	—	—	—	"	"
"	1 in 30	×	×	—	—	—	—	"	"
"	1 in 40	×	×	×	×	—	—	"	"
Phenol	1 in 100	×	×	×	×	—	—	"	"

Carbolic Acid Coefficient, $\frac{1}{10} = 0.4$.

etc., used in making dilutions were previously rigorously sterilized.) Into four sterile test-tubes were put dilutions of sanitas of the several strengths of 1 in 1, 1 in 2, 1 in 3, 1 in 4, and in a fifth sterile test-tube a solution of phenol of the strength of 1 in 100. In each case 5 c.cm. were used.

Now into each of these five disinfectant solutions of 5 c.cm. content five drops of a 24 hours' bouillon culture of *B. typhosus* are allowed to fall at intervals of exactly 30 seconds, and the mixture in each case well shaken. Continuing the 30 seconds' interval, a loopful is removed in serial order from each of the medicated cultures and sown in a bouillon tube, care being taken that the platinum loop is thoroughly sterilized in the flame after each inoculation. This manipulation is continued until the last of the thirty tubes is inoculated. The tubes are held during inoculation, withdrawal and replacement of plugs, etc., in such an oblique position that any extraneous organisms floating in the atmosphere are excluded.

Table I shows that an undiluted sample of sanitas prevented the growth of the bacillus when the time of contact with the culture was 2½ minutes. A dilution of 1 in 2, however, was not able to prevent the growth of the bacillus until a contact of 10 minutes' duration had been effected. The same result exactly was attained by phenol diluted to 1 in 100. Thus it is seen that 1 in 100 pure phenol solution performs the same amount of bactericidal work as a solution of sanitas of the strength of 1 in 2. The carbolic acid coefficient of sanitas is therefore $\frac{1}{100} = 0.02$ (See Table I.)

The bouillon tubes after inoculation, each with a loopful of the serial mixtures of antiseptics, were incubated for 48 hours at 37° C. The temperature of medication was 15 to 18° C. At the end of 48 hours each tube was individually examined for the presence of bacterial life. No adventitious organisms were at any time found.

Table II gives the results of experiments made in the same manner, and under exactly similar conditions with formalin (40 per cent. formic aldehyde in H₂O). (See Table II.)

By this it is seen that 1 in 100 phenol performs the same bactericidal work as 1 in 40 formalin.

The carbolic acid coefficient for formalin is therefore $\frac{1}{10} = 0.4$.

A friend informs me that in working, by the same method, with formalin of strength 39.2 per cent., he obtained a carbolic acid coefficient of 0.3. These figures strikingly demonstrate at once the accuracy and delicacy of this bacteriological test.

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REPORT LXXXVII.

THE EFFECT OF CERTAIN DYES UPON THE CULTURAL CHARACTERS OF THE BACILLUS TYPHOSUS AND SOME OTHER MICRO-ORGANISMS.*

BY

E. W. AINLEY WALKER, and W. MURRAY,
M.A., D.M. Oxon. M.B. Edin., D.P.H. Lond.

Fellow of University College, Oxford.

(From the Gordon Laboratory, Guy's Hospital, and the Pathological Department of the University of Oxford.)

In the course of a series of experiments upon the *intra vitam* staining of bacteria, which we began about 18 months ago, remarkable changes were observed in the cultural characters of the bacteria employed when they were grown in media to which certain small percentages of various dyes had been added. These changes we have found to occur with constancy, and they appear to possess sufficient interest to be worth recording.

Briefly, the change consists in the conversion of a normal short bacillus into a form which presents itself almost entirely or entirely as long filaments or threads. These may be twenty or more times the length of the ordinary bacillus, and often show no sign of segmentation. They frequently present the appearance of true branching when grown on solid media.

We have worked chiefly with a number of different strains of the bacillus typhosus, but have also obtained similar results with some other organisms, such as the bacillus coli and the cholera vibrio. The effect to be described has been produced in greater or less degree with all the following dyes: Grubler's methyl violet (6B), ordinary gentian violet, fuchsin,

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