THE EXAMINATION OF SOME COMMERCIAL CARBOLIC ACIDS AND DISINFECTING POWDERS.

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THE bacteriological testing of disinfectant powders is a subject which has received very little attention. In comparing the relative values of powders it is not sufficient to make a watery solution of the powder and filter or allow to stand, for in this way only the value of those substances which are completely soluble in water is obtained, while those more or less insoluble oils, which have a high germicidal value when brought in contact with micro-organisms either in the form of an emulsion or in a state of very fine division as in powder, are prevented from coming in contact with the organism. A solution of the powder in water has been used by many people in this country, and has also been used by Robertson and Severn (1904—1905) in Cape Colony. These authors fully recognised that such a method was unsatisfactory, for they say, "The objections to this method as giving any clue to the operations of powders in practice are many and obvious, but it is difficult to imagine a fairer method of procedure."

The method they employed was as follows:—

"One hundred grammes of the powder are thrown into a litre stoppered measure, made up to the mark with sterile distilled water shaken at frequent intervals for four hours, and then left to subside till the same hour next day at which the water was added. The supernatant fluid is syphoned off and 10 c.c. of it regarded as equal to one gramme of the powder."

The function of a disinfectant powder, as I understand it, is to disinfect the surface of more or less solid infected matter. It is essentially a surface disinfectant. If a disinfectant be required to penetrate into the body of a substance either a liquid disinfectant should be chosen or possibly a powder containing a readily soluble substance such as phenol.

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The action of the two classes of powders, soluble and insoluble, is obviously quite different, and according to the purpose for which the powder is to be used so one or the other should be chosen. The chemical and bacteriological examination of all powders is of the highest importance owing to the large amount used each year and the enormous amount of cheap and worthless powder which is sold as carbolic powder, or as equivalent in germicidal value to some stated quantity of carbolic acid. It is necessary to take as our standard, when testing powders, a carbolic acid powder containing the same quantity of oil per cent. as the one we are testing. For instance, if a powder A, containing 10% of an oil, kills the organism with a dilution of 1 : 200, and a 10% pure carbolic acid powder kills the organism with a dilution of 1 : 10, it is obvious that our powder A is 20 times more effective than is the same quantity of carbolic acid *placed under the same conditions.* It is not possible to keep a standard carbolic acid powder for comparison—the powder must be made up fresh for each determination.

Messrs Calvert and Co. of Manchester very kindly supplied me with various powders and acids of definite composition. Their powder marked A, composed of 10% pure phenol mixed with a silica base, gave with a particular organism when fresh a dilution figure of 1 : 130; after the bottle (of clear glass) had been standing in the light for some weeks it gave a dilution figure of 1 : 150, and when last tested a dilution figure of 1 : 160; freshly distilled phenol in distilled water giving a dilution figure of 1 : 110 on each occasion with the same organism. Further, the phenol when extracted by repeated exhaustion with petroleum ether gave at first a beautifully crystalline mass of phenol; when last extracted it was in the form of a yellow oil which refused to crystallize. Again, a 1% solution of phenol was prepared and divided into two parts, one being placed in a tightly corked yellow bottle and kept in the dark, the other in a clear bottle and kept in the light. The following results were obtained:—

	Dilution figure	Fresh solution
Phenol solution fresh	1 : 110	
Kept in the dark for 1 month	1 : 130	1 : 110
In light ,, 1 ,,	1 : 130	1 : 110
Kept in dark ,, 2 months	1 : 130	1 : 110
In light ,, 2 ,,	1 : 150	1 : 110

In a previous paper attention was called to the increase in germicidal value of cresylic acids on standing in the light, and it was then said that it was possible that the same thing might happen with phenol. From the above experiments and many others it is evident that in any experiments on germicidal value, taking phenol as a

standard, it is absolutely necessary to take a freshly distilled phenol and to make up the solution immediately before use, unless it is proved by repeated experiments that the phenol solution gives the same results over a long period of time. In testing powders I take silica as the base and add to this the same quantity of freshly distilled phenol as there is oil in the sample to be tested; for instance, in testing a 10% carbolic powder (cresylic powder), the standard is 90 grammes of dry silica and 10 grammes of pure phenol; for a 15% powder the standard is 85 grammes silica and 15 grammes of phenol, and so on.

The increase in germicidal value observed in old samples of commercial Carbolic Acids, in powders, and indeed in comparatively pure ortho, meta- and para-cresol and in phenol, makes it impossible to say from a direct estimation of germicidal value how much cresylic or carbolic acid the acid or powder contains. To do this it is necessary to make both a chemical and a bacteriological estimation.

The direct estimation of phenol in the presence of the cresols is a matter of great difficulty. The ordinary method of estimating it by fractional distillation is far from accurate, giving only approximate figures. The methods given in various text books for drying with lead oxide, sodium acetate, etc. are all unsatisfactory, and after a prolonged trial I have abandoned them as useless. It is impossible to get an accurate estimation by extracting with dry volatile solvents, as I find the phenol cannot be freed from the solvent without loss even with the greatest care and working at a low temperature. The only method which I have found applicable to powders containing *only* phenol is to extract with water and estimate the phenol in the water solution by means of bromine. With powders containing small quantities of phenol, the total oil may be estimated with accuracy by extracting with petroleum ether boiling at 40° C., and distilling off the bulk of the ether at a temperature not exceeding 80° C., then allowing the oil to stand for 24 hours over sulphuric acid in a large desiccator. With blast furnace oils and coke oven oils, such as izal, the experiments are not altogether satisfactory, as the following show:

100 grammes powder 10% izal oil extracted with pure pentane and allowed to stand at ordinary temperature for 24 hours:

Weight of oil found	Theoretical	Loss
9·79 grammes	10·0	2·1 %
After 3 days weight of oil found		
9·43 grammes	10·0	5·7 %

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Izal powder extracted with pure pentane and then mixed with oxide of lead:

	Grammes found	Theoretical	Loss
24 hours	9·88	10·0	1·2 %
3 days	9·55	10·0	4·5 %

These results were confirmed by taking definite weights of izal oil kindly sent to me by Messrs Newton, Chambers and Co. It was found impossible to estimate the oil within 1% by the use of any volatile solvent, and direct distillation was found even more unsatisfactory. These methods are only accurate enough to tell us whether we are dealing with a 10%, 15% or other approximate quantity of oil in such a substance as Izal Powder.

The estimation of water and phenol in commercial carbolic acids and the phenol in carbolic powders may be carried out as follows:— at least 100 grammes of the powder, if it contains as little as 10% of oil, are extracted with petroleum ether, the ether driven off and the total oil weighed. If the powder contains slime or soap, or anything that combines with the acids, sulphuric acid is added before extracting with the ether. For distillation I use a short condenser, filled at first with cold water, but through which water is not kept running. If the oil or powder contains less than 10% of phenol it will only be necessary to distil one-third of the bulk, if more, it may be necessary to distil a further quantity, and in some cases the distillation must be carried to dryness. The distillate is weighed, then well mixed and divided into two parts, to one of which lumps of freshly ignited calcium chloride are added, and both are tightly corked and put in a dark cupboard for at least 12 hours. The germicidal value of both portions of the distillate is then determined as described in *The Analyst*, May 1907, and compared with freshly distilled phenol and ortho-cresol.

The use of calcium chloride in this way enables us to dispense with the use of milk, for in the first place we are determining water cresol and phenol, and in the second cresol and phenol. In the case of good powders extracted by petroleum ether, the use of calcium chloride is unnecessary. With carbolic acids however there is always a little water.

Example 1. Carbolic acid C, weight taken 28·44 grammes, distillate 11·319 grammes.

	Distillate with water	Distillate without water
Dilution figures	1 : 260	1 : 270
	With cresol	Phenol
Controls	1 : 270	1 : 130

The sample is free from phenol, but contains water; the quantity in the distillate being such that a dilution of 1 : 260 is equivalent to 1 : 270, in other words our dilution of 1 : 260 is really a dilution of $\frac{260}{270}$ in 260, and every 1 gramme of distillate contains only ·963 gramme of oil and ·037 gramme of water.

Our whole distillate contains 11·319 × ·037 grammes water, and this is obtained from 28·44 grammes of our sample, therefore the sample contains $\frac{11{\cdot}319 \times 3{\cdot}7}{28{\cdot}44}$ grammes of water.

Example 2. 50 grammes of a carbolic acid guaranteed to contain 10 % of real phenol. Distilled 25 grammes.

	With water	Without water D_3
Distillate	1 : 250	1 : 260
	Cresol D_1	Phenol D_2
Controls	1 : 270	1 : 130

Then percentage of phenol in the water-free distillate

$$\frac{100\,(D_1 - D_3)}{D_1 - D_2} = \frac{100\,(270 - 260)}{270 - 130} = 7{\cdot}14 \text{ grammes.}$$

Total oil in distillate $= \frac{2}{2}\frac{5}{6} \times 25 = 24{\cdot}04$ grammes.
Total water in distillate = ·96 gramme.

This is derived from 50 grammes of the sample, therefore the percentage of water is 1·92. The total phenol in the distillate is $\frac{7{\cdot}14 \times 24{\cdot}04}{100} = 1{\cdot}72$, and this quantity is contained in 50 grammes of the sample, therefore the sample contains 3·44 % of phenol. All samples of carbolic acids and carbolic powders which contain only phenol or crude carbolic acid and cresol may be examined in this way. The germicidal value of such samples, together with the temperature at which the fractions distil over, their specific gravity, solubility in alkali, and the amount of tarry matter left behind in the still, gives us all the information necessary with regard to these powders and acids.

With powders made with blast furnace oil, coke oven oils, and other coal tar distillates, which either contain very little carbolic or cresylic acids, or none, the question arises as to their value as germicides under the conditions of some standard test, as compared with carbolic acid. This value is one which it is also necessary to ascertain in the case of commercial carbolic acid powders.

Method for testing the germicidal value of disinfecting powders against naked organisms.

Organism. Twenty-four hours' old culture of *B. typhosus.*

Broth. Reaction + 15 to phenol-phthalein. Composition, Liebig's extract of meat 20 grammes, peptone (Witte's) 20 grammes, sodium chloride 10 grammes, tap water to make 1000 c.c.

Temperature of experiments, 17° C. exactly (this refers to the actual temperature of the tubes and not to the room temperature).

Time of comparison, 10 to 12½ minutes. (All apparatus and distilled water and sterilised before use.)

1. Estimate the total quantity of oil present in the sample to be tested, and weigh out a corresponding quantity of silica base and pure freshly distilled phenol.

For example, 9 grammes silica, 1 gramme phenol made up to 100 c.c. with distilled water = 10% solution of a 10% powder.

The powder and water are shaken vigorously together and poured out in small quantities at a time (shaking at intervals) into a narrow measuring glass graduated into one-tenths of a c.c.; the required quantity of distilled water is added to bring the strength down to the dilution required and the various mixtures shaken and poured into glass stoppered tubes. It is best to calculate the dilution on the actual phenol present. This way of making dilutions is only possible when we have a soluble substance such as phenol.

2. Various quantities of the powder to be tested are now weighed out into glass stoppered tubes, which if we have a very active oil present must have a capacity of at least 50 or 100 c.c., and the requisite amount of distilled water run into each tube to bring the mixture up to the dilution required. Each gramme of powder being taken as occupying 1 c.c., as in the case of the control. The tubes are placed in a small tank containing water at 17° C. The bottom and sides of the tank are covered with felt, a thermometer being fitted by means of a perforated cork through one of the sides. The top of the tank is of thin copper in the form of a tray partly covered at one end to prevent the tubes falling out (or a rubber band may be passed round the tank to keep the tubes in position). The tank is so fixed that it may be rocked either by hand or foot or by some mechanical means. The rocking must be done so that the tubes are completely and gently turned right on end once during a period of at least every three seconds, so as to ensure the powders being kept in intimate contact with the organism during the

whole of the time. The actual speed of the rocking, providing it is kept within reasonable limits, has little, if any, effect on the results obtained.

The tubes are inoculated by allowing five drops of broth culture for every 5 c.c. of powder and liquid present in each tube. The tubes can be taken out and replaced without stopping the rocking. Inoculations are made into broth in the ordinary way at intervals of $2\frac{1}{2}$ minutes up to 15 minutes, and the dilution figure is taken as that which gives a + value at the end of 10 minutes and a − value at the end of $12\frac{1}{2}$ minutes. The process may be simplified by taking inoculations at the end of 10 minutes, or any other agreed definite limit. In the following example the dilution figures refer to the actual amount of phenol, or any other oil present in the dilution.

I. A sample of carbolic acid A, specially prepared for this research by Messrs Calvert and Co., containing 10% crystal carbolic acid fusing at 40° C. silica base.

	Time in minutes					
Dilution	$2\frac{1}{2}$	5	$7\frac{1}{2}$	10	$12\frac{1}{2}$	15
1 : 220	+	+	−	−	−	−
1 : 230	+	+	+	+	−	−
1 : 240	+	+	+	+	+	+
Fresh phenol and silica base control :						
1 : 180	+	+	−	−	−	−
1 : 190	+	+	+	+	−	−

The 10% carbolic A is equivalent to $10 \times \frac{230}{190}$ or a 12% pure carbolic powder when freshly prepared.

The above carbolic powder, with an organism of the coli group, gave the following values:—

(1) Shaken dilution figure ... ... 1 : 130
(2) Not shaken ... ... 1 : 130
(3) Fresh distilled carbolic acid and silica base shaken ... 1 : 120
(4) Ordinary fresh distilled phenol in water without silica 1 : 100

The influence of shaking and the change that takes place on standing are not so marked with the more resistant *B. coli* as it is with the more delicate *B. typhosus*.

II. A sample of silica base powder containing 7% liquid carbolic acid (practically pure cresylic acid) and 3% crystal carbolic acid fusing at 40° C. specially prepared by Messrs Calvert and Co. (Note the

3% carbolic acid was readily detected by extraction and dilution as already described.)

(*a*) Dilution figure found 1:460

(*b*) Phenol and silica dilution figure ... 1:200

The 10% powder is equivalent to $10 \times \frac{46}{20} = 23\%$ pure carbolic acid powder.

III. Experiments on a powder containing an insoluble oil. Samples of 10% and 15% izal powder were bought in the open market (these powders are commercially known as No. 1 and No. 2). The exact quantities of oil present in each sample could only be determined, as already described, approximately. The control powders were made up with 10% fresh phenol for the 10% izal powder and 15% fresh phenol for the 15% izal powder. The powders were weighed out, the water added, and the experiments proceeded with at once; this is important, as somewhat higher results will be obtained if the water and powder be left in contact for some time (24 hours).

		Time in minutes					
	Dilution	2½	5	7½	10	12½	15
15% izal powder	1 : 1300	+	+	+	−	−	−
(The dilutions refer to the actual quantity of oil)	1 : 1500	+	+	+	+	−	−
	1 : 1700	+	+	+	+	+	−
Fresh phenol and silica control	1 : 190	+	+	−	−	−	−
	1 : 200	+	+	+	+	−	−

The 15% izal powder is equivalent to $15 \times \frac{1500}{200}$, or a powder containing 112% pure carbolic acid.

In the same way the 10% powder was found to be equivalent to a 70% pure carbolic acid powder.

A large number of experiments were made on an organism of the *B. coli* group, with the powder shaken as in the method described above and with the extracted oil, which was then saponified by resin and soft soap. The result being that for this particular organism the same coefficient was obtained for the oil both when shaken in powder form and when saponified. Showing that when powders are well mixed, and are kept in intimate contact with the organism, they act in the same way as emulsions. When powders of this class, containing more or less insoluble oils, are not shaken, as in the method of Robertson and Severn, very different results are obtained, and results which I do not think can in any way represent the action of the oil when actually brought in contact with the organisms, depending as they do simply on the solubility of the oil. This solubility being far from desirable in a

powder except for special purposes, when a powder containing carbolic acid should be chosen.

Organic matter, such as faeces-emulsion, milk, etc., may be introduced into this test in exactly the same manner as it can be introduced into the ordinary test for liquid disinfectants, and the introduction of organic matter is in my opinion quite as essential in testing the value of disinfectant powders as it is with liquid disinfectants.

This research deals only with those powders containing silica base. The influence of various other bases, such as Kieselguhr, lime, road sweepings, destructor refuse and other substances, on the germicidal value of powders is being studied at present, and I hope to report the results in a future paper.

Summary and conclusions.

1. The phenol solutions used as a standard in the bacteriological testing of disinfectants should be made from pure freshly distilled phenol.

2. The actual quantity of phenol, cresylic acid and water present in commercial carbolic acids and in carbolic powders may be estimated by extracting the oils and testing their germicidal power.

3. The comparative germicidal value of disinfectant powders may be estimated by keeping the powder and organism in contact by mechanical means during the whole period of the experiment.

REFERENCES.

ROBERTSON, G. W. and SEVERN, W. D. (1904—1905). *Report of the Medical Officer of Health for Cape Colony.*

BLYTH, M. W. and GOODBAN, L. (v. 1907). The estimation of Phenol and Cresol by a bacterial method. *The Analyst.*

BLYTH, M. W. (1906). The Standardisation of Disinfectants. *Journ. Soc. Chem. Industry*, XXIII. 1183.

LUNGE, G. (1900). *Coal Tar and Ammonia*, p. 516.