

## SUMMARY.

1. The toxicity of amyl-meta-cresol and hexyl resorcinol has been determined on rats and mice. Both compounds are relatively non-toxic, the cresol being less toxic than the resorcinol compound.

2. Neither compound has any marked cumulative action, as it is possible to administer large doses daily without toxic symptoms.

3. Toxic absorption occurs when an oily solution of either substance is applied to the skin of mice, amyl-meta-cresol being about half as toxic as hexyl resorcinol.

4. Amyl-meta-cresol is rapidly absorbed and eliminated.

5. Amyl-meta-cresol has no deleterious action on the lungs when inhaled daily by rats for long periods.

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THE DISINFECTANT AND ANTISEPTIC PROPERTIES OF  
AMYL-META-CRESOL.

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IN their paper, "The Variation of Phenol Coefficients in Homologous Series of Phenols," Coulthard, Marshall and Pyman (1930) state, "Of the n-amyl cresols, 4-n-amyl-m-cresol has been studied in some detail, and has been found to have high phenol coefficients. Since in addition its toxicity is comparatively low, it may prove to be of value in medicine." The following paper places on record some of the experiments upon which this statement was based, and also some results obtained subsequently.

Amyl-meta-cresol is relatively insoluble in water, hence in carrying out

tests it is necessary to use dilute alcohol, dilute alkali or some other menstruum. In these tests a weighed amount was dissolved in the necessary amount of diluted alcoholic solvent or in N/10 NaOH and the solution so obtained diluted with distilled water. It is difficult to get a solution by shaking the pure amyl-meta-cresol with dilute solvent.

#### A. DETERMINATIONS OF THE RIDEAL WALKER COEFFICIENT USING VARIOUS ORGANISMS.

Similar results are obtained whether the solvent used is alcohol or alkali. The exact technique adopted in preparation of the solutions was :

(a) Addition of a 1 per cent. solution in rectified spirit to N/10 NaOH and subsequent dilution with sterile distilled water, until at a dilution of 1/25,000 the NaOH concentration was N/250 to N/500.

(b) Addition of a 1 per cent. solution in rectified spirit to more alcohol and subsequent dilution with sterile distilled water, until at a dilution of 1/25,000 the concentration of alcohol was 16 to 20 per cent.

The dilution of disinfectant used in calculating the Rideal Walker coefficient is that which kills the test organism in  $7\frac{1}{2}$  minutes but not in 5 minutes ; this is compared with the phenol dilution having the same effect. Tests were carried out to ensure that any percentage of solvent used would not by itself be lethal in anything like this period. It was found that 25 per cent. ethyl alcohol was not lethal under test conditions in 30 minutes and N/100 NaOH (several times stronger than test dilutions) not in 20 minutes.

Coefficients obtained in a series of tests were :

(i)	Test organism	<i>B. typhosus</i>	.	.	.	.	250 to 300
(ii)	„	<i>B. coli</i>	.	.	.	.	203
(iii)	„	<i>Staphylococcus aureus</i>	.	.	.	.	222
(iv)	„	<i>Streptococcus faecalis</i>	.	.	.	.	200

In all four cases a 1/10,000 dilution of the disinfectant in N/200 NaOH was diluted with sterile distilled water as required.

#### B. DETERMINATIONS OF THE INHIBITIVE EFFECT.

These have been carried out using the following technique : Calculated amounts of solutions of amyl-meta-cresol in alcohol or alkali were added to 10 c.c. tubes of sterile trypsin broth. A loopful of a 24-hour-broth culture of the organism concerned was then added and the presence or absence of growth noted after 48 hours.

The smallest amounts of amyl-meta-cresol preventing growth were :

For	<i>B. coli</i>	.	.	.	.	.	1/20,000
„	<i>B. typhosus</i>	.	.	.	.	.	1/30,000
„	<i>B. pyocyaneus</i>	.	.	.	.	.	1/10,000
„	<i>Staphylococcus aureus</i>	.	.	.	.	.	1/50,000 to 1/75,000
„	streptococci (faecal)	.	.	.	.	.	1/40,000
„	<i>B. smegmatis</i>	.	.	.	.	.	1/100,000

## C. DETERMINATION OF THE EFFECT OF THE ADDITION OF VARIOUS SUBSTANCES UPON THE EFFICIENCY OF AMYL-META-CRESOL.

(1) *Addition of Serum.*

(a) *Lethal efficiency.*—A solution of 1/10,000 amyl-meta-cresol in 20 per cent. isopropyl alcohol under test conditions killed a strain of faecal streptococci in under 1 minute; the addition of 2.5 per cent. of ox-serum increased the killing-time to 3 minutes.

(b) *Inhibitive efficiency.*—A dilution of 1/75,000 amyl-meta-cresol in trypsin broth prevented the growth of a strain of *Staphylococcus aureus*, 1/20,000 of a strain of *B. coli*. Under otherwise identical conditions the addition of 10 per cent. ox-serum rendered the addition of 1/20,000 and 1/15,000 respectively necessary to prevent growth.

(2) *Addition of Acid or Alkali.*

The addition of more than a little alkali to solutions of amyl-meta-cresol appears to reduce lethal efficiency considerably. Thus when two solutions of 1/20,000 were prepared, one in N/400 NaOH and one in N/200, the former solution killed the test organism (*B. typhosus*) much more rapidly.

(3) *Addition of Emulsifying Agents.*

In our experiments the preparation of amyl-meta-cresol emulsions has considerably lowered the phenol coefficient. This is in accordance with the finding by B. Hampil (1928) and others that the addition of soaps to some similar cresols and phenols markedly lowers their efficiency.

## D. AMYL-META-CRESOL VAPOUR: DETERMINATION OF THE EFFICIENCY OF THIS AS AN INHIBITANT OF GROWTH.

*Technique of tests.*—The organisms under test were heavily sown from young cultures, usually 24-hour cultures in broth, on to the surface of nutrient agar in 3-inch Petri dishes. Small sterile filter-papers were then inserted into the lids of the dishes and a standardized three drops of antiseptic dropped on each filter-paper. The bottom halves of the dishes were then inserted in the lid in the usual manner, and after incubation at 37° C., agar above, filter-papers below, the presence or absence of growth was read off. Controls were put on without antiseptic and with acetone only.

Dilutions were prepared in acetone. Amyl-meta-cresol gives similar results with acetone, rectified spirit, or isopropyl alcohol. Thirty-eight volatile antiseptics were compared by this test. The five most potent substances tested were amyl-meta-cresol, formaldehyde, oils of cinnamon and cassia and thymol, the actual order of preference varying with the organisms tested.

Amyl-meta-cresol was found to be markedly superior when tested against three cultures of acid-fast bacteria. The three cultures used were: (a) *B. smegmatis*, (b) *B. phlei*, (c) *B. "Mist" Moeller*. Table I gives the results of these experiments. It will be noted that although growth occurred with

25 per cent. eucalyptus oil and guaiacol, 10 per cent. creosote and phenol, and 1.25 per cent. oil of cassia, thymol and liq. formaldehydi, 1 per cent. amyl-meta-cresol prevented it.

TABLE I.—*Inhibition of Growth of Acid-Fast Bacteria by Vapour.*

Phenol, oil, etc.	3 drops (25%).	3 drops (10%).	3 drops (2.5%).	3 drops (1/80).	3 drops (1%).
None (control) . . . . .	+	+	..	+	+
Eucalyptus oil . . . . .	+	+	+	..	..
Guaiacol . . . . .	+	+	+	..	..
Phenol . . . . .	—	+	+	..	..
Creosote . . . . .	—	+	+	..	..
Formaldehyde (B.P. sol. 36–38 per cent.)	—	—	—?	+	+
Oil of cassia . . . . .	—	—	—?	+	+
Thymol . . . . .	..	..	—	+	..
Amyl-meta-cresol . . . . .	—	—	—	—	—

? denotes that there was possibly slight growth.

+ indicates growth of one or more of the three organisms.

— indicates complete absence of growth.

1 in 120 amyl-meta-cresol in acetone usually shows growth.

Confirmatory evidence as to the great value of the vapour as an antiseptic was obtained in a series of tests in which organisms were exposed to the vapours obtained by heating amyl-meta-cresol and, as a control, creosote, over a small bunsen flame in a fume cupboard. The capacity of the cupboard was about 6 cubic feet, definite amounts of disinfectant were heated, and so far as possible all was vaporized during the period of the test.

The following organisms were sown upon nutrient agar in Petri dishes, exposed to the vapour for the specified time and then incubated at 37° C. : *B. pyocyaneus*, *B. coli*, *B. typhosus*, *B. smegmatis*, *B. phlei*, *Staphylococcus aureus* (2 strains), *Streptococcus faecalis* (3 strains).

With 0.5 gm. creosote vaporized over a period of 1 hour all organisms grew. Using 0.25 gm. amyl-meta-cresol vaporized over a period of 30 minutes the acid-fast bacteria did not grow and the cocci were either prevented from growing or grew very weakly.

Using 0.5 gm. amyl-meta-cresol vaporized over a period of 1 hour only *B. coli* and *B. pyocyaneus* grew.

Agar plates sown with *Aspergillus niger* and blood-agar plates sown with pneumococci types 1, 2 and 3, three strains of *Streptococcus hæmolyticus* or *Neisseria catarrhalis* failed to develop after 1 hour's exposure to amyl-meta-cresol vapour, although creosote treatment usually failed to affect growth.

These fume cupboard tests were carried out in conjunction with Mr. W. A. Broom, B.Sc., A.I.C., and toxicity tests were carried out by him simultaneously (*cf.* p. 327).

## E. AMYL-META-CRESOL AS A URINARY DISINFECTANT.

(1) *Freedom from Toxicity.*

Gelatine capsules were prepared each containing 0.15 gm. of amyl-meta-cresol in olive oil. A gradually increasing number of these capsules were taken per day until ten was reached without ill-effect. The ten capsules were taken in pairs at 9.30 a.m., 11.50 a.m., 2.30 and 11.30 p.m.

(2) *Evidence of Disinfecting Effect.*

Samples of urine passed on the day the ten capsules were taken were collected in sterile receptacles and tested for disinfecting efficiency. The samples were—

U2 collected 7.30 a.m. (no capsule taken).  
 U3     ,,     5.30 p.m. (six capsules taken).  
 U4     ,,     4.30 a.m. next day (ten capsules taken).

These were tested both at the pH at which they were passed and neutralized to litmus, against a series of other samples from three sources: 10 c.c. amounts of each urine were sown with equal volumes of a broth culture of *B. typhosus* and plated after 24 hours' incubation at 37° C.

These results are given in Table II:

TABLE II.—*Effect of Amyl-meta-cresol as a Urinary Disinfectant.*

Tube.	Contents.	Capsules taken.	pH.	Colonies, 24 hours.
1 .	Control urine, R. G. P—	0 .	5	. Uncountable
2 .	,,     ,, P. H. D—	0 .	5 to 6	.     ,,
3 .	,,     ,, C. E. C—	0 .	5	.     ,,
4 .	,,     ,, R. G. P—	0 .	Neutral to litmus	.     ,,
5 .	,,     ,, P. H. D—	0 .	,,     ,,	.     ,,
6 .	,,     ,, C. E. C—	0 .	,,     ,,	.     ,,
7 .	,,     ,, U2	0 .	5 to 6	.     ,,
8 .	A.-M.-C. urine, U3	6 .	5 to 6	.     0
9 .	,,     ,, U4	10 .	5 to 6	.     0
10 .	Control urine, U2	0 .	Neutral to litmus	. Uncountable
11 .	A.-M.-C. urine U3	6 .	,,     ,,	.     0
12 .	,,     ,, U4	10 .	,,     ,,	.     0

## F. AMYL-META-CRESOL AS A PREVENTIVE OF MOULD.

Amyl-meta-cresol in varying amounts was added to tubes of trypsin broth and the tubes sown with various moulds, including species of *Sterigmatocystis*, *Aspergillus*, *Penicillium* and one *Thamnidium*. All the moulds tested were inhibited by a dilution of 1/30,000.

Using the vapour efficiency test as detailed in Section D, three drops of a 2.5 per cent. solution in acetone showed restraining power on mould growth,

three drops of a 5 per cent. solution prevented the growth of all species of mould tested.

#### SUMMARY.

1. These results appear to justify the claim that amyl-meta-cresol is a disinfectant showing high lethal and inhibitive efficiency against all the bacteria used in our tests, also against moulds.

2. Rideal Walker (phenol) Coefficients of 200 and over have been obtained using *B. typhosus*, *B. coli*, *Staphylococcus aureus* and *Streptococcus faecalis*.

3. In broth amyl-meta-cresol dilutions up to or beyond 1/70,000 show inhibitive effect, and dilutions of 1/20,000 to 1/30,000 prevent the development of many bacteria and moulds.

4. Amyl-meta-cresol vapour, either vaporized by heat or allowed to vaporize spontaneously, is effective in extreme dilution in preventing the growth of and ultimately killing many organisms. Amongst bacteria so affected are *B. smegmatis* and other acid-fast organisms, pneumococci types 1, 2 and 3, *Streptococcus haemolyticus* and moulds.

5. Amyl-meta-cresol appears to confer bactericidal properties on urine.

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