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A NOTE ON THE TOXICITY OF AMYL-META-CRESOL.

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ACCORDING to Leonard (1924), hexyl resorcinol with a phenol coefficient of 46–52 is one of the most powerful non-toxic organic germicides ever described. Coulthard, Marshall and Pyman (1930), however, described the preparation of amyl-meta-cresol, a relatively non-toxic substance, having a phenol coefficient of about 250. It is proposed in this paper to give an account of some of the experiments on which the claim for the relative non-toxicity of amyl-meta-cresol is based, and also to include some results obtained in an investigation of the toxicity of hexyl resorcinol.

EXPERIMENTAL.

The median lethal dose (M.L.D.) as defined by Trevan (1927) was determined for both compounds when administered orally and by subcutaneous and intraperitoneal injection. Mice were used except where the compounds were given orally, when rats were found to be more convenient. The results are given in Table I:

TABLE I.—*Toxicity of Amyl-meta-cresol and Hexyl Resorcinol.*

Substance.	Form.	Method of administration.	Animal.	Median lethal dose. gm./Kg.
Amyl-meta-cresol . 25 per cent.	emulsion	Oral	Rat	2·5
,, . 25 ,,	solution in olive oil	,,	,,	4–4·5
,, . 20 ,,	,, ,,	Subcutaneous	Mouse	Approx. 7·5
,, . 1 ,,	emulsion	Intraperitoneal	,,	0·15
,, . 5 ,,	solution in olive oil	,,	,,	1·25
Hexyl resorcinol . 25 ,,	emulsion	Oral	Rat	0·25
,, ,, . 25 ,,	solution in olive oil	,,	,,	1·5
,, ,, . 5 ,,	,, ,,	Subcutaneous	Mouse	0·75–1·0
,, ,, . 1 ,,	emulsion	Intraperitoneal	,,	0·05
,, ,, . 5 ,,	solution in olive oil	,,	,,	0·2

A post-mortem examination of the rats which died following the oral administration of these compounds revealed only a slight reddening of the intestines and stomach lining. Histological sections showed a slight sub-epithelial infiltration of the mucosa of the stomach and duodenum, which was so slight as to be negligible. The heart, liver, spleen and kidneys were also examined, but, with the exception of the kidneys, no definite changes were observed. The kidneys showed very slight damage to the tubules in the case of amyl-meta-cresol and a little more in the case of hexyl resorcinol.

Intravenous Injection.

Mice were found to tolerate a dose as large as 1 c.c. per 20 gm. of a saturated solution in saline, whilst up to 100 c.c. of the same solution had no depressor action on rabbits arranged for recording blood-pressure.

Inunction.

Strong solutions of both amyl-meta-cresol and hexyl resorcinol when applied to small areas of depilated skin on the backs of mice produced a wrinkling and reddening of the epidermis which was followed by local necrosis and the formation of a dry scab. After a few days, the period varying with the concentration of the solutions, the mice died without any marked change in the painted area. Toxic solutions of lower concentration produced no necrosis and no scab formed, the only change being the slight wrinkling and reddening of the skin. The average survival period for groups of four mice is given in Table II.

TABLE II.—*Toxicity by Absorption Through the Skin of Mice.*

Substance.	Strength of solution.	Average survival period.
Amyl-meta-cresol	100 per cent.	2 days
,,	40 ,,	5 ,,
,,	20 ,,	7½ ,,
,,	10 ,,	13 ,,
,,	5 ,,	Over 14 days
Hexyl resorcinol	40 ,,	2 ,,
,, ,,	20 ,,	5 ,,
,, ,,	10 ,,	7½ ,,
,, ,,	5 ,,	14 ,,

DAILY DOSES OVER LONG PERIODS.

A. *Rabbits.*

Leonard (1924) was able to feed 0·5 gm. of hexyl resorcinol daily to rabbits without toxic effect. We have been able to confirm this, having fed 0·6 gm. and 0·3 gm. respectively for 25 days without observing any toxic symptoms. The urine and fæces were examined daily for blood, albumin, pus-cells, casts, etc., with negative results. In a parallel test in which 0·6 gm. of amyl-meta-cresol was fed under exactly similar conditions no toxic symptoms were observed and the urine findings were negative.

B. *Rats.*

Rats in groups of six or over received daily doses of amyl-meta-cresol or hexyl resorcinol in olive-oil solutions. The doses used were 1·0 gm., 0·5 gm.,

0·25 gm. and 0·1 gm. per kgm. body-weight. The solutions were administered by stomach-tube under light ether anæsthesia, and the urine and fæces separately collected and examined for albumin, blood, epithelial cells, pus-cells and casts.

Of the animals receiving 1 gm./kg. amyl-meta-cresol daily, three died after 10, 10 and 12 days respectively, whilst the remaining three were still alive after 37 days, when the treatment was discontinued. All those receiving smaller doses survived the whole experimental period of 37 days. Hexyl resorcinol was slightly more toxic ; the rats receiving 1 gm. kg. all died after 3, 5, 5, 7, 8 and 8 days respectively. The rats receiving 0·5 gm./kg. survived 8, 9, 12, 17, 17 and 19 days. The smaller doses proved non-lethal even after 37 days' treatment.

The daily urine examination suggested that there was some very slight renal damage with the largest doses of both substances, there being present after the 1·0 and 0·5 gm. doses slight traces of albumin and minute traces of blood-pigment. No pus-cells, casts or blood-cells were seen in any animal and only a few squamous epithelial cells.

The fæces of the rats receiving the 1·0 gm. dose showed a trace of blood-pigment.

At intervals during the investigation animals were killed, and the liver, spleen, kidneys, heart, stomach and intestines removed for histological examination. The organs of those rats which died were also examined. This examination showed a very slight sub-epithelial infiltration of the mucosa of the stomach and duodenum in the rats receiving the large doses of both substances,although the damage was so slight as to be almost negligible. The kidneys of the animals which died following the daily dose of 1·0 gm. per kg. of amyl-meta-cresol showed very slight damage to the tubules, and a little more damage was observed following the same dose of hexyl resorcinol.

ABSORPTION AND ELIMINATION.

The fact that amyl-meta-cresol can be taken over long periods in very large doses without harmful effect suggested that it must be rapidly absorbed and eliminated. To get some direct evidence on this point, a number of rats were given, by stomach-tube, a dose of 250 mgm. in 25 per cent. aqueous emulsion. Then at intervals varying from 5 minutes to 96 hours, two or three of these animals were killed, the stomach and intestines immediately removed and their amyl-meta-cresol content estimated chemically.

The method of estimation, which was devised by Mr. G. F. Hall, B.Sc., is as follows :

Shred the stomach and intestines finely with scissors, wash into a steam distillation apparatus and steam distil until the fluid passing over is no longer opalescent. This requires 500–800 c.c. of distillate. Add to the distillate 1 c.c. N/1 NaOH per 100 c.c. to retain the fatty acids and extract four times with 75 c.c. of petrol ether. Wash combined extracts with 25 c.c. of water and extract this wash-water with petrol ether. Evaporate the combined petrol ether extract at 30–40° C. to about 5 c.c., at which volume complete the evaporation *in vacuo* to constant weight of amyl-meta-cresol.

It was found that rapid absorption and elimination had taken dlace, less

than 50 per cent. of the amyl-meta-cresol administered remaining at the end of 24 hours, and less than 10 per cent. at the end of 70 hours. The average values obtained by using a large number of animals are shown graphically in Fig. 1. This result is of particular importance when it is remembered that amyl-meta-cresol promises to be a useful urinary antiseptic, and it demonstrates the necessity of repeated doses at frequent intervals if it is desired to maintain a high concentration in the urine.

Separate analyses of the stomach, small and large intestines showed that the absorption was principally from the stomach.

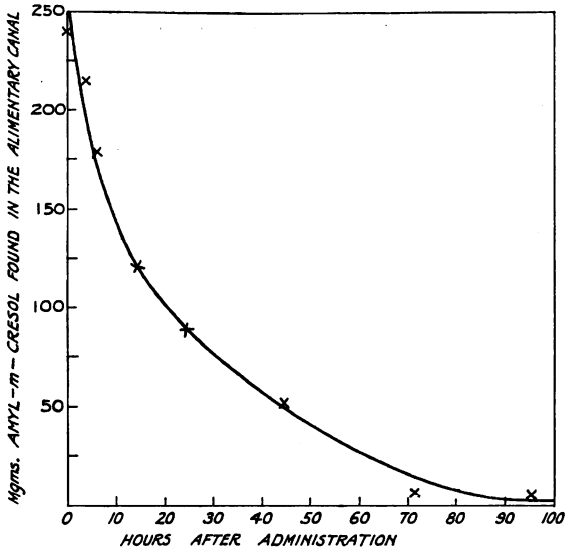

FIG. 1.—The rate of absorption of amyl-meta-cresol from rats' alimentary canal. Dose 250 mgm.

INHALATION.

In the previous paper Coulthard reports the action of the vapour of amyl-meta-cresol on various organisms. The plate cultures discussed by him were exposed to the vapour in a fume chamber, which also contained a number of rats in an open wire cage. The fume chamber had a capacity of 6 cubic feet, and the amyl-meta-cresol was vaporized in an open dish over a small bunsen flame at a standard rate of 0·5 c.c. in 60 minutes. Rats were exposed to this atmosphere for ½, 1 and 2 hours daily for periods of 5, 10, 20 and 30 days. The animals were killed and the lungs examined microscopically. The lungs from one of the animals exposed to the largest dose for 30 days showed a thickening of the alveolar walls, and in one other animal from the same group there was evidence of a very slight inflammation of the bronchial tubes. The lungs from the rest of the animals showed no abnormality.

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.

My thanks are due to Prof. E. C. Dodds, of the Courtauld Institute of Biochemistry, for his advice and for carrying out the histological examinations; to Dr. Pyman for his advice and criticism, and to my colleagues in the Pharmacological Department, Boots Pure Drug Co. Ltd., who have assisted at various stages in the work.

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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