

9. The amino acids appear to act as auxoamylases toward the pancreatic enzyme also. Hence the amino acids produced in the intestine by digestive proteolysis will act as hormones in starch digestion and this factor should be taken into account in the study of normal digestion.

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## PREPARATION OF A PRESERVATIVE FROM CRESOL.

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Schering's "Trikesol" has been extensively used in this country as a preservative for biological products such as antitoxins, vaccines, etc. Since the beginning of the European war, however, no more "Trikesol" being available, our laboratory has been compelled to find a substitute of the same quality.

Commercial cresol is a mixture of all three isomeric cresols—ortho, meta and para. The boiling point of *o*-cresol is 188–190.8°, *m*-cresol 201–202.8°, and *p*-cresol 198–201.8°. Nördlinger<sup>1</sup> states that *o*-cresol is less poisonous than its isomers. Working on the supposition that the lower fractions of commercial cresol contained mostly *o*-cresol, we prepared a product with the boiling point 190–193° from Cresol U. S. P. The toxicity of this preservative, which until recently was used in our laboratory in the place of "Trikesol," was 0.00045 g. per body weight of a white mouse and the germicidal power was 1 : 150 in 5 minutes and 1 : 180 in 10 minutes.

In order to get a more desirable product and to confirm our supposition as to the toxicity of different fractions of cresol we decided to subject commercial cresol to a more thorough fractional distillation and to investigate the different fractions as to their toxicity and germicidal power.

Commercial cresol was distilled over a free flame and the distillate collected while the thermometer in the vapor recorded 188–205°.

**First Fractional Distillation.**—1000 cc. of this product were distilled, giving:

Fractional distillate.	Boiling point.	Quantity in cc.	Specific gravity.
1.....	188–192°	25	...
2.....	192–196°	250	1.039
3.....	196–199°	400	1.035
4.....	199–204°	250	1.030

Owing to the small amount of the first distillate no further work was done with it.

<sup>1</sup> Allen's "Commercial Organic Analysis," 3, 313.

**Second Fractional Distillation.**—The fractions 2, 3, and 4 were separately redistilled.

200 cc. of Fraction 2 gave, after a second distillation, 130 cc. of material with a boiling point of 192–196° and a specific gravity of 1.040 at 25°. The residue had a higher boiling point.

Fraction 3 gave, after a second distillation:

80 cc. with a boiling point 192–196° and sp. gr. 1.039 at 25°.

85 cc. with a boiling point 196–199° and sp. gr. 1.034 at 25°.

Fraction 4 gave, after a second distillation:

20 cc. from 192–196°.

80 cc. from 196–199°, sp. gr. 1.033 at 25°.

90 cc. from 199–204°, sp. gr. 1.028 at 25°.

**Third Fractional Distillation.**—After the third distillation 200 cc. of the fraction from 192–196° were again distilled. 125 cc. went over from 192–196°. This fraction, which we designated as Fraction A, had a specific gravity of 1.041.

After a third redistilling, 200 cc. of the fraction with the boiling point of 196–199° gave 110 cc. of a product with a boiling point of 196–199° and sp. gr. of 1.034 at 25°. This fraction was called Fraction B.

After a third distillation the fraction with the boiling point of 199–204° gave 125 cc. of cresol with a boiling point of 199–204° and sp. gr. 1.030 at 25°. This fraction was called Fraction C.

These three fractions were then tested for their toxicity and germicidal action as compared with "Trikesol" of Schering and with phenol.

#### Toxicity Test.

Considering the fact that these preservatives were to be used for biological products to be inoculated into the human body, we considered it most necessary to accurately determine their toxicity. After consulting the literature, we came to the conclusion that the method used by Hale<sup>1</sup> was the most desirable.

Hale's method consisted in injecting white mice subcutaneously with the same volume of an aqueous solution of the preservative, but of a different concentration, the weight of the mouse being not less than 15 g. and not more than 30 g. The dose was figured out per g. body weight of the animal.

In our tests it frequently happened that animals of the same lot and approximately of the same weight, receiving the same dosage, varied in resistance to the toxic action of the preservative. It was therefore necessary to use many animals for the same dosage to form an estimate from the majority.

The results of the test can be seen on Table I.

<sup>1</sup> Hyg. Lab., *Bull.* 88.

TESTS OF TOXICITY OF DISINFECTANTS.  
Tested on White Mice by Subcutaneous Injection of 1 cc. Dilution.

Disinfectant.	Mouse weight in g.	Dose per gram body weight, cc.	Total amount of pure disinfect. used.	Results.
Phenol.....	22	0.00035	0.0077	Lived
	26	0.0004	0.0104	Died in 1 hr.
	26	0.00045	0.0117	Died in 1/2 hr.
	26	0.0005	0.0130	Died in 8 hrs.
Trikesol (Schering).....	18	0.0003	0.0054	Lived
	24	0.0004	0.0096	Lived
	27	0.00045	0.0121	Died in 20 min.
	27	0.0005	0.0135	Died in 15 min.
Cresol, Fraction A, 192-196°.	17	0.0002	0.0034	Lived
	18	0.00025	0.0045	Lived
	20	0.0003	0.0060	Died in 30 min.
	22	0.0003	0.0066	Lived
	18	0.0003	0.0054	Died in 1 hr.
	19	0.0003	0.0057	Lived
	24	0.00035	0.0084	Died in 30 min.
	29	0.0004	0.0116	Died in 20 min.
Fraction B, 196-199°.....	19	0.0002	0.0038	Lived
	19	0.00025	0.0047	Lived
	22	0.0003	0.0066	Died in 1 hr.
	23	0.0004	0.0092	Died in 1 hr.
Fraction C, 199-204°.....	19	0.0003	0.0057	Lived
	22	0.00035	0.0077	Lived
	24	0.0004	0.0096	Died in 22 hrs.
	24	0.0004	0.0096	Died in 1/2 hr.
	23	0.0004	0.0092	Died in 1 hr.

The phenol control showed a toxicity of 0.0004.

Since the toxicity is estimated as the least amount per gram body weight killing a white mouse within 24 hours, we found the toxicity of Fractions A and B to be 0.0003 (phenol toxicity coefficient 133.33), of Fraction C to be 0.0004 (phenol toxicity coefficient 100), of "Trikesol" (Schering) to be 0.00045 (phenol toxicity coefficient 88.88). As can be seen, the highest boiling fraction was the most favorable from the point of toxicity.

The standard method in general use for the determination of the phenol coefficient is that of McClintic.<sup>1</sup> This method calls for the use of a 24-hour culture of *B. typhosus* (Hopkins) and beef extract broth.

The method used in our laboratory differs slightly from that of McClintic, namely beef infusion broth is used for the seeding tubes as it offers a more favorable medium for the growth of bacterial in general—and *B. coli* is the test organism, on account of its being more generally present as a contaminant and also on account of its greater resistance to the action of germicides.

The results of the tests are seen in Table II.

<sup>1</sup> Hyg. Lab., Bull. 82.

TABLE II.  
Germicidal Tests of Disinfectants.

Disinfectant.	Dilution.	Exposure of culture to disinfectant.				
		2 <sup>1</sup> / <sub>2</sub> min.	5 min.	7 <sup>1</sup> / <sub>2</sub> min.	10 min.	15 min.
Phenol.....	I : 70	—	—	—	—	—
	I : 80	+	—	—	—	—
	I : 90	+	+	+	—	—
	I : 100	+	+	+	+	—
	I : 110	+	+	+	+	+
	I : 120	+	+	+	+	+
Trikresol.....	I : 180	—	—	—	—	—
	I : 200	+	+	+	+	—
	I : 225	+	+	+	+	—
	I : 250	+	+	+	+	+
	I : 300	+	+	+	+	+
Fraction A.....	I : 150	—	—	—	—	—
	I : 180	—	—	—	—	—
	I : 200	+	+	—	—	—
	I : 225	+	+	+	+	+
	I : 250	+	+	+	+	+
Fraction B.....	I : 150	—	—	—	—	—
	I : 180	—	—	—	—	—
	I : 200	—	—	—	—	—
	I : 225	+	+	+	—	—
	I : 250	+	+	+	+	+
Fraction C.....	I : 250	—	—	—	—	—
	I : 180	—	—	—	—	—
	I : 200	—	—	—	—	—
	I : 225	+	+	—	—	—
	I : 250	+	+	+	+	+

*B. coli* 24-hr. culture used.

48-hour readings.

+ = growth.

— = no growth.

The coefficient of a disinfectant is the figure that represents the ratio of the germicidal power of the disinfectant to the germicidal power of phenol, both having been tested under the same conditions. This is determined by the figure representing the dilution of the weakest strength of the disinfectant that kills within 2<sup>1</sup>/<sub>2</sub> minutes, being divided by the figure representing the degree of dilution of the weakest strength of the phenol control that kills within the same time. The same is done for the weakest strength that kills in 15 minutes. The mean of the two is the coefficient. Following this technique (see Table II), we estimated the phenol coefficient of the disinfectant as follows:

“Trikresol” (Schering).....	2.41
Fraction A.....	2.28
Fraction B.....	2.55
Fraction C.....	2.55

As can be seen, Fractions B and C show a germicidal coefficient slightly higher than "Trikrisol" (Schering), and Fraction A is lower.

#### Summary and Conclusions.

Purified and redistilled cresol can be used as a preservative for biological products. The fraction from 199–204°, with a sp. gr. of 1.030 at 25°, is the best for this purpose. It has the same toxicity as phenol, only slightly lower than "Trikrisol," but a germicidal coefficient of 2.55, which is higher than that of "Trikrisol."

Investigations are under way as to the practical value of preservatives in immune sera and purified antitoxin.

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#### NEW BOOKS.

**Practical Chemistry for Medical Students.** By ALEXANDER CHARLES CUMMING. Second edition. 165 + 8 pages. Edinburgh: James Thin, 1917.

The first edition of this little book was published in 1911. It contains a series of simple experiments in general chemistry, some work in qualitative analysis, a few pages on volumetric analysis and a very few experiments on a number of physiologically important substances.

There is nothing in the book which would indicate that it is especially suited to the needs of medical students.

J. H. LONG.

**Palotay's Chemistry and General Science Chart.** By JULIUS A. PALOTAY. Published by the author, 405 So. Hill St., Los Angeles, California. Price, \$3.00, postpaid.

This wall-chart consists of a table of the common elements with their physical constants and properties, confirmatory qualitative tests, etc.; miscellaneous anatomical and physiological data; and a collection of odds and ends such as the following: "coal, which is chiefly carbon;" "fluorine—has a green flame, dissolves glass;" "color-heat and light-producing waves;" "gaseous state—has absolutely no self-subsistent figure;" hardness—resists force that passes between their particles."

H. G. DEMING.