It is believed that the "N" group represents the maximum intensity of color which good quality oils on the American market possess. A "Co–Fe" blend was accordingly prepared to match this color. Curve No. 3 shows the transmission of this blend, which consists of 68 cc. of $M/6$ $FeCl_3 \cdot 6H_2O$, 3.5 cc. of $M/4$ $CoCl_2 \cdot 6H_2O$ and 48.5 cc. of distilled water.

If this standard is adopted, the directions for matching should be similar to that specified under cod liver oil.

III. CASTOR OIL.

The U. S. P. X specifies that castor oil shall be a pale yellowish or almost colorless transparent liquid. The majority of the nine oils studied were practically colorless.

Figure 3 shows the curves of the five distinct hues into which these oils could be grouped.

As before, the directions for matching should be stated as under cod liver oil.

SUMMARY.

1. Fifty-four samples of cod liver oil were examined and color standards suggested for a lighter and a darker product.
2. Eleven samples of almond oil were examined and a standard proposed for maximum permissible color.
3. Nine samples of castor oil were examined and a standard proposed for maximum permissible color.
4. Directions have been given for preparing the permanent color standards, making use of the Arny "Co–Fe–Cu" color system.

DEPARTMENT OF CHEMISTRY,
COLLEGE OF PHARMACY,
COLUMBIA UNIVERSITY.
August 1932.

THE BACTERICIDAL EFFICIENCY AND TOXICITY OF CREOSOTE AND ITS COMPONENTS.*

BY LOUIS GERSHENFELD, RALPH PRESSMAN[1] AND HORATIO C. WOOD, JR.

The purpose for which this study was undertaken may be stated as follows: (1) to determine the variation in bactericidal power of creosote, (2) to determine whether the standards mentioned in the U. S. Pharmacopœia for specific gravity and boiling point are an indication of the therapeutic value of the drug, (3) to determine whether creosote depends largely for its therapeutic efficiency on the guaiacol and creosol as is implied in the official definition, (4) to determine if there are any one or two constituents to which the value of the drug may be justly attributed and (5) to determine whether different samples of creosote vary in their toxicity and if so can the variation be attributed to any special constituent.

---

* Scientific Section, A. PH. A., Toronto meeting, 1932.

[1] Maltbie Chemical Co., Research Fellow, Philadelphia College of Pharmacy and Science, 1929–1931.

The medicinal uses of creosote are, as far as we know, attributable to two properties, namely, its bactericidal power and its expectorant action. Various authors have attributed the value of the drug in tuberculosis to its anti-bacterial properties, but most recent authorities believe that it is highly improbable that the quantities employed therapeutically can exert any antiseptic action in the lungs and are inclined to assign such benefit as may result from its use to some expectorant effect. As we know of no method for the quantitative estimation of expectorant action we have used entirely the bactericidal potency as the criterion of efficiency.

Creosote, being the product of a rather crude chemical process (the destructive distillation of wood), it is to be expected that its composition will vary considerably according to the sort of wood from which it is obtained and the conditions of its manufacture. Sickman and Fischelis (1) in the examination of 16 commercial samples found considerable variations in the physical properties and in the proportion of methoxyl phenols (the methoxyl content represents the combined guaiacol and creosol).

Bactericidal Powers.—Éwe (2) reported that creosote has a phenol coefficient (by Hygienic Laboratory) of 3.62. Sternberg (3) found 1:200 killed micrococci of pus in 2 hours. Guttman (quoted by Sternberg) states that 1:300 killed *B. pyocyan.* and *B. anthracis* in 1 minute. Marfori (4) mentions that guaiacol, 1%, kills *B. tubercul.* in 2 hours and many other bacteria in 20 to 30 minutes. DeWitt, Benyenga and Wells (5) found that 0.5% of creosote destroyed all tubercle bacilli in one hour and 0.1% in 24 hours. Guttman reached the conclusion that creosote is definitely superior to phenol in antiseptic action.

While the literature leads to the conviction that creosote has a stronger bactericidal action than carbolic acid, there is singularly little definite information on the exact disinfectant properties of creosote, and still less of its variability.

Experimental.—Our own tests were made by the Hygienic Laboratory Method[1] employing as test organisms *B. typhosus* and *Staphylococcus aureus.* Twenty-four-hour growths which have been transplanted for at least three consecutive days were used. The procedure was as follows: Into 5 cc. of an aqueous solution of the sample of designated dilution introduce 0.1 cc. of a filtered bouillon culture of organisms (24 hours old); shake and allow to remain in contact with the sample. The test is carried out in a standard water-bath at 20 degrees C. At intervals of $2^1/_2$, 5, $7^1/_2$, 10, $12^1/_2$ and 15 minutes a standard loopful (4 mm. diameter) of this mixture is transplanted into a test-tube containing 10 cc. of sterile bouillon. The transplants are incubated at body temperature for forty-eight hours. Table I gives the details of tests of one sample.

TABLE I.—SHOWING DETAILS OF TEST OF PHENOL COEFFICIENT OF A SAMPLE OF CREOSOTE.

Time of Exposure.		$2^1/_2$.	5.	$7^1/_2$.	10.	$12^1/_2$.	15.
	1:100	–	–	–	–	–	–
Phenol	1:110	+	–	–	–	–	–
Dilution	1:120	+	+	–	–	–	–
	1:130	+	+	+	–	–	–
	1:140	+	+	+	+	+	+

[1] This research was begun before the publication of the recent change in technique (using 0.5 cc. of test organism culture) and it was deemed advisable to continue by the original technique so that results would be more accurately comparable.

TABLE I.—SHOWING DETAILS OF TEST OF PHENOL COEFFICIENT OF A SAMPLE OF CREOSOTE. (*Continued.*)

Creosote	1:390	−	−	−	−	−	−
Sample No. 16	1:400	+	−	−	−	−	−
	1:410	+	−	−	−	−	−
	1:420	+	+	+	−	−	−
	1:430	+	+	+	+	+	+

Weakest dilution of phenol killing in $2^1/_2$ minutes: 1:100
Weakest dilution of creosote killing in $2^1/_2$ minutes: 1:390
Weakest dilution of phenol killing in 15 minutes: 1:130
Weakest dilution of creosote killing in 15 minutes: 1:420

$$\frac{\frac{390}{100}+\frac{420}{130}}{2} = 3.56.$$ Phenol coefficient, 3.56.

Studies were made on seventeen samples of creosote. All samples except No. 14 (which was a semi-refined creosote) and No. 16 (which was marked B. P. 1882) were offered as U. S. P. In most instances simultaneous tests were made with phenol, so that the results could be given as "phenol coefficients."

The phenol coefficients for the 17 samples ranged between 2.2 and 3.9 for both organisms, with an average of 3.0. The variation in strength is not the same for the two species of bacteria; that is, a sample of creosote might be relatively potent against the typhoid bacillus but less efficient against the *Staphylococcus aureus* or vice versa. There is, however, in the majority of cases a considerable degree of similarity in the two sets of tests. The strongest sample showed a coefficient of 3.9 for the typhoid bacillus and 3.8 for the *Staphylococcus aureus;* the weakest gave 2.45 for both species of bacteria. The widest discrepancy was found in a sample of semi-refined creosote which showed a coefficient of 3.8 for the *Staphylococcus aureus* but only 3.0 for the typhoid bacillus.

TABLE II.—SHOWING THE RELATION BETWEEN BACTERICIDAL POWER AND PHYSICAL CONSTANTS.

Phenol Coefficient. *B. typh.*	*Staph. aureus.*	Specific Gravity.	Per Cent Distilling at 200–210° C.	210–220° C.
3.9	3.8	1.073	37	45
3.6	3.0	1.075	54	30
3.4	3.0	1.077	55	36
3.15	2.2	1.078	56	37
3.1	3.4	1.081	50	42
3.05	3.0	1.079	55	36
3.0	2.45	1.077	57	34
3.0	2.4	1.075	53	39
3.0	3.8	1.083	32	49
2.95	3.1	1.082	10	74
2.8	3.25	1.076	46	41
2.8	2.5	1.078	36	55
2.75	2.6	1.083	46	44
2.6	3.3	1.080	50	42
2.6	2.45	1.080	23	64
2.45	2.45	1.083	57	37

Sixteen of these samples were those whose physical constants were reported by Sickman and Fischelis.[1] A study of these physical constants in comparison

[1] We desire to express our thanks to Dr. Robert Fischelis for his coöperation.

with disinfectant properties failed to show any constant relationship. Table II, arranged in descending order of efficiency against the typhoid bacillus, shows also the *Staphylococcus aureus* phenol coefficient and the specific gravity and distilling temperatures as reported by Sickman and Fischelis.

Although a comparison of these samples of creosote failed to show any consistent relationship between the proportions distilling at a given temperature and the bactericidal powers, it was thought advisable to study more carefully the comparative effects of the fractional distillates. This we did on 2 samples of creosote which were fractionated for us by Mr. Sickman. One of these was made up of fractions distilling between 200° C. and 220° C. and the other of fractions distilling from 187° C. to 225° C. (the U. S. P. states that creosote "begins to distil at about 200° C."). The phenol coefficients of these samples against both test organisms are given in the following tables.

TABLE III.—CONCENTRATION OF FRACTIONAL DISTILLATES OF CREOSOTE (FIRST SAMPLE) REQUIRED TO KILL BACILLUS TYPHOSUS.

Fraction (° C.).	Kills in $2^1/_2$ Min.	Kills in 15 Min.	Fraction (° C.).	Kills in $2^1/_2$ Min.	Kills in 15 Min.
200–201°	1:250	1:300	210–211°	1:200	1:250
201–202°	Not tested		211–212	1:200	1:250
202–203	Not tested		212–213	1:200	1:250
203–204	1:200	1:250	213–214	1:250	1:300
204–205	1:200	1:250	214–215	1:250	1:300
205–206	1:200	1:250	215–216	1:250	1:300
206–207	1:150	1:200	216–217	1:250	1:350
207–208	1:150	1:200	217–218	1:250	1:350
208–209	1:150	1:200	218–219	1:300	1:350
209–210	1:150	1:200	219–220	1:300	1:350

Second Sample.

Distilling at (° C.).	Phenol Coefficients. *B. typh.*	Phenol Coefficients. *Staph. aureus.*	Distilling at (° C.).	Phenol Coefficient. *B. typh.*	Phenol Coefficient. *Staph. aureus.*
Below 187	2.25	1.7	208–210	2.75	2.7
187–195	2.4	1.9	210–213	2.75	2.9
195–200	2.5	2.1	213–216	2.75	2.9
200–202	2.5	2.1	216–218	2.75	2.9
202–204	2.25	1.9	218–220	2.75	2.9
204–206	2.4	2.1	220–225*	3.2	3.5
206–208	2.5	2.3	Whole Creosote	3.2	3.0

* The residue, not distilling at 225° C. which represents about 8.5 per cent of the whole creosote, was not tested because of its insolubility.

While there are some minor divergences in the two series, it will be noted that there is in both of them an obvious indication that the most active fractions are those boiling above 210° C. The greater activity of the fractions between 190° C. and 202° C., as compared to those of a few degrees higher, we believe may be attributed to the presence of cresols, which boil at these temperatures.

Unfortunately, we did not have any samples of the original creosote from which the fractions in No. 1 were obtained, the whole quantity having been used in the distillation. In No. 2, however, the whole creosote showed a phenol coefficient for both bacteria of 3.2 which is higher than any of its fractions distilling below 220° C. The residue left after distillation at 225° C. was so insoluble that we could not accurately determine its phenol coefficient.

Is Guaiacol the Chief Constituent?—The Pharmacopœia defines creosote as "a mixture of phenols principally guaiacol and creosol." The investigations of Sickman and Fischelis have shown that from a chemical standpoint this statement is somewhat exaggerated. They found that in 16 samples the methoxyl content ranged from 10.98 to 16.30% with an average of 12.92%. If all the methoxyl compounds are calculated as guaiacol this would correspond to an average guaiacol content of 51.7%. It seems to us inaccurate to speak of a mixture of which approximately one-half is guaiacol as consisting "principally" of guaiacol.

DeWitt, Benyenga and Wells report that guaiacol is about one-half as efficient a bactericide for the tubercle bacillus as creosote. Kuprianow (6) found that guaiacol was not only inferior to creosote but also to carbolic acid in its bactericidal powers. We have not been able to find any bacteriologic studies of creosol.

The Pharmacopœia recognizes two forms of guaiacol, one which is derived from creosote, occurring as a liquid; and the other, which is crystalline, is manufactured synthetically. Obviously, the liquid natural guaiacol is not as pure as is the synthetic. We have tested the bactericidal efficiency of a commercial liquid guaiacol, a synthetic crystalline guaiacol obtained from Eastman Kodak Company and a synthetic creosol, made by Sickman. The following table shows the results.

	Phenol Coefficient.	
	B. typh.	*Staph. aureus.*
Natural guaiacol (liquid)	1.62	1.7
Synthetic guaiacol (crystals)	1.05	1.1
Creosol (synthetic)	1.4	0.7

It will be noted in this table that the natural is about half as active as the average commercial creosote and that the purer, synthetic, guaiacol is only about $^1/_3$ as efficient as creosote. Creosol has about the same disinfectant powers as guaiacol.

It is obvious that the bactericidal action of creosote does not depend principally either on guaiacol or creosol.

The Other Constituents of Creosote.—Having found that guaiacol and creosol were not the most active constituents we have tested a considerable number of the other ingredients in the hope of finding one with outstanding bactericidal properties. A study of the efficiency of the fractional distillates indicates that the most active constituents are those with a boiling point of 215° C. or over. An exception to this was noted in one set of distillates examined in which the fractions below 202° C. were very active. These low boiling fractions are composed chiefly of cresol.

The following table shows the phenolic bodies generally recognized to be components of creosote together with the boiling points given in the International Critical Tables:

	° C.		° C.
Cresols	191–203	Dimethyl-guaiacol (traces)	230
Guaiacol	205	Xylenols (1:3:4 and 1:3:5)	218, 219
Ethyl-phenol (1:2)	207	Pyrogallol derivatives	265–290
Ethyl-guaiacol (1:3:4)	?	Coerulignol (traces)	241
Creosol	222	Catechol	245

Of these compounds the germicidal efficiency of cresol is too well established to need further study; and we have reported above on the activity of guaiacol and creosol. Of the other components we were unable to obtain either ethyl-phenol[1] or dimethyl-guaiacol. We were kindly furnished by Mr. Sickman samples of the 4-ethyl-guaiacol, 4-*n*-propyl-guaiacol (this is supposed to be the same as the somewhat mysterious "coerulignol"), and butyl-guaiacol. In addition to these we were able to purchase from the Eastman Co. samples of three of the dimethyl-phenols including one of those reported in creosote.

Klarmann, Shternov and von Wowern (7) have reported a phenol coefficiency for xylenol (not stating which one of the isomers they tested) of 5 for *B. typhosus*, and 5.6 for *Staph. aureus;* this agrees closely with our results given in the following table:

Sample.	Phenol Coefficient. *B. typhosus.*	Phenol Coefficient. *Staph. aureus.*
1,2,4-Dimethyl-phenol	5	5
1,2,5-Dimethyl-phenol	5	5
1,3,5-Dimethyl-phenol	3	3
4-Ethyl-guaiacol	6.1	5.1
n-Propyl-guaiacol	8.5	1.07
4-*n*-Butyl-guaiacol	15	1.07

While we did not know of any study indicating the proportion of these phenols occurring in creosote it seems to us that our results suggest very strongly that the superiority of creosote over guaiacol and creosol is probably attributable to the xylenols and the ethyl guaiacol; if one can transfer the figures for para-ethyl-phenol to the ortho compound this would also enhance the bactericidal activity of creosote. Propyl-guaiacol occurs in creosote in too small an amount to be a factor, indeed the U. S. Pharmacopœia gives a test to insure its absence. Butyl-guaiacol has not been reported as a constituent of creosote.

Toxicity.—Tests of the toxicity of (1) two samples of creosote, (2) of guaiacol (both natural and synthetic) and (3) of various fractional distillates of creosote were made upon guinea pigs. The method employed was to determine the M. L. D. by intraperitoneal injections of a 10% solution of the sample in sterile olive oil.

The M. L. D. of two samples of creosote was 0.54 and 0.55 cc. per kilo, respectively; the guaiacols, synthetic and natural, were fatal in doses of 0.55 per kilo. The M. L. D. of the fractional distillates are shown in the following table:

B. P. (° C.)	M. L. D. Cc. per Kilo.	B. P. (° C.)	M. L. D. Cc. per Kilo.	B. P. (° C.)	M. L. D. Cc. per Kilo.
187° C.	0.2–0.3	207–210	Not tested	216–218	0.5–0.6
188–195	0.4–0.5	210–213	0.5–0.6	218–220	0.5–0.6
200–202	0.4–0.5	213–216	0.5–0.6	220–225	0.5–0.6
204–206	0.4–0.5			Whole creosote	0.55

It will be noted that the low boiling fractions are more toxic than the high boiling fractions. Attention may be called to the fact that the fraction distilling at 187° C. (which is below the U. S. P. limit) is twice as poisonous as the whole creosote.

When we compare the bactericidal and toxic powers of the various fractions, the comparative advantages of the higher boiling portions become quite evident.

[1] Coulthard, Marshall and Pyman (6) have reported a phenol coefficiency of 7.5 for para-ethylphenol. Although it is the ortho-ethylphenol which occurs in creosote it is probable that the germicidal powers of the isomers are not widely different.

If we make a table of what we might call "therapeutic efficiency," assuming that the therapeutic value is due to the disinfectant properties, by multiplying the phenol coefficient by the fatal dose (P. C. × M. L. D.) we have the following figures:

TABLE IV.

B. P. (° C.)	Efficiency.	B. P. (° C.)	Efficiency.	B. P. (° C.)	Efficiency.
187° C.	0.56	204–206	1.08	210–219	1.51
188–195	1.08	206–208	1.25	220	1.76
195–202	1.12	208–210	1.37	Entire	1.76
202–204	1.01				

These figures seem to us to indicate strongly that the fractions of creosote boiling above 206° C. are decidedly preferable to the lower boiling fractions, at least as far as it concerns their disinfectant properties.

CONCLUSIONS.

1. Creosote is an active bactericide. The phenol coefficients of various samples ranged from 2.4 to 3.9 for both *Bacillus typhosus* and *Staphylococcus aureus*, with an average of 3.2.
2. Both guaiacol and creosol are less than half as powerfully bactericidal as creosote.
3. No reliable criterion of bactericidal power was observed in any physical property but the high boiling fractional distillates (215–220° C.) were the most efficient.
4. The minimal lethal dose for the guinea pig of either creosote or guaiacol is about 0.55 cc. per kilo intraperitoneally. The high boiling fractions are less toxic than those distilling below 210° C.
5. The dimethyl-phenols (xylenol) and ethyl-guaiacol are more active bactericides than either creosote or guaiacol. It is suggested that the antibacterial efficiency of the high boiling fractions of creosote is due largely to these compounds.
6. Propyl-guaiacol and butyl-guaiacol are very efficient against the *typhoid bacillus* but decidedly less active than creosote against *Staphylococcus aureus*.
7. We believe the quality of creosote would be improved by raising the temperature at which it begins to distil.

REFERENCES.

(1) Sickman and Fischelis, JOUR. A. PH. A., 18 (1929), 1145.
(2) Ewe, "Proceedings of Pennsylvania Pharmaceutical Association," 41, 166.
(3) Sternberg, "Textbook of Bacteriology."
(4) Marfori, *Chemisches Centralblatt*, 1890, page 155.
(5) DeWitt, Benyenga and Wells, *J. Infectious Diseases*, 28 (1920), 115.
(6) Coulthard, Marshall and Pyman, *J. Chem. Soc.* (Feb. 1930), 280.
(7) Klarmann, Shternov and von Wowern, *J. Bact.*, 17 (1929), 423.

The University of North Carolina has secured the W. W. Ashe Herbarium, a collection very valuable for its large number of type specimens from the southeastern states. William Willard Ashe (1872–1932), a graduate of the University of North Carolina and Cornell University, was the first forester employed by the state of North Carolina. The acquisition of the Ashe Herbarium by the University of North Carolina was made possible through the generosity of George Watts Hill, of Durham, N. C.