short-lasting exposure with rapid excessive rise in temperature. This observation would indicate that some time must elapse before the cells of the various parenchymatous organs show evidence of damage, and that fever over a brief period is apparently not very harmful to those cells although it produces death of the organism from some other cause. The difference in the cellular changes occurring in animals exposed to fatal and non-fatal doses of fever is very striking. No parenchymatous necrosis or replacement fibrosis was observed in animals who survived repeated short-lasting exposures to 106.5°. Since this is the condition we are meeting daily in the fever therapy of the human, we may emphasize the rather encouraging conclusions which can be drawn from these observations.

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Calcium Creosotate: V. Nature of Phenols Eliminated in Urine.*

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Investigation[1-4] of calcium creosotate appeared desirable on account of its admission to U.S.P. XI without published reports on its clinical and experimental behavior. Volatile phenols could not be detected in sputum of hospitalized patients or in expired air of rabbits after oral administration of calcium creosotate.[2] It has been found, however, that calcium creosotate phenols are eliminated in rabbit urine[1] and also in human urine[2] after oral administration. Although calcium creosotate is advocated as a urinary antiseptic no clinical or experimental data have been found in the literature to indicate that its administration by mouth produces a bacteriostatic urine. It seemed, therefore, that the ability of *calcium creosotate* to produce a bacteriostatic urine, and the *form* in which it is eliminated should be investigated; especially since Knapp[5] reported that

* I wish to express my thanks to Dr. A. E. Livingston for the helpful suggestions received during these experiments.

1 Fellows, *J. Pharm and Exp. Therap.*, 1937, **60**, 183.

2 Fellows, *Am. J. Med. Sci.*, 1939, **197**, 683.

3 Fellows, *J. Pharm. and Exp. Therap.*, 1937, **60**, 178.

4 Fellows, *J. Am. Pharm. A.*, 1939, **26**, 609.

5 Knapp, *Schweiz. Wchschr.*, 1911, **49**, 229, 245, 257.

creosote was not eliminated as free phenolic material but largely in the form of glycuronates and ethereal sulfates. Statements have not been found in the literature concerning the *nature* of the phenolic material appearing in urine after creosote administration. It appeared desirable to determine if creosote was eliminated, as such, in urine, particularly in view of experiments[4] which have suggested the possibility that certain of its constituent phenols might be more readily destroyed in the body than others.

Experiments first were carried out in which large single doses of calcium creosotate were given to rabbits and urine specimens withdrawn at 1-hour intervals for 10 hours. On examination all of these specimens were found to be devoid of bacteriostatic activity. Normal human subjects were given single doses of calcium creosotate varying from 2 to 8 0.25 g tablets. Also a group of 8 normal individuals, after control urine specimens had been collected, received 2 0.25 g tablets every 2 hours during the waking period for 3 days. Urine specimens were collected every 2 hours throughout the day. The plates poured from inoculated urine specimens collected during the period of drug administration failed to show evidence of diminished growth when compared with those poured from inoculated specimens before the drug was given. The method of Davis and White[6] was used in testing urine specimens for bacteriostatic action. The test organisms used were *S. aureus* and *E. typhi.*

The following experiments were carried out to determine the amounts of free as compared with the amounts of conjugated phenols appearing in urine after administration of calcium creosotate. Twelve male rabbits were maintained in metabolism cages on a constant daily diet and 24-hour urine specimens collected. The 24-hour urine specimens were measured and filtered. Two cc portions of the filtrate were treated with 15 cc of diazotized paranitraniline reagent and allowed to stand several minutes. Two cc of 2% acacia solution were added, the mixture was made alkaline by the addition of 10 cc of 7% NaOH solution and the volume adjusted to 100 cc with distilled water. A known creosote solution was treated in the same manner and the urine specimens compared with it in a colorimeter. The results obtained during these experiments have been summarized in Fig. 1. Milligrams of phenols, in terms of creosote, found in the 24-hour urine specimens have been plotted against days of excretion. The first part of the figure (A to B) shows the values obtained before administration of calcium creosotate. At B the first dose of calcium creosotate was adminis-

[6] Davis and White, *J. Urol.*, 1918, **2**, 107.

tered to 8 of the 12 rabbits in the series. The drug was given orally in a solution containing either 0.25 g or 0.5 g of phenols. On the first day all but one of the animals receiving the drug were given the 0.25 g dose, the other received 0.50 g. The next day 6 of the animals received the 0.25 g dose and 2 received 0.50 g. This procedure of decreasing the number of animals receiving the 0.25 g dose and increasing the number receiving the 0.5 g dose was continued until the eighth day of drug administration (C) when all animals except one were given the 0.5 g dose. It is to be noted that only a very slight increase in the free phenols excreted could be detected during this period (B to C). These determinations were all made on *non-hydrolyzed* urine specimens. After a 3-day control period (C to D) calcium creosotate was again administered to the same 8 animals in the same manner as before but the urine phenol determinations were made on *hydrolyzed* urine specimens (D to E). The marked increase in phenol values, obtained *after hydrolysis* as compared with the values obtained for the *non-hydrolyzed* specimens clearly shows that the portion of calcium creosotate which is excreted in rabbit urine is present almost entirely in conjugated form. Marenzi and Banfi[7] as well as Barrac[8] have reported that histidine and other imidazoles

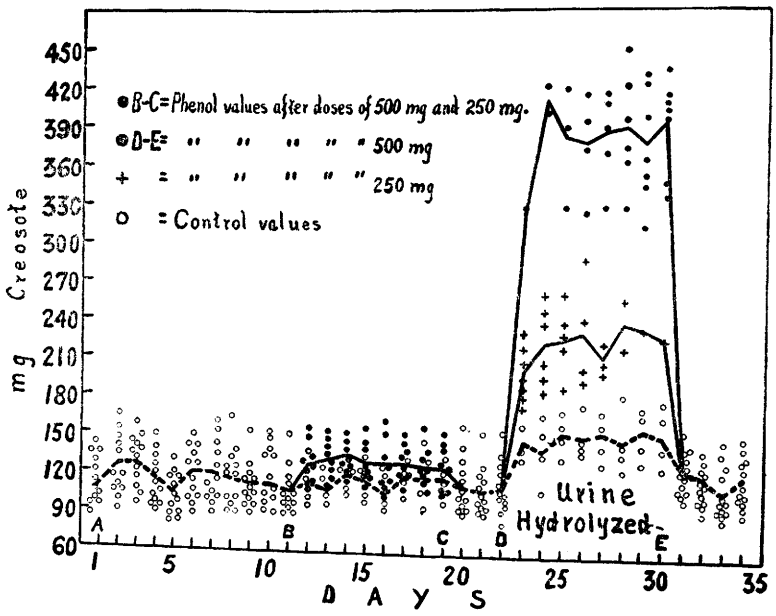

FIG. 1.

[7] Marenzi and Banfi, *Rev. Soc. argentine biol.*, 1936, **2**, 62.

[8] Barrac, *J. pharm. Belg.*, 1936, **18**, 534.

react with the nitraniline reagent to give a color similar to that produced by phenols. For this reason direct reaction between urine and the diazo reagent is not entirely a measure of phenol content. In the present experiments it is to be emphasized that the animals were maintained on a constant weighed amount of food and therefore the amounts of non-phenolic constituents of the urine which react with the diazo reagent should be approximately the same during the time of drug administration as during the control period.

In previous experiments[4] evidence was obtained that a portion of the methoxy compounds of creosote were destroyed. It was suspected that perhaps the methoxy compounds might be more readily "oxidized" in the body than the other creosote phenols. If this happened the volatile phenols excreted in urine should exhibit analytical constants at variance with those for creosote. The following experiments, therefore, represent an attempt to determine if oral administration of creosote to rabbits would result in elimination of this mixture *per se* in urine.

Approximately 0.5 g of creosote (1.8% suspension in 0.5% acacia) was administered to each of 14 rabbits and 24-hour urine specimens collected. These specimens were pooled, acidified with HCl, heated to boiling and allowed to remain in a boiling water bath for 15 minutes. The urine was allowed to cool, saturated with sodium chloride, and then extracted with ethyl ether. This procedure was undertaken on each of 12 successive days. The combined ether extracts were allowed to stand over anhydrous sodium sulfate for several days, filtered and the ether removed by distillation. The residue of approximately 20 g was distilled in a 50 cc distilling flask and after traces of moisture were driven off it was found to distill between 192-215°C. Considerable solid residue remained in the distilling flask. This material was identified as benzoic acid. It probably arose from the hydrolysis of hippuric acid. The material distilling between 192-215°C was extracted with hot water and redistilled. As shown in Table I the boiling point of this material is somewhat lower than that of the creosote administered but previous experience[4] has indicated that creosote placed in aqueous solution, acidified and extracted with ether will boil in this range. It is to be noted that the specific gravity and methoxyl data correspond with those of the creosote administered. From these data it is apparent that after hydrolysis of the conjugates a portion of orally administered creosote can be recovered in rabbit urine. This indicates that the methoxy compounds are no more readily destroyed in rabbits than the other creosote phenols.

TABLE I.

Material	B.P. °C	Sp. gr., 25°C	MeO %
Creosote	200-215	1.085	12.5
Phenols extracted from rabbit urine after creosote administration	192-215	1.085	11.6

Summary. 1. Calcium creosotate administered either in single doses of 0.5 to 2.0 g or in amounts and at intervals approximating its therapeutic application (0.5 g every 2 hours) did not produce bacteriostatic urine in normal human subjects. 2. Evidence was obtained that practically all of orally administered calcium creosotate which is excreted in rabbit urine is present in conjugated form. 3. Data are presented to show that after hydrolysis of the conjugates a portion of orally administered creosote could be recovered in rabbit urine. This indicates that the methoxy compounds are not more readily destroyed in the body than the other phenols of the mixture.

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Some Effects of Testosterone on Sexual Differentiation of Female Albino Mice.

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Early investigators observed that the persistence of testicular transplants in oöphorectomized rodents produced hypertrophy of the clitoris. Since male sex hormone became available in crystalline form, it has been demonstrated that varying degrees of intersexuality may be induced experimentally in guinea pigs,[1, 2] mice,[3, 4, 5] rats,[6–10] and rabbits.[10] Although the previous findings of Raynaud indicated that a pronounced degree of masculinization of genetic

1 Dantchakoff, V., *Compt. Rend. Soc. de Biol.*, 1936, **123**, 823.

2 *Ibid.*, 1937, **124**, 195.

3 Lacassagne, A., and Raynaud, *A.*, *Compt. Rend. Soc. de Biol.*, 1937, **125**, 351.

4 Raynaud, A., *Compt. Rend. Soc. de Biol.*, 1937, **126**, 866.

5 Raynaud, A., and Lacassagne, A., *Compt. Rend. Soc. de Biol.*, 1937, **126**, 868.

6 Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200.

7 Greene, R. R., Burrill, M. W., and Ivy, A. C., *Science*, 1938, **87**, 396.

8 *Ibid.*, PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 4.

9 *Ibid.*, 1938, **38**, 1.

10 Hamilton, J. B., and Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 570.