

CONCERNING CERTAIN AROMATIC CONSTITUENTS OF URINE.

III. THE NON-PHENOLIC VOLATILE OILS OF HORSE AND HUMAN URINE.

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INTRODUCTION.

It was reported by Stadeler¹ that the distillates from acidified urines from horses and human beings contained certain alkali-insoluble volatile oils similar to the oil obtained from cow urine. Other investigators have observed and confirmed this report of Stadeler. Hoppe-Seyler² states that in horse urine there occur in addition to phenols also volatile camphor-like substances and Baumann,³ in his exhaustive investigations concerning the conjugated sulfuric acids in urine observed a similar substance. This author states, "Destilliert man das durch Zersetzung einer grosseren Menge Pferdeharn erhaltene, auf dem Wasser meist schwimmende Oel mit Ätzkali, so erhält man im Destillat auf dem Wasser schwimmende gelbe oelige Tropfen die frei von phenolartige Körper sind. Dieselben besitzen einen eigentümlichen, an gewisse ätherische Oele erinnernden Geruch." Brieger⁴ describes a similar oil obtained from human urine. This substance is described as a light yellow colored oil of pleasant peppermint-like odor. By cooling, it neither solidified nor crystallized. It contained nitrogen. It gave red colorations with fuming nitric acid, concentrated sulfuric acid, and with Millon's reagent. With concentrated hydrochloric acid it gave a fine red color which changed into blue and after a while into a dirty violet. Bromine water gave a resinous precipitate but the oil gave no color reaction with ferric chloride. Given to a rabbit it caused no visible disturbance.

¹ Stadeler, G., *Ann. Chem.*, 1851, lxxvii, 17.

² Hoppe-Seyler, E., *Handb. Phys. u. Path. Chem. Analyse*, Berlin, 4th edition, 1875, 109.

³ Baumann, E., *Arch. ges. Physiol.*, 1876, xiii, 285.

⁴ Brieger, L., *Z. physiol. Chem.*, 1880, iv, 204.

It has been claimed by Fricke⁵ that both horse and human urine contain the substance "Urogon" described by Mooser⁶ as existing in cow urine. We⁷ have shown, however, that the substance "Urogon" is not homogeneous but a mixture consisting of *p*-cresol and a non-phenolic oil having the composition $C_{10}H_{16}O$, our results confirming the opinion expressed by Neuberg and Czapski.⁸

Dehn and Hartman⁹ have recently reported the discovery in human urine of a substance which they call "Urinod." This substance is supposed to be the cause of the characteristic odor of urine and it is described as possessing other remarkable properties. It reacted with concentrated nitric acid with explosive violence and when treated with fixed alkalis a terpene-like odor was produced. The substance combined with bromine with liberation of hydrobromic acid. It gave no definite compounds with semicarbazide or hydroxylamine hydrochlorides but a crystalline dinitro derivative was obtained.

In the present investigation we have confined ourselves entirely to the non-phenolic or alkali-insoluble portion of the oils obtained on distilling acidified horse urine and human urine. In the preparation of the so called neutral or alkali-insoluble oils from the distillates of urine it is necessary to bear in mind that the distillates contain large quantities of phenols principally *p*-cresol and a very small percentage of really neutral or alkali-insoluble oils. We have previously referred to the difficulty of completely removing phenols from an ethereal solution by shaking the latter with aqueous alkali⁷ and Neuberg and Czapski⁸ have shown that both phenol and cresol may be extracted by petroleum ether from a strongly alkaline solution. Various authors like Mooser⁶ Fricke,⁵ and Dehn and Hartman⁹ apparently have not taken the importance of this matter into account and have failed to assure themselves that phenols and cresols had been completely removed from the so called neutral oils which they finally obtained and analyzed.

We cannot believe that the oil described under the name of "Urinod" by Dehn and Hartman⁹ is any more of a chemically homogeneous substance than the "Urogon" of Mooser and Fricke.

⁵ Fricke, E., *Arch. ges. Physiol.*, 1914, clvi, 225.

⁶ Mooser, W., *Z. physiol. Chem.*, 1909, lxxiii, 155.

⁷ Anderson, R. J., *J. Biol. Chem.*, 1916, xxvi, 387, 401.

⁸ Neuberg, C., and Czapski, L., *Biochem. Z.*, 1914, lxxvii, 28.

⁹ Dehn, W. M., and Hartman, F. A., *J. Am. Chem. Soc.*, 1914, xxxvi, 2136.

The properties ascribed to "Urinod" are practically identical with those which we have observed in the case of all so called neutral oils obtained from cow and goat urines. The peculiar chemical reactivity of these oils, *viz.*, the violent reaction with concentrated nitric acid and the absorption of bromine with liberation of hydrobromic acid, is due to the cresol contained in such oils and we feel quite certain that the substance called "Urinod" must also have been largely contaminated with phenol or cresol since it would have been practically impossible, in accordance with our experience, completely to remove all of the phenols by the method employed by Dehn and Hartman.

The peculiar and disgusting odors possessed by all of the crude neutral oils obtained from urine distillates are apparently caused by very minute quantities of powerfully odorous substances. These odors, moreover, differ according to the kind of urine from which the oils are isolated. The oil from goat urine, for instance, has a powerful and disgusting odor of goats, that from cow urine smells like the odor peculiar to cow stables, and the oil from human urine has a strong and persistent odor which is very similar to that of human urine. These odors are destroyed by nitric acid, as stated by Dehn and Hartman, and they are also immediately destroyed by concentrated alkali when brought into direct contact with the crude oils. We have noticed also that these odors are lost on repeated fractionation of the oils in high vacuum. For this reason we believe that these highly odorous substances are very volatile bodies which are merely dissolved in the crude oils. The crude oils like "Urogon" and "Urinod" contain therefore not less than three substances; *viz.*, (1) principally cresol; (2) a small percentage of alkali-insoluble neutral oils which vary according to the terpene-like bodies contained in the food; and (3) the disgusting odorous bodies.

The first two can be separated by means of sufficiently concentrated alkali which dissolves out the cresol leaving the alkali-insoluble oil but the odorous substances become destroyed by this treatment, because the residual insoluble oil possesses an agreeable terpene-like odor and the cresol isolated after acidifying the alkaline solution has a pure phenolic odor without any trace of the disgusting odor of the original substance.

It would be interesting to study further these odorous sub-

stances. Such studies, however, are very difficult because these substances are apparently present in exceedingly minute quantities. Although we have tried to obtain some knowledge concerning their nature we have been unable to secure any other evidence of their existence than the smell.

EXPERIMENTAL.

The Non-Phenolic Volatile Oil from Horse Urine.

About 67 liters of mixed horse urine were collected during the winter. The fresh urine was evaporated to about one-eighth of its volume, strongly acidified with sulfuric acid, and distilled with steam until no more oil went over. The distillates were extracted with ether and the ethereal solution was repeatedly shaken with 20 per cent sodium hydroxide and then with dilute sulfuric acid, finally washed with water, and dried with sodium sulfate. After evaporating the ether the oily residue was distilled in vacuum. There were obtained 2 gm. of a light yellow oil which in odor resembled the product previously isolated from cow urine, but it differed in composition by containing nearly 3 per cent more hydrogen.

0.1353 gm. of substance gave 0.1251 gm. H₂O and 0.3831 gm. CO₂.
Found: C = 77.22; H = 10.35 per cent.

The substance reacted slightly with concentrated nitric acid which indicated traces of cresol.

The oil was, therefore, thoroughly shaken with 20 per cent sodium hydroxide until a milky emulsion was formed. This was extracted with ether and after drying and evaporating the ether the residue was again distilled in vacuum. At about 1 mm. pressure it went over at 100°. It was a light yellow colored oil of pleasant aromatic or terpene-like odor which weighed 0.7 gm. It was free from nitrogen and sulfur.

The alkaline solution, after extracting the above alkali-insoluble oil, was acidified with sulfuric acid, the oily substance was extracted with ether, and finally distilled in vacuum. The distillate went over at 76° and 2 mm. pressure. It was a practically colorless oil of distinct phenolic odor which when suspended in water

gave a fine blue coloration with ferric chloride and it reacted with great violence with concentrated nitric acid. There appears to be no doubt, therefore, that this substance was cresol.

This shows again how difficult it is to extract all of the phenols from an ethereal solution by aqueous alkali. Traces of cresol remained in this oil in spite of the fact that the ethereal solution had repeatedly been shaken thoroughly with 20 per cent sodium hydroxide.

Properties of the Above Alkali-Insoluble or Neutral Oil.

The aqueous suspension of the oil gives no color reaction with ferric chloride. It does not solidify in a freezing mixture of ice and salt. When mixed with cold concentrated nitric acid the oily globules assume a red color and on warming, the oil dissolves, giving a yellow solution which on dilution with water turns cloudy. With cold concentrated hydrochloric acid no change takes place but on heating the oil turns red in color. In cold concentrated sulfuric acid the oil dissolves giving a dark red solution. In chloroform solution bromine is absorbed with liberation of hydrobromic acid. Potassium permanganate solution is immediately decolorized. Ammoniacal silver nitrate is reduced on heating. It is soluble in all proportions in the ordinary organic solvents but insoluble in alkali or water. On analysis the following result was obtained.

0.1247 gm. of substance gave 0.1224 gm. H_2O and 0.3423 gm. CO_2 .

Found: C = 74.86; H = 10.98 per cent.

The percentage composition agrees with the formula $C_7H_{12}O$.

Calculated for this: C = 75.00; H = 10.71 per cent.

The Non-Phenolic Oil from Human Urine.

The fresh urine which was acid in reaction was made slightly alkaline by adding sodium carbonate and then evaporated to about one-eighth of its volume. The residue was acidified with sulfuric acid, distilled with steam, the distillate extracted with ether, and the ethereal solution shaken repeatedly with 20 per cent sodium hydroxide and then with dilute sulfuric acid. In

all 111 liters of urine were evaporated and distilled. A small amount of yellow colored oil remained on evaporating the ether. The oil possessed a strong and persistent odor of human urine and it reacted slightly with concentrated nitric acid. In order to remove phenols completely the oil was shaken for some time with 20 per cent sodium hydroxide and distilled from the alkaline solution with steam. The distillate was extracted with ether, the latter dried with sodium sulfate, filtered and evaporated, and the residue distilled in vacuum when about 0.15 gm. of a light yellow oil was obtained. It did not contain nitrogen.

This oil had an agreeable terpene-like odor, the odor of urine of the original crude oil having been lost in the treatment with sodium hydroxide. In other respects it gave exactly the same reactions as described for the oil isolated from horse urine and it had practically the same composition.

0.1005 gm. of substance gave 0.0909 gm. H_2O and 0.2777 gm. CO_2 .
Found: C = 75.36; H = 10.12 per cent.

This substance differs in composition from the "Urinod" of Dehn and Hartman⁹ by containing about 2 per cent more hydrogen. It also differs from the above product by giving no reaction with concentrated nitric acid. The violent reaction produced when "Urinod" was treated with concentrated nitric acid was undoubtedly due to the presence of phenols.

Unfortunately the quantity of neutral oil obtained from horse urine and from human urine was too small to permit of a more extensive investigation. Of all the urines examined that from human beings contained the smallest percentage of neutral oil. Städeler¹ also reported that the amount of oil obtained from human urine was very slight.

As a result of our investigation of the neutral alkali-insoluble oils obtainable from the urine of different animals we have found only two different kinds of oil as far as composition is concerned, as is shown in the résumé below.

Composition:		Corresponding to the formula:	
	C	H	
Cow urine.....	78.25	10.84	$C_{16}H_{16}O$
	78.90	11.12	Calculated: C = 78.94; H = 10.52
	78.30	10.46	
	79.15	10.49	
Goat urine.....	78.24	11.17	
	78.68	11.31	
Cow urine.....	74.77	10.61	Corresponding to the formula:
Horse urine.....	74.86	10.98	$C_7H_{12}O$
Human urine.....	75.36	10.12	Calculated: C = 75.00; H = 10.71.

In conclusion we venture to express the opinion that the kind of neutral alkali-insoluble oils excreted in the urine of different animals will be found to a large extent to depend upon the nature of the terpene-like bodies contained in the food and for this reason the nature of the oil will vary in accordance with the season and the available food supply.