

ON THE TOXICITY OF NORMAL URINE.

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The purpose of this article is to present the results of some experiments undertaken at the Ohio State University to determine, first, the degree of toxicity possessed by normal urine, and, secondly, the nature of the substance or substances lending toxic properties to this fluid. The importance of this line of work was pointed out by Prof. Bleile, and the investigations were carried on under his supervision.

Before giving the results of these experiments, however, it may be stated, as a matter of history, that poisonous substances were long ago believed by some to exist in normal urine. There were many, on the other hand, who denied the presence of such toxic bodies in this excretion, and, as a consequence, experimental evidence was sought to support each side of the question. Investigations along this line were instigated, in the first place, by the discussion, in the early part of this century, of the cause or causes of uræmia. The first work of any importance was done about that time by Vauquelin and others, who made actual demonstrations of the toxicity of normal urine by intravenous injections. Frerichs, however, claimed that death resulting from such procedures was due to suspended solid elements in the urine and to poisonous ammonium carbonate formed in it by fermentation. Voit was the first to point out that potassium salts, on account of their toxicity, could play an important part in uræmia, and Feltz and Ritter, and Astaschewsky concluded that these salts were the chief toxic bodies in normal urine. Among those who opposed this view were such workers as Schiffer, Pouchet and Bouchard, who satisfied themselves that the toxicity was due largely to the presence of organic compounds of an alkaloidal nature.

Lépine, Guerin, Griffiths, Feltz and others performed experiments

showing that these alkaloidal bodies were increased in certain morbid states, as in measles, diphtheria, pneumonia, cholera, etc. Griffiths claims to have isolated toxins from the urine of pleurisy, influenza, cancer, and epilepsy. Prof. Bleile found that the urine was decidedly more toxic after epileptic attacks, but the alkaloid which Griffiths claims to have isolated from such urine was not found, although his methods were closely followed. Observations on the urine of pathological conditions have lately been very numerous, but satisfactory experimental elucidation of the degree and cause of the toxicity of normal urine is wanting.¹

A complete account of the methods which yielded the results to be stated presently is omitted from this article, as those methods have been published elsewhere. It will suffice to outline the work merely, and to give the more important results.

Urine was collected, usually to the amount of 4 litres, from healthy adults who were not, and never had been, users of tobacco. It was then extracted by Brieger's method,² which after various trials was

¹The following are among the works which may be consulted for a consideration of the subject of the toxicity of the urine, and for references to the authors cited and the other rather voluminous literature:

C. Bouchard. *Leçons sur les auto-intoxications dans les maladies*, Paris, 1887, also *Lectures on auto-intoxication in disease*. Translated by Thomas Oliver. Philadelphia, 1894.

Neubauer and Vogel (Huppert). *Analyse des Harns*, Wiesbaden, 1898.

H. Roger. "Les intoxications" in Bouchard's *Traité de path. gén.*, T. i, p. 773, Paris, 1895.

Hymans van den Bergh. Ueber die Giftigkeit des Harns. *Zeitschr. f. klin. Med.*, 1898, xxxv, p. 53.

A. Gottheiner. Die Lehre von den Harngiften in pathologischen Zuständen. *Monatsb. üb. d. Gesamtleist. d. Krankh. d. Harn- u. Sexual-Apparates*, 1897, ii, pp. 649; 713.

A. Beck. Ueber die Giftwirkung des Harnes. *Pflüger's Archiv*, 1898, lxxi, p. 560.

W. P. Herringham. An account of some experiments upon the toxicity of normal urine. *Trans. Path. Soc.*, London, 1899, l, p. 293.

Forchheimer and Stewart. On the toxicity of the urine. *Am. Journ. Med. Sc.*, 1899, cxviii, p. 297.

²Brieger's method (Ueber Ptomaine and Weitere Untersuch. üb. Ptomaine, Berlin, 1885) is described in Vaughan and Novy's *Ptomains, Leucomains, Toxins and Antitoxins*, Philadelphia and New York, 1896, as well as in other works treating of the same subject. The method, as followed by the writer, was essentially as follows:

Four litres of urine were treated with lead acetate (sugar of lead) until no precip-

found to be the best for extraction of normal urine. The product thus obtained was then injected in a concentrated watery solution subcutaneously into white mice in doses varying from three to seven minims. The following are in brief some of the effects of these injections:

1. Mouse injected with 5 minims of extract of 4 L. normal urine. Somnolence came on in 10 minutes. In 45 minutes severe spasmodic movements appeared. At one time the spasm was general and very violent. These symptoms decreased gradually and recovery took place in about 24 hours.

Seven minims of the same material killed a mouse in 10 minutes, the animal dying in violent convulsions.

2. Seven minims of an extract from another urine killed a mouse in 16 minutes. Respiration became very irregular, the mouse dying in clonic convulsions.

3. Six and one-half minims of another extract caused spasms in the legs. Sometimes the legs would all jerk together, or would be jerked separately, first the front legs together, then the hind legs together. Respiration was irregular and difficult. There was exophthalmus. The symptoms had all disappeared in three hours.

4. Two litres of urine gave two separate masses after removal of the mercury with H_2S , the one soluble in alcohol and the other in water. The alcohol was evaporated from the one part and the mass extracted several times with more alcohol, which was finally evaporated and the residue taken up with water, the slight residue remaining being filtered off. Six minims of this produced in 20 minutes deep somnolence and dilatation of the external arterioles and capillaries. No other symptoms.

itate was formed. The precipitate was then filtered off and the filtrate concentrated carefully to a syrup. This syrup was then extracted with 96% alcohol and the filtered extract was treated with an alcoholic solution of the lead acetate. Any precipitate that followed was filtered off, and the filtrate again concentrated and extracted with 96% alcohol. This alcoholic extract was then concentrated again and taken up with water, the lead in the watery solution being removed by hydrogen sulphide. After filtering off the precipitate of PbS the filtrate was acidified with hydrochloric acid and once more concentrated to a syrup and extracted with alcohol. To this extract a saturated alcoholic solution of mercuric chloride was added till no further precipitate was formed. This precipitate was dissolved in a large quantity of hot water and the mercury was removed by hydrogen sulphide. The water was evaporated and the residue taken up finally in alcohol, which was evaporated when material in solution was to be injected.

Six minims of the original mass soluble in water produced the opposite condition in the external vessels. In 20 minutes the respiration was very weak. In one hour the reflexes were much heightened. These conditions lasted about 6 hours. Mouse recovered.

5. Six minims of extract of 4 L. of urine caused drowsiness only.

6. Four and one-half minims of extract from 4 L. of another sample caused twitching in $2\frac{1}{2}$ minutes. This developed into severe clonic spasms which occurred every few minutes. Respiration was very irregular and finally dyspnoea became pronounced. The reflexes were greatly heightened in 21 minutes, a snap of the finger causing spasm. The exophthalmus was extreme. In 25 minutes the reflexes were abolished. He died in clonic convulsions 26 minutes after injection. The action of this material was much like that of strychnine.

It is apparent, as might be expected, that these urines varied in their toxicity. There was likewise variation in the symptoms. The nervous system was affected most strongly in nearly every case. Respiration was often very irregular and labored. In one case a disturbance of respiration was the only symptom observed. Sometimes the arterioles of the extremities were dilated; at other times they were not. The condition of the heart and pulse was not observed, but it is reasonable to suppose that they were disturbed.

We particularly desired to learn whether these extracts contained any mineral matter. To determine this, in each case a quantity of the extract equal to that injected was evaporated to dryness. Usually a large residue remained. This residue, however, could be easily destroyed by heat, practically no ash remaining after incineration with a Bunsen flame. There was, therefore, no mineral matter in these extracts. Tests for xanthin compounds, it may be stated, gave also negative results.

Evidently we obtained from normal urine a substance, or substances, of an organic nature, possessing marked toxic properties. The effects described above could have been due neither to potassium salts nor to any of the organic bodies usually included in the list of compounds contained in normal urine. No attempt was made to isolate the substance, or substances, that produced the results presented, the intention being to undertake that work at the earliest opportunity.