ON THE OCCURRENCE OF METHYL GUANIDINE IN THE URINE OF PARATHYROIDECTOMIZED ANIMALS.

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The experiment described in this paper was designed to determine whether guanidine could be detected in the urine of animals subjected to thyro-parathyroidectomy.

On May 8, the thyroid and parathyroid glands were removed from a dog weighing 18.5 kgms. On May 10, at 11 a.m., fibrillary twitchings were noted in his body muscles. The symptoms increased in violence until at 9.15 p.m. he died in severe tetany. During this period he excreted 2250 cc. of urine, which was examined as follows:

The kynurenic acid was removed with hydrochloric acid. The protein-like substances were precipitated from the acid filtrate with 20 percent tannin solution. The tannin excess was removed with barium hydroxide, the barium excess with sulphuric acid and the excess of the latter with lead oxide. The resulting filtrate was then treated with a hot saturated solution of sodium acetate and mercuric chloride until no more precipitate formed, according to the method of Engeland. 1 The precipitate was then filtered off; washed with a cold saturated solution of mercuric chloride and sodium acetate, treated with hot dilute hydrochloric acid and the soluble portion filtered from the insoluble. The filtrate was then decomposed with hydrogen sulphide and filtered. The resulting filtrate was evaporated to a thick syrup and extracted with methyl alcohol. The extract was then filtered from inorganic salts. The methyl alcohol was evaporated from the filtrate, the residue extracted with ethyl alcohol, filtered from insoluble, residue and again extracted with alcohol. This process was repeated until no more creatinine and ammonium salts were dissolved. The resulting alcoholic solution was treated with 20 percent platinic chloride until no more precipitate occurred. The precipitate was then filtered off.

The filtrate was decomposed with hydrogen sulphide, the platinum sulphide removed, the filtrate evaporated to a thick syrup and treated with a 30 percent solution of gold chloride.

The platinic precipitate was suspended in water, decomposed with hydrogen sulphide and the platinum sulphide filtered off. To the resulting liquid a 30 percent solution of gold chloride was added.

From the gold solution from the platinic filtrate, after standing in the desiccator for several days, a rich crop of beautiful needles formed and were filtered off. In the course of another day a second precipitate of needles and cubes occurred. These were separated mechanically as far as possible and fractionally crystallized. From the mother liquid, on further standing, another precipitate, apparently only of cubes and plates, formed. Both fractions of needles on recrystallization showed the same melting point, namely, 198°C. (uncorrected). They possessed also

similar solubilities in water and ether and were therefore combined.

From the gold solution of the platinum precipitate a crop of needles crystallized. These were filtered off. In the course of twenty-four hours another precipitate of both needles and rhomboid plates was obtained. The needles and plates were fractionally crystallized and both yields of needles, which possessed the same melting point (198°C.), were united.

Owing to the similarity in the melting points and the solubilities of the needles of both the platinic filtrate and precipitate, they were united and weighed, the total yield being 4.3 grams. They were recrystallized from hot dilute hydrochloric acid and a portion dried in the air and later at 90° for melting point and gold content determinations. At 198° they melted to a red brown liquid.

ANALYSIS:
(a) 0.084 gram substance gave 0.0402 gram Au.
(b) 0.1635 gram substance gave 0.078 gram Au.

	Calculated for C2H7N3.HCl.Au.Cl:	Found (a)	(b)
Au	47.7	47.86	47.71

Complete analyses have not been made but the melting point and gold percentage identify the substance as methyl guanidine aurochloride.

From the remaining gold solution of the platinic precipitate after standing several days in the desiccator and after cooling at a temperature of 30° for twelve hours a yield of about 2 grams of crystals was obtained. These were found to melt in a peculiar manner after recrystallization and drying. At about 130° they gradually became cloudy and between 207° and 208.5° melted to a brown oil. Finally about a gram of prisms melting at 310°C. were obtained. They will be further studied.

Methyl guanidine was first isolated from normal human urine by Kutscher and Lohman. 2 From 100 liters they obtained about 4 grams as the aurochloride. Later Engeland 3 isolated 2.1 grams of the gold salt from 28 liters of normal urine. From 11 liters of normal dogs' urine Achelis 4 isolated 0.122 gram as the picrolonate; corresponding to about 0.04 gram of the free base per liter of urine. These results correspond to about 0.07 gram of the gold salt per liter of normal urines, both of man and dog. In the case noted above the yield was considerably in excess, namely, 1.9 grams as the gold salt per liter. The greater part was probably excreted on the day of the animal's death since no symptoms were noticeable until ten hours before death. During the last five hours he passed only a few cubic centimeters of urine. On microscopic examination of the kidney, the cortex was found to be very hyperemic and hemorrhagic. Many of the glomeruli had become blood islands with complete loss of Bowman's capsule: It is not surprising that under these conditions little urine was passed during the hours just preceding death, a probable indication that, since the kidney function had been inhibited, whatever methyl guanidine might have been formed must have accumulated in the blood.

Further investigation is being carried on concerning the extent to which methyl guanidine is responsible for the symptoms and death of the parathyroidectomized animals and concerning the of the other bases which our work has shown to be present.

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1. Zeitschr. F. physiol. Chem., lvii, p.49.

2. Zeitschr. F. physiol. Chem., xlix, p. 81.

3. Ibid., lvii, p. 49.

4. Ibid., l, p. 10.