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TOXIC BASES IN THE URINE OF PARATHYROIDECTOMIZED DOGS.

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In a recent paper[1] I demonstrated the presence of methyl guanidine in the urine of a parathyroidectomized dog, and called attention to the presence of some other bases. It is the purpose of this paper to report the occurrence of methyl guanidine in the urines of each of five other parathyroidectomized dogs, and to describe some of the other bases more fully. Experiments have also been made to determine in what manner mercuric chloride might best be used as a precipitant for their isolation.

Since the isolation of toxic bases from a urine involves a large number of manipulations and often large precipitates, quantitative results are hardly to be expected. The urines were therefore run in pairs, and the same technique so far as possible applied to each. I hoped in this way to be able to attribute any pronounced differences in the bases of two urines, similarly examined, to variations in metabolism. Moreover, substances that agree in a number of their properties when present in small quantities in each urine, could be combined.

The urines of the first two parathyroidectomized dogs were treated separately and similarly as follows: After each micturition the samples, collected by drainage from the cage, were acidified with HCl to precipitate the kynurenic acid. After standing in the cold until complete precipitation occurred, the precipitate was filtered off and the filtrate treated with tannin, barium hydroxide, sulphuric acid, and lead oxide according to Kutscher's method[2] for

[1] This *Journal*, xii, p. 313, 1912.

[2] *Zeitschr. f. physiol. Chem.*, xlviii, p. 1, 1906.

the removal of proteins. The urines thus purified were treated with mercuric chloride and sodium acetate according to the method of Engeland.[3] The mercury salts of the bases thus obtained were taken up in dilute hydrochloric acid, decomposed with hydrogen sulphide, and the mercuric sulphide filtered off. The filtrate containing the hydrochlorides of the bases was evaporated to a syrup, and the organic substances extracted with methyl alcohol and the insoluble portion filtered off. After evaporation of the methyl alcohol, those substances easily soluble in ethyl alcohol were taken up in this solvent and treated with an alcoholic solution of platinum chloride. The precipitate that formed was filtered off, taken up in hot water and decomposed with hydrogen sulphide. The platinum sulphide was filtered off, and the filtrate concentrated and treated with gold chloride (Fraction A). Likewise the platinic filtrate after evaporation of the alcohol was decomposed with hydrogen sulphide and the platinum sulphide filtered off. The aqueous solution thus obtained was concentrated and treated with gold chloride (Fraction B). Those substances difficultly soluble in alcohol were taken up in water and treated with absolute alcohol until no more precipitate formed. The precipitate was filtered off, taken up in water and treated with picrolonic acid in aqueous solution (Fraction C). The filtrate after removal of the alcohol was likewise treated with picrolonic acid (Fraction D).

From fraction A of urine 1, 0.7 gram of yellow needles melting after two recrystallizations at 200°C. was obtained. They appeared similar to those mentioned in my preliminary report. From urine 2, 0.5 gram of the similar needles melting after two recrystallizations at from 200° to 205°C. was obtained. A number of crystals from each sample intimately ground together became soft at 200°C. and did not melt until 206°C. They were very insoluble in cold water and not readily soluble in hot water. After the removal of the gold each gave the diazo reaction with diazobenzene-sulphonic acid and sodium carbonate. A weighed portion of the combined crystals was taken up in water and the gold removed as the sulphide. This was converted into free gold and weighed. The filtrate containing the bases was treated with picrolonic acid.

[3] *Zeitschr. f. physiol. Chem.*, lvii, p. 49, 1908.

The precipitate thus obtained after recrystallization melted at 266°C.[4]

The gold salt.

0.4301 gm. gave 0.2132 gm. Au.
0.6008 gm. gave 0.2997 gm. Au.
0.4453 gm. at 21°C. and 732.5 mm. gave 23 cc. N.

The picrolonate.

0.2000 gm. at 22.5°C. and 739 mm. gave 43.8 cc. N.
0.1609 gm. gave 0.2762 gm. CO_2 and 0.0576 gm. H_2O.

	Calculated for $C_5H_{11}N_3 2(AuCl_4)$:	Found: *a*	Found: *b*
Au	49.9	49.67	49.88
N	5.32	5.68	

	Calculated for $C_5H_9N_3 \cdot 2(C_{10}H_8N_4O_8)$:	Found:
N	24.14	24.13
C	46.92	46.81
H	3.91	4.01

This substance appears from the diazo reaction, the melting points, and analyses, to be β-imidazolylethylamine. I am surprised at its presence among the substances readily soluble in alcohol since the hydrochloride of this substance is described as being sparingly soluble in alcohol.[5]

There next occurred in each urine fraction, a crop of rhomboid plates. They were recrystallized twice, and melted slowly between 241° and 245°C. Portions of the crystals from each urine were intimately mixed. These melted at 243°C. After another recrystallization their form changed to needles but the melting point was not altered. From each urine 0.3 gram was obtained.

From urine 1, 0.2259 gm. substance gave 0.1019 gm. Au.
From urine 2, 0.1845 gm. substance gave 0.0831 gm. Au.

	Calculated for $C_5H_{14}NOCl \cdot Au\,Cl_3$:	Found: *a*	Found: *b*
Au	44.52	45.16	45.04

[4] Thermometer readings are not corrected. The nitrogen was collected over 40 per cent KOH; barometer readings are taken from an instrument with a glass scale, reading directly; direct readings are given not corrected except where indicated.

[5] *Zeitschr. f. physiol. Chem.*, lxv, p. 504, 1910.

The melting point is that of choline aurichloride and the gold content indicates a substance of equal molecular weight.

After the solutions became quite concentrated, a large quantity of needles and some plates separated out. After three recrystallizations they began to soften in the melting tube at 262°C., became almost fluid at 286°C. and melted rising in the tube with bubble formation at 292°C. to 296°C. On analysis the substance was found to be free from carbon and proved to be the gold salt of ammonium chloride.

Fractions B of urines 1 and 2, the filtrates from the platinum precipitates, gave after concentrating and treatment with gold chloride, each, a precipitate of yellow needles. These after one recrystallization melted sharply at 198°C. Urine 1 gave 2.3 grams; urine 2 gave 2.9 grams. They were more soluble in alcohol and ether than in water. The gold was removed from a portion of the united needles, and weighed; the filtrate was treated with picrolonic acid. The picrolonate melted sharply at 275°C. with decomposition and bubble formation.

The gold salt.

0.1602 gm. gave 0.0763 gm. Au.

The picrolonate.

0.2023 gm. at 22.5°C. and 745 mm. gave 53.5 cc. N.
0.0950 gm. gave 0.1490 gm. CO_2 and 0.0369 gm. H_2O.

	Calculated for $C_2H_8N_2(AuCl_4)$:	Found:
Au	47.7	47.6

	Calculated for $C_2H_7N_3(C_{10}H_8N_4O_5)$:	Found:
N	29.14	29.31
C	42.7	42.78
H	4.45	4.35

This substance is evidently methyl guanidine as shown by the properties and analyses of these derivatives. After no more methyl guanidine aurochloride separated out, the gold was removed as the sulphide from the mother solution and the filtrates united and treated with picrolonic acid. A dark red, very insoluble precipitate formed. By burning in a crucible it left magnetic iron oxide. A voluminous precipitate was next obtained which melted after

two recrystallizations at 346°C. The yield was slightly over 1 gram.

0.1126 gm. substance at 23.5°C. and 738 mm. gave 22.2 cc. N.
0.1129 gm. substance at 16.25°C. and 745 mm. gave 21.0 cc. N.
0.1439 gm. substance gave 0.2327 gm. CO_2 and 0.042 gm. H_2O.

	Found:	
	a	*b*
N	21.54	21.21
C	44.1	44.14
H	3.27	3.49

On burning in a porcelain crucible the substance emitted a strong peach-blossom odor and left no ash. It needs further study.

Fraction C, the alcoholic precipitate of the substances difficultly soluble in alcohol, contained a large quantity of inorganic salts. However from this fraction of urine 2 a picrolonate was obtained. After several recrystallizations, it darkened at 340°C. and did not melt below 360°C. The yield was about 2 grams.

0.1912 gm. substance at 22.1°C. and 741 mm. gave 35.1 cc. N.
0.1713 gm. substance at 23.5°C. and 740.5 mm. gave 31.2 cc. N.
0.2347 gm. substance gave 0.0644 gm. H_2O and 0.3704 gm. CO_2.
0.1822 gm. substance gave 0.0530 gm. H_2O and 0.2847 gm. CO_2.

	Found:	
	a	*b*
N	20.25	20.04
C	42.9	42.6
H	3.07	3.25

This substance has about the same solubility as the picrolonate obtained by treating ammonium chloride with picrolonic acid. The latter however melts at 278°–280°C.

Fraction D gave a small precipitate with picrolonic acid which upon an attempt at recrystallization did not precipitate until the solution had almost evaporated to dryness. It cannot be studied until more substance is at hand.

The urines obtained from dogs 3 and 4 were treated similarly to those of dogs 1 and 2, with the exception that the removal of the proteins was omitted. After the removal of kynurenic acid, the urines were evaporated to a syrup, taken up in methyl alcohol,

filtered and again evaporated to remove the excess of HCl, and then taken up in water. This solution was treated with mercuric chloride and sodium acetate according to Engeland's method to precipitate the bases. The urine fractions were similar to those of urines 1 and 2. Fractions A are again the platinum precipitates of substances soluble in alcohol. Fraction B of each urine is the filtrate from this platinum precipitate. Fraction C is the alcoholic precipitate of substances insoluble in alcohol. Fraction D, the filtrate from this precipitate.

Fraction A of urine 2 upon concentration and treatment with gold chloride gave 0.6 gram of rhomboid plates which after two recrystallizations melted at 248°C.

0.1901 gm. substance gave 0.085 gm. Au.

	Calculated for $C_5H_{14}NO\,AuCl_4$:	Found:
Au	44.52	44.7

From urine 4 only a few needles melting at 238°C. were obtained. The quantity was too small for purification and analyses. As the solutions concentrated a large quantity of the aurochloride of ammonia was obtained.

Fractions B, the filtrates from the platinum precipitates, were found to reduce a test portion of gold chloride. After study they revealed the presence of ferrous iron. They were therefore made alkaline with Ag_2O. (Ag_2O was used instead of other alkalies because it could be removed so readily.) The precipitate obtained was decomposed with H_2S and gave upon removal of the silver sulphide, acidifying with HCl and concentrating, a large crop of beautiful translucent green rhomboid plates. These were very soluble in water and alcohol, and gave with potassium ferricyanide, the reaction for ferrous iron. They contained a large quantity of water of crystallization. They were dried by standing in a desiccator over H_2SO_4 for several days. In a vacuum tube under a slow stream of dry air and at 120°C. they lost after one-half hour a portion of their water of crystallization.

2.0298 gms. substance lost 0.3611 gm. H_2O at 120°C.
0.2729 gm. substance gave 0.1350 gm. AgCl.
0.3500 gm. substance gave 0.1728 gm. Fe_2O_3.

	Calculated for $FeCl_2$:	Found:
Fe	44.08	34.53
Cl	55.92	45.8

Evidently all of the water of crystallization was not lost by the drying. The substance appears to be ferrous chloride. The form in which the iron was excreted into the urine is not known, but it appears that it might, in part, have been part of a protein molecule since urines 1 and 2, from which the proteins were removed, contained much less iron.

The filtrates from the silver precipitates, after the removal of the silver, gave upon treatment with gold chloride crops of beautiful needles. These after recrystallization melted sharply at 198°C. They were soluble in water and more readily soluble in alcohol and ether. Urine 3 gave 2.2 grams, urine 4 gave 1.7 grams. After recrystallizing from alcohol ether and water, a portion was freed from gold and converted into the picrolonate. This melted sharply, rising in the tube at 275°C. with decomposition instead of at about 270° as described by Achelis.

The gold salt.

0.1031 gms. gave 0.4930 gms. Au.

The picrolonate.

0.1024 gms. at 25°C. and 742 mm. gave 27.4 cc. N.

	Calculated for $C_2H_8N_2(AuCl_4)$:	Found:
Au	47.7	47.81

	Calculated for $C_2H_7N_3(C_{10}H_8N_4O_5)$:	Found:
N	29.14	29.29

Their solubilities and melting point, the analyses, and the urine fraction from which they were obtained, characterize them as the gold salts of methyl guanidine. These crystals were followed in urine 4 by a small quantity of cubes and short rectangular prisms of a brown color. The quantity was too small for purification. The mother solutions from both urine fractions were united and the gold removed with hydrogen sulphide. The filtrates thus obtained were treated with picrolonic acid. About 0.8 gram of a substance precipitated which after two recrystallizations melted at 260°C. The substance was quite insoluble in water.

0.1783 gm. substance at 22.0°C. and 748.5 mm. gave 44.2 cc. N.

	Calculated for $C_3H_9N_3(C_{10}H_8N_4O_5)$:	Found:
N	27.97	27.73

This substance agrees in melting point and nitrogen content with symmetrical dimethyl guanidine.

Fraction C, the alcoholic precipitate of substances insoluble in alcohol, gave with picrolonic acid a precipitate which after several recrystallizations exploded at 348°C.

0.0752 gm. substance at 23°C. and 732 mm. gave 14.9 cc. N.
0.1619 gm. substance gave 0.2474 gm. CO_2 and 0.0490 gm. H_2O.
0.1070 gm. substance gave 0.1635 gm. CO_2 and 0.0364 gm. H_2O.

	Found:	
	a	*b*
N	21.56	
C	41.7	41.74
H	3.43	3.81

This substance requires further study. It is not very insoluble in water and may not be pure. Other derivatives are being prepared.

Urine 5. In order to determine if the choline present in the preceding urines might have arisen from phosphatides in the urine, an attempt was made to isolate such a substance. The urine was therefore extracted with an equal volume of ether. Upon evaporation of the ether only a trace of a lipoid substance was found. This gave a precipitate with cadmium chloride, but was too small to examine further. The urine was then carefully neutralized, evaporated to a syrup and again extracted with ether. Upon evaporation of the ethereal extract no better result was obtained. Since the choline bases are known to give mercuric chloride salts which are less soluble in alkaline alcohol than in alkaline water, the syrupy residue of this urine was taken up in a small quantity of warm alcohol and the hot solution saturated with mercuric chloride and potassium acetate.

It was then treated with a hot saturated alcoholic solution of mercuric chloride and potassium acetate. While warm the mixture, in a wide mouthed bottle, was permitted to evaporate and then placed in the cold. When precipitation was complete, the precipitate was filtered off, taken up in dilute HCl, and decomposed with H_2S. The mercury sulphide was filtered off and the filtrate strongly acidified with HCl. After standing two days the

kynurenic acid had all precipitated and was then filtered off. The filtrate was evaporated to a syrup. The residue was now extracted with methyl alcohol, filtered, the alcohol evaporated, and the residue again extracted with methyl alcohol. After another repetition of the process no more inorganic salts were present in the extract, with the exception of ammonium chloride. The methyl alcohol was removed and the residue taken up in ethyl alcohol. The alcohol was evaporated off and the process repeated until only those substances easily soluble in alcohol were dissolved. The solution was then treated with alcoholic platinum chloride and the precipitate, which formed, filtered off (Fraction A). The alcohol was evaporated from the filtrate, the residue taken up in hot water and the platinum removed as the sulphide. The filtrate thus obtained was concentrated and treated with gold chloride (Fraction B). As in the previous urines the substances not dissolved by the extracting alcohol were taken up in water and precipitated with alcohol. This gave a precipitate (Fraction C) and a filtrate (Fraction D).

Fraction A, the platinum precipitate, was taken up in hot water and the substances readily soluble filtered off, as division 1. Substances left undissolved were again extracted with hot water, and the solution filtered from the insoluble portion (division 2). Those substances still undissolved were suspended in hot water and the three divisions decomposed with H_2S. The platinum sulphide was filtered from each. Division 1 was treated with gold chloride and gave a precipitate of rhomboid plates weighing about 2 grams. After two recrystallizations it melted at 238°C. The gold was removed as the sulphide, converted into free gold and weighed. The filtrate from the gold sulphide upon treatment with picrolonic acid gave a precipitate. This after one recrystallization melted cloudy at 178°C. and decomposed at about 230°C. The substance was dried in a vacuum. At 115°C. it lost water of crystallization.

Gold salt.

0.7392 gm. substance gave 0.3393 gm. Au.

	Calculated for $C_5H_{12}N \cdot AuCl_4$:	Found:
Au	46.4	45.9

The picrolonate.

0.0979 gm. substance at 15°C. and 748.5 mm. gave 17.2 cc. N.
0.2253 gm. substance at 21.5°C. and 740 mm. gave 40.8 cc. N.
0.2107 gm. substance gave 0.1140 gm. H_2O and 0.3978 gm. CO_2.
0.1630 gm. substance gave 0.0884 gm. H_2O and 0.3088 gm. CO_2.

	Calculated for $C_5H_{11}N(C_{10}H_8N_4O_8)$:	Found:	
		a	*b*
N	20.05	20.26	20.07
C	51.57	51.49	51.67
H	5.44	6.05	6.06

The substance is neurine as shown by the properties and analyses of the two derivatives.

The second platinic division was treated with picrolonic acid. It gave a precipitate weighing 2.1 grams which upon recrystallization was fractionated into two portions. The first and larger portion after recrystallizing several times melted between 284°C. and 286°C. The substance is quite insoluble in water.

0.1779 gm. substance at 22.5°C. and 740.8 mm. gave 41 cc. N.
0.1596 gm. substance at 22.0°C. and 742.0 mm. gave 36.8 cc. N.
0.1526 gm. substance gave 0.0609 gm. H_2O and 0.2583 gm. CO_2.
0.1715 gm. substance gave 0.0648 gm. H_2O and 0.2895 gm. CO_2.

	Calculated for $C_5H_9N_3O(C_{10}H_8N_4O_8)$:	Found:	
		a	*b*
N	25.2	25.45	25.54
C	46.00	46.17	46.06
H	4.35	4.46	4.23

The second and smaller fraction after three recrystallizations softened and drew together in the melting tube at 240°C. and melted at 264°C.

0.0979 gm. substance at 18°C. and 736.5 mm. gave 22.5 cc. N.
0.1038 gm. substance gave 0.0391 gm. H_2O and 0.1732 gm. CO_2.

	Calculated $C_5H_{10}N_2(C_{10}H_8N_4O_8)$:	Found:
N	25.74	25.85
C	45.84	45.51
H	3.97	4.21

These substances freed from picrolonic acid did not give the diazo reaction and their structure is unknown. When more material is obtainable they will be studied further.

The third division was treated with gold chloride. A large precipitate of needles was obtained. These after recrystallization melted at 292°C. and appear to be the gold salt of ammonium chloride. After the solution had become quite concentrated a small quantity of flat yellow needles was obtained. They were not identified.

Fraction B, the alcoholic platinic filtrate from the platinum precipitate after the removal of the alcohol and platinum, gave a solution which reduced gold chloride with avidity. It reacted with potassium ferricyanide for ferrous iron. It was therefore made alkaline with silver oxide, the precipitate filtered off, and the silver removed from both precipitate and filtrate. The solution obtained from the precipitate upon concentration gave about 2 grams of ferrous chloride, as shown by its solubility in alcohol and water and the ferricyanide reaction. The silver filtrate after removal of the silver was slightly acidified with HCl, concentrated and treated with picrolonic acid. A precipitate was obtained in the form of fine needles which after several recrystallizations came to a constant melting point at 284°C. after previous sintering at 247°C. The weight was nearly 2 grams.

0.1993 gm. substance at 23°C. and 743.8 mm. (corr.) gave 50.1 cc. N.[6]
0.1009 gm. substance at 23°C. and 730.0 mm. (corr.) gave 26 cc. N.[6]

After another recrystallization the nitrogen content did not change.

0.1514 gm. substance at 18°C. and 754 mm. gave 37.8 cc. N.
0.0947 gm. substance gave 0.0453 gm. H_2O and 0.1592 gm. CO_2.
0.1003 gm. substance gave 0.0473 gm. H_2O and 0.1670 gm. CO_2.

	Calculated for $C_5H_{14}N_4(C_{10}H_8N_4O_5)$:	Found:		
		a	*b*	*c*
N	28.48	28.36	28.54	28.63
C	45.65	45.85	45.41	
H	5.58	5.38	5.27	

It would seem that a substance containing both a guanidine and an amino group, such as the calculated substance, should form a salt with two molecules of picrolonic acid. However the acidity of the solution, and the careful addition of the picrolonic acid,

[6] These nitrogen determinations were made by Mr. Jiklin.

may account for the precipitation of the substance as a monopicrolonate. In order to determine its molecular weight, an attempt was made to extract and weigh the picrolonic acid from a weighed portion after acidifying with HCl. But the hydrochloride of the base is also soluble in ether and sufficient came over with the picrolonic acid to make the result valueless. The picrolonate is very insoluble in cold water, and not readily soluble in hot water. The nitrogen and hydrogen contents place it among the substituted guanidines. A small quantity was taken up in hot water and treated with a saturated solution of picrolonic acid. A precipitate formed during the cooling. This was collected, dried and used for a nitrogen determination.

0.1169 gm. substance at 22.5°C. and 742 mm. gave 27.2 cc. N.

	Calculated for $C_5N_4H_{14}2(C_{10}H_8N_4O_5)$:	Found:
N	25.59	25.86

This substance is no doubt the dipicrolonate of the above substance. The analyses point to the mono- and dipicrolonate of either guanidine-butylamine or perhaps methylguanidine-propylamine. What the structural formula may be can be decided, definitely, only after further study.

After standing a few days the solution gave another precipitate in the form of small mounds, orange colored on the surface, light yellow inside. Some green-yellow microscopic crystals were also present. After six recrystallizations they melted at 276°C. The yield of pure substance was about 1 gram.

0.1065 gm. substance at 17.5°C. and 747 mm. gave 27.6 cc. N.
0.1454 gm. substance gave 0.0558 gm. H_2O and 0.2291 gm. CO_2.

	Calculated for $C_2H_7N_3(C_{10}H_8N_4O_5)$:	Found:
N	29.14	29.52
C	42.7	42.98
H	4.25	4.30

This substance agrees in melting point and analyses with the picrolonate of methyl guanidine. The filtrates, remaining from the recrystallizing methyl guanidine solutions, were concentrated. From them, besides more methyl guanidine, a picrolonate was obtained in the form of yellow-green microscopic needles. These

were recrystallized, and melted at 272°C.–277°C. They were very insoluble in water. The yield was more than 0.5 gram.

0.1427 gm. substance gave 0.0672 gm. H_2O and 0.2332 gm. CO_2.
0.1337 gm. substance at 16.5°C. and 750.8 mm. gave 32.2 cc. N.

	Calculated for $C_3H_9N_3(C_{10}H_8N_4O_5)$:	Found:
N	27.97	27.68
C	44.42	44.57
H	4.84	5.26

The analyses show it to be dimethylguanidine picrolonate and the melting point distinguishes it as being asymmetrical dimethylguanidine which melts between 275°C. and 277°C. The original solution from which the above three substances came gave another precipitate of microscopic crystals. After three recrystallizations, it came down in the form of larger red crystals. The yield was about 0.4 gram. They melted at 272°C.

0.1702 gm. substance at 17°C. and 747.5 mm. gave 45.5 cc. N.
0.1107 gm. substance gave 0.0414 gm. H_2O and 0.1650 gm. CO_2.

	Calculated for $CN_3H_5(C_{10}H_8N_4O_5)$:	Found:
N	30.35	30.49
C	40.83	40.65
H	4.02	4.19

This substance is doubtless guanidine picrolonate, as melting point and analyses show. The mother solution next gave about 0.2 gram of a picrolonate that decomposed in the melting tube at 119°C. It gave the reaction for neutral sulphur. A portion of the substance was allowed to stand in the sunlight for a day, after which it turned dark green. It decomposed in the air bath at 80°C. It is remarkable that this substance could have escaped decomposition from the manipulation, and reach this fraction. Finally a voluminous precipitate was obtained in the mother solution. After recrystallizing a few times it exploded in the melting tube at 348°C.

0.1094 gm. substance at 19.25°C. and 736.5 mm. gave 21.5 cc. N.
0.1023 gm. substance gave 0.0324 gm. H_2O and 0.1707 gm. CO_2.

After one more recrystallization the contents altered but slightly.

0.1100 gm. substance gave 0.0391 gm. H_2O and 0.1806 gm. CO_2.

	Found.	
	a	*b*
N	21.83	
C	45.52	45.1
H	3.55	3.98

This substance is not very insoluble in water. I am not certain as to its purity. The presence of ammonium chloride is suspected since upon drying in the vacuum tube at 120°C. a small white sublimation took place forming a film on the roof of the tube. Ammonium chloride would hardly be expected to be present in this fraction. The solution always had an acid reaction, with the exception of the time of its short exposure to Ag_2O; and ammonium chloride was previously removed quantitatively with platinum chloride. Its presence can however be explained by absorption of ammonia from the air.[7]

Fraction D, the alcoholic precipitate of substances insoluble in alcohol, after treatment with picrolonic acid gave a voluminous precipitate which after nine recrystallizations came down as long silky needles. They exploded at 355°C. and contained 21.38 per cent N, 44.14 per cent C and 3.5 per cent H. The substance which was very insoluble in water was not identified.

Histological changes.

The histological observations are mentioned here in so far as they appear to contribute to the interpretation of the presence of the bases found. The most striking histological changes occurred in the blood, liver, kidney and brain. The blood of the vena cava and heart of all animals showed extensive ante mortem coagulation. White clots in several cases were continuous from within the heart chambers down the vena cava to its iliac bifurcation. They nearly filled the lumen of the vessel. Upon section of the liver, the vessels showed fragmented erythrocytes, many normoblasts, erythroblasts with mitotic nuclei, and a small proportion of ery-

[7] Seven or eight dogs were stored in the room in which this work was carried on. The atmosphere was generally ammoniacal and often very strongly so. The acid solutions during manipulation had great opportunity to absorb ammonia. Sulphuric acid was spread about for a time but there was not sufficient room for these safeguards and they had to be abandoned.

throcytes that stained brilliantly in eosin; the remaining red cells in large areas were blood shadows. Each section of the liver and lung showed a number of large mononuclear cells with eosinophile granules. There were also present a larger number of large flat cells staining very intensely in eosin. These showed no definite granulation. In places they were found to line the smaller veins like endothelial cells. In these places no endothelial cells could be observed. The cells of the hepatic cords showed advanced fatty degeneration of the protoplasm. The nuclei of large areas had disappeared entirely in places where the cell form was fairly well preserved. Such areas were surrounded by circular areas of cells in which the nuclei had become densely stained clumps of chromatin. In the livers of four of the dogs only a diffuse chromatolysis could be observed.

All kidneys showed marked congestion and hemorrhage in the cortex, some anaemic, and others, congested medullae. Some glomeruli had lost Bowman's capsule and were hemorrhagic, others were markedly congested. In some of the convoluted tubes the epithelium had degenerated.

The spleen contained a large quantity of pigment. Some of the cells showed chromatolysis.

The lung showed oedema, congestion and the blood changes mentioned.

The brain sections, which I prepared in Professor Barrett's laboratory, showed cells in the motor areas with partial loss of Nissl substance and typical tetany nuclei. Various degrees of chromatolysis were also observed in these nuclei.

The intestinal tract besides marked congestion showed in the duodenum and pyloric end of the stomach disintegrating epithelial cells. Their nuclei were converted into solid deeply staining clumps. These appeared like those in the process of extrusion from the normoblasts.

Symptoms.

After the operations the dogs lived from three to five days. The wounds showed beginning healing and no infection. The postoperative period can be divided into two stages. The first stage was free from symptoms. The first portion of the second stage showed mild symptoms. The dogs were uneasy and excit-

able, and at times appeared markedly depressed. Their pupils were sometimes unevenly dilated, and their limbs showed tremors especially after slight exertion. The last portion of the second stage was introduced by mild convulsions. The animal would lie on its side, its limbs extended and rigid. The breathing was rapid and also deep. His trunk muscles showed tremors. At times, he was oblivious to his surroundings and at other times wide awake, and appeared anxious. Such a convulsion was generally followed by a period of lassitude and fatigue, but the limbs were always more or less rigid and showed intermittently violent tremors. Some animals recovered and for a number of hours appeared quite normal. The latter part of this stage was marked by severe tetany and clonic convulsions in which the animal struggled as if to free himself. Salivation always occurred at this stage. The breathing became difficult, as from severe constriction of the air passages, and the inspirations and expirations produced high pitched and loud sounds that could easily be heard in a neighboring room. The salivation, and breathing which gradually assumed the Cheyne-Stokes type, generally was followed by death within a few hours. In some cases the bladder was distended and full of urine, in others it was constricted until the cavity was nearly obliterated.

Discussion.

The occurrence of toxic bases in large quantities in the urines of parathyroidectomized dogs, observed for the first time in my work, warrants a discussion, especially since the current views regarding the function of the parathyroids make no allowance for their presence. All the urines studied contained methylguanidine. Where this substance was found in smaller quantities other guanidine bases were present, so that the excreted guanidine nitrogen approached a constant in all animals. In addition to these bases others were observed although not uniformly distributed. β-Imidazolylethylamine was found in three urines out of six, choline in three out of five and neurine in large amounts in one urine. In urine 5 two unidentified bases were found in the fraction where β-imidazolylethylamine was previously observed.

In order to determine whether any of these substances could have been split from larger molecules during analysis, the following experiments were performed.

Fifty grams of Witte's peptone were taken up in 95 per cent alcohol and the solution treated with a hot saturated alcoholic solution of potassium acetate and mercuric chloride. Some mercuric oxide formed. The mixture was hydrolyzed on the water bath under a return condenser for one-half hour, then treated with a hot saturated alcoholic solution of mercuric chloride and potassium acetate, and allowed to stand in a wide mouthed bottle for three days. A large proportion of the alcohol evaporated off. It was then placed in the cold for one day. The precipitate was filtered off and treated like that obtained from urine 5. No bases except ammonium chloride could be isolated. In order to learn if Witte's peptone would yield any bases if the hydrolysis were prolonged, the experiment was repeated, the hydrolysis lasting eight hours instead of one-half hour. Otherwise the manipulations were like those of urine 5. Besides ammonium chloride and ferrous chloride two bases were isolated from the fraction corresponding to fraction A of the urines, and another substance was found in fractions B, C, and D. There is evidence of the presence of other bases.

The picrolonate of the first substance decomposed at 130° C.

0.0965 gm. substance gave 0.0351 gm. H_2O and 0.1721 gm. CO_2.
0.0893 gm. substance at 22.5°C. and 734 mm. gave 20.2 cc. N.

	Calculated for $C_4H_6N_2(C_{10}H_8N_4O_5)$:	Found:
N	24.34	24.76
C	48.53	48.64
H	4.04	4.07

These analyses agree well with those calculated for methyl imidazol but more data is necessary for a positive identification.

The picrolonate of the second substance softened at 192°C. and decomposed at 238°C. It was freed from picrolonic acid and converted into the gold salt; these were needles, quite insoluble in water. They melted at 232°C.

0.0261 gm. substance gave 0.0120 gm. Au.

	Calculated for $C_5H_{12}N(AuCl_4)$:	Found:
Au	46.4	45.98

The substance appears to be neurine but requires further study.

The third substance became more insoluble in water with each evaporation of the alcohol during the removal of the inorganic

salts and ammonia, so that it became sparingly soluble in water. It formed a yellow-green solution from which it crystallized in long slender yellow needles. It is soluble in hot chloroform, soluble in alcohol and ether. It gives no insoluble gold, platinum or cadmium derivatives. It gives the iso-nitrile reaction for a primary amine but no insoluble benzoyl derivative. When the aqueous solution is made alkaline with sodium hydroxide cherry red develops which is intensified by standing or more rapidly by heating. If this test solution is heated a small precipitate forms, also an oil that smells strongly of mustard oil. A solution of the substance can be heated with mercuric oxide without change, but upon rendering it alkaline and again heating the odor of mustard oil is produced and the mercury blackens. The substance is auto-oxidizable with change of the sulphur containing group. Boiling water or hot dilute acids split it into an oil, soluble in the usual organic solvents but not in water, and a substance soluble in water and the ordinary solvents from which it crystallizes in needles. The latter give a copper derivative which melts at 237°C. Sufficient substance is at hand for perhaps a proper identification and the analyses will be reported when the study is completed. The substance is of interest because of its probable genetic relation to the guanidines. The presence of a similar substance in the urines of parathyroidectomized rabbits suggested to me the search for guanidines in these urines.

The interesting investigations of Vaughan and his co-workers demonstrate that bacterial protein, egg white, and Witte's peptone after alkaline hydrolysis yield a toxic substance soluble in alcohol.[8] These observers found that the substance was precipitable by platinum chloride though not in crystalline form. They could isolate no bases from it[9] and regard it a peptone.[10] Although the work of these and other investigators has not yet demonstrated that the above substances can be split from Witte's peptone I can find no other reason for their occurrence than that they must have been present in combination in the original peptone. However the uniformity in composition of various samples of Witte's peptone may perhaps be questioned.

[8] *Zeitschr. f. Immunitätsforschung*, i, 1909.
[9] *Journ. Amer. Med. Assoc.*, April 22, 1905; *American Medicine*, x, p. 145
[10] *Trans. Amer. Assoc. Physicians*, xxvi, p. 198, 1911.

It is to be noted that the treatment given Witte's peptone in these experiments was much more vigorous than that which the urines received. The first experiment with its comparatively vigorous treatment yielded no bases as are found in these urines. In the second experiment the prolonged hydrolysis produced what are apparently neurine and methyl imidazol, and a substance which may be related to the guanidines genetically. These experiments show that prolonged alcoholic alkaline hydrolysis of a fairly large quantity of a protein produces minimal quantities of basic substances. It is therefore evident that the small quantity of protein in the urines after its comparatively mild exposure during the manipulations, could not materially alter the yields of the bases found. It is regretted that imidazol derivatives were not suspected in these urines and that the diazo reaction was neglected both before and after the removal of the proteins. The bases found may be considered to have been excreted into the urine as such nevertheless.

The histological picture of cellular disintegration may account for the excretion of these bases as products of passive protein disintegration. But something must account for the initiation of the changes. For this reason two feeding experiments were performed, none of the other dogs having been fed the day before the operation or at any time after the operation. Experiment I was performed with the dog that gave urine 1. This animal after having recovered from a violent convulsive attack and appearing quite well was fed 300 cc. of fresh milk. He drank about 200 cc. Within thirty minutes he was again in convulsions which increased in violence with exceeding rapidity and lasted two hours, proving fatal.

Experiment II was performed upon dog 5. For two days this dog had no symptoms. I then gave him some fresh sterile beef broth. He drank about 50 cc. and showed no symptoms for thirty-six hours, when they were very mild. The dog showed no stupor, but irritability. He was again fed 25 cc. of the same broth diluted, forty-eight hours after the first feeding. Within one-half hour he had symptoms of stupor with the legs in tetany and the respiration labored. The tetany was so general that the heart beat was transmitted to the abdomen in such a way that this could be seen to throb with each systole. All the muscles

were in severe tetany within two hours after feeding. The dog was in a stupor and had lost all volition and appreciation of his surroundings. The eyes were bulged out, and the pupils dilated. Six hours after this feeding he had passed 300 cc. of urine, and two hours after this was wide awake and panting, his jaws snapped involuntarily and rigidity of his limbs gave way to tremors. He gradually recovered, could stagger about and drink small quantities of water. This he did quite often. I left him at about one o'clock a.m. in this condition. By morning he had passed 200 cc. more urine and appeared quite well. His breathing was rapid and he appeared much fatigued and excited. By noon the tetany again set in and the convulsions increased in violence until four o'clock p.m. when he died.

These experiments show that digested proteins taken into the body have very toxic effects after parathyroidectomy. These toxic effects are due to products of intestinal and perhaps also products of parenteral digestion. Such products of digestion are normally placed in some cell molecule or stored up in some form. In the case of these animals they are free and act as toxins. In other animals where no feeding occurred the symptoms increased in violence with short intermissions until death. The violence of the symptoms doubtless followed the rate of disintegration of the body protein. This disintegration had perhaps two sources, the preparation of units to supply cells for regeneration (these could no more be used than those received from the food) and the disintegration of the famished cells. The pathological condition would thus appear to be a failure upon the part of the cells to build up their protein. This part of the metabolism of the cell is regarded as a function of the nucleus. These indications together with the formation of free nuclein elements point to a nuclein atrophy. The histological findings moreover show an active nuclein degeneration. The extensive coagulation of the blood coming from organs rich in cells and nuclei, indicates the presence of free nucleic acid in the circulation, since nucleic acid coagulates blood plasma in acid solution. The acidity of the blood is indeed indicated by the absence of iron in the erythrocytes of the blood of this region as well as by the presence of a small proportion of erythrocytes that stain intensely in eosin.

The parathyroid secretion, therefore, appears to be concerned

with anabolic processes closely related with the building of nucleins. When facilities permit these investigations will be continued.

I take pleasure in expressing my thanks to Dr. Vaughan and to Dr. Novy for facilities for carrying on this work and I am also greatly indebted to Dr. Kollig, Dr. Beyer and Dr. Huber for operating on the animals under observation.