THE EFFECTS OF CONTINUED INJECTIONS OF METHYL GUANIDIN SULPHATE ON THE TOTAL NITROGEN EXCRETION AND URINE VOLUME

by

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Approved by:

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O. O.﻿#518
Head of Department
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CONTENTS

Introduction 1
Historical 2
General Discussion 14
Procedure 17
Protocols 21
Charts 25
Discussion of Results 30
Tracings 34
Conclusion 39
Bibliography 40
INTRODUCTION

In recent years a great deal of work has been done in an experimental line on the various aspects concerned in the removal of the parathyroid glands and more particularly the symptoms which appear following their removal and the relation of these symptoms to the symptoms observed following toxic doses of various poisons.

It would seem logical, therefore, that it might be worth while to study some of the effects which were demonstrated by continued injection of Methyl Guanidin Sulphate.
HISTORICAL

The close similarity of parathyroid tetany to so-called guanidin tetany has been observed by Paton and Findlay (1). They worked on a great number of animals and found that the difference in the symptoms was merely a difference in degrees. Fuhner, (2) found that the frog's muscle when placed in a modified Ringer's solution would soon develop twitchings upon the addition of guanidin in various concentrations. Camis (3) in reporting the work of Fuhner found that the Ringer solution employed by Fuhner would itself cause muscular twitching due to the low calcium concentration. By employing a solution with twice the concentration of calcium, Camis was able to cause muscular concentration by adding guanidin, the amount and the time of onset of the contractions varied according to the contraction of the guanidin added. Camis also found the twitching to occur in frogs after de-inervation of the muscles. Findlay and Paton found that guanidin solution up to .1 gram per kilo. caused tetany which could be relieved by calcium chloride. Burns and Watson (4) found that with venous injection of guanidin into cats, rabbits and dogs caused a partial paralysis of the vagus endings. These workers also reported that guanidin poisoning and parathyroid tetany differed in de-
Major and Stephenson (5) working on the effect of guanidin on blood pressure found a prolonged rise with a lowering of heart rate and a decrease of the respiratory. These findings have been confirmed many times in our laboratories here. They also found that this action could be abolished by calcium salts alone or accompanied by potassium salts. They found too, that the substances excreted normally have a prolonged and marked pressor effect. This is in agreement with the work of Burns and Watson (6).

Koch (7) noted the presence of guanidin in the urine of parathyroidectomized dogs.

Paton, and Findlay (8) described an increase urinary output of guanidin compounds after parathyroidectomy during tetany.

Sinalnicoff and Bovshik (9) found that if the guanidin was injected as the chloride in doses of .005 gm. per kilo, it would cause first a decrease in blood pressure then a sustained increase. If both vagi were cut the pressure rose still higher and the heart was accelerated. This effect they found to be obtained after section of the cord below the bulb, showing the effects to be on the peripheral nerves and muscular elements of the vessels.
The rise could be maintained after section of splanchnic nerves and ligation of abdominal aorta and inferior vena cava.

Burns and Watson (6) in the above experiments find that if guanidin is produced in excess, during tetany might cause a rise in blood pressure if not excreted by the kidney.

Collip (10) on experimenting with dogs, compared the results of animals in tetany from parathyroidectomy and those poisoned with guanidin, concluded that his results were opposed to the theory that the two were the same.

Teigs (11) gave a color test for detection of guanidin compounds.

Swingle and Nicholas (12) state that the symptoms occurring after subcutaneous injection of guanidin were hardly comparable to reactions of animals suffering with parathyroid tetany. They used cats and these animals after subcutaneous injection of guanidin showed spasms, convulsions and tremors but no tetany.

Dragstedt and Sudan (13) state there was little change in blood calcium after repeated injection of guanidin subcutaneously and that animals would show retching,
vomiting, tremors, depression, weakness and usually death. These results were also verified in our experiments.

McCallum and Vogel (14) state that tetany after parathyroidectomy may be due to a lack of calcium in the blood or the presence of a circulating poison like an oxalate that takes up the calcium or a substance like strychnine that renders the nerves hyperirritable. They were unable to maintain a high calcium level by the use of parathyroid extract nor were they able to lower the blood calcium in normal dogs by the use of an oxalate without poisoning the animal by the oxalate.

Greenwald (15) found that in parathyroid tetany the excretion of nitrogen was increased only after the appearance of tetany and that there was a marked phosphorus retention on the first few days after the removal of the parathyroids which was followed by an increased excretion of phosphorus during tetany.

Cooke (16) ran total nitrogen, ammonia nitrogen, volume and specific gravity on thyroparathyroidectomized dogs. He fasted the dogs after the operation and took the urine by a catheter, giving the animals only water by stomach tube. He found one animal that showed no changes in the urine, but at the death of this animal, a small nodule of parathyroid tissue was found.

Wilson, Stearns and Janney (17) found that para-
thyroidectomy there was usually a sudden diminution in the excretion of acids and ammonia and a decrease in ammonia to total nitrogen ratio. During tetany, ammonia excretion increased together with the increased value of the ammonia ratio.

Baknez (18) found that guanidin convulsions in rabbits were accompanied by a fall in blood sugar. Intravenous injections of glucose postponed but did not prevent these symptoms. Paton (19) in the same year stated that guanidin has a part in maintaining the tone of visceral muscles and that the secretion of the parathyroids regulate this action and also regulates the conversion of guanidin into creatinine, hence the lack of parathyroids causes guanidin poisoning.

De Waele and Blucke (20) state that guanidin injected into dogs and rabbits causes protein shock followed by vagal excitation, incoagulability of the blood, and increased metabolism. The fall in pressure was compensated for by vaso constrictor action which has the same localization as pituitrin and adrenalin. They found the vagal effect to be peripheral and of short duration. The effect of guanidin on striated muscle was investigated by Frank, Northman and Gultman (21), in 1923. They found that guanidin acts by increasing the excitability of striated muscle to drugs like choline if injected before hand. The action of guanidin is peri-
pheral as the phenomenon was obtained after section of nerves four days previous. Guanidin was said to produce effects similar to parasympathetic drugs. Sabovics (22) found the guanidin to produce symptoms in frogs, (tetany) as Paton and others found in mammals.

Komarrow (23) using dogs, found that guanidin was a secretory hormone to the salivary, gastric, mucous glands of the trachea and intestinal glands. It had a wide range of action in addition to exerting specific effects on respiratory and circulatory systems. Doses of 5-7 mg. per kilo, acted as a secretagog while 20-30 mg. per kilo, produced toxic effects as well. Creatine and creatinine up to a dosage of 1 gram per kilo, were devoid of these effects.

Working on the low calcium theory as the cause of tetany, Fuchs, (24) did not verify Paton's work that the symptoms of guanidin poisoning and parathyroid tetany were identical. He found the symptoms produced in cats by guanidin were not relieved by calcium injection. Nelken (25) found that during guanidin tetany there was not a diminution of calcium but rather an increase in the blood together with an increase of phosphate in whole blood. A year prior to this Bayer (26) found that guanidin poisoning causes in rabbits, cats and guinea pigs in a few hours a decrease in blood
calcium. This decrease he stated to be less than the decrease caused by extirpation of the parathyroid glands.

Klinger (27) confirmed the similarity of the symptoms of parathyroid tetany and guanidin poisoning but found the latter could not be relieved by calcium.

Watanabe (28) fixed the normal phosphate in rabbit serum as 2-4 mg. per 100 c.c. Following a large dose of guanidin this level rose to as high as 5 times normal. The calcium content he placed at 11-13 mg. per 100 c.c. of blood. Where guanidin was given the calcium content usually decreased. During guanidin tetany however, the calcium-phosphate ratio decreased and hypoglycemia occurred.

Burns (29) working along another line attributed anaphylactic shock to over activity of the vagus. He showed that since guanidin blocks the cardio-inhibitory mechanism, this drug was given to guinea pigs and rabbits either before or just following the development of anaphylactic shock with a marked diminution of the death rate.

Burns (30) in 1916 found a decrease in the total nitrogen excretion following complete parathyroidectomy in five dogs. However, he made observation on his dogs for a period of from 4-13 days, including the time prior
to operation. We have observed that in that length of time, the level of total nitrogen excretion would vary within wide limits. He doesn't state whether he ran the ammonia excretion on the twenty-four hour sample or on the sample taken daily by catheter. He found it almost impossible to prevent ammonia formation in the twenty-four hour sample collected from metabolism cages. In case I, Burns showed the total nitrogen for a three day period prior to injection of guanidin and then made one intramuscular injection. He found the average to rise from an excretion of 5.3 grams nitrogen per day to 6.5 grams a day. This rise has been verified by our work.

In another dog he obtained an increase of .3 gram per day above the excretion prior to injection, observations being taken for three day intervals before and after injection. In another case, his dog wouldn't eat in the cage so was placed in the kennels and was well fed. Good feeding for a few days would in itself increase the total nitrogen excretion. After analyzing the guanidin injected for nitrogen content, he found the total nitrogen excreted to contain a rise over and above the amount of nitrogen injected in the guanidin.

He made the same determinations on dogs that were parathyroidectomized instead of injected with
guanidin and concluded that the metabolic disturbance induced in the dog by the removal of the parathyroid glands and by the administration of a salt of guanidin to a fasting animal supports the evidence already given by Paton, Findlay and others that these two states are exactly identical.

Camus (3) as stated above, found that guanidin produces spontaneous twitchings in frog muscles but not a constant effect since it can be observed on denervated muscles. He thought that guanidin acts by combining with two different substances in the muscle, one substance being responsible for the twitches and the other for the changes in contractility.

Swingle and Nicholas (31) working on the effects of chemical compounds on the tetany syndrome, injected methyl guanidin sulphate, nitrate, trimethyl amine, ammonia, ergot, strychnine and creatinine, subcutaneously. These drugs if given orally were without effect. They gave from .2 to .5 gram of guanidin per kilo., subcutaneously to cats and death followed within twenty-four hours with tremors and spasms preceding death. Using normal animals they obtained no symptoms of tetany from any of the above drugs. They concluded that the symptoms produced by these drugs are comparable to symptoms following parathyroid removal. They studied the effects of injections of these drugs on
four cats that had been parathryoidectomized but had developed no tetany. These animals could not be thrown into tetany by the use of guanidin.

Dragstedt (32) in his work on the effects of diet on parathyroid tetany found that if a dog had an aciduric flora of the intestinal contents, the animal would not develop tetany, though such a derangement of body functions would eventually cause death. He thought the parathyroids keep down the toxemia of the body and if after their removal the toxins would not be allowed to develop, tetany would not develop.

Greenwald and Gross (33) conducted experiments over a long period of time. They placed animals on standard diet and injected parathyroid extract and measured the calcium and phosphorus excretion. They found these substances to be excreted over and above the amount injected. The rise in total nitrogen excretion was only 2.5%.

Salveson (34) states tetany is always due to low calcium. He thought milk would prevent tetany because it contains 1.78 grams calcium per liter. By precipitating the calcium from milk it is ineffective in parathyroid tetany. He concluded that the parathyroids control the calcium level of the blood,
so they function to influence muscle, nerve and all organs.

Luckhardt and Goldberg (35) found that dogs could be kept alive by massive doses of calcium lactate on a heavy meat diet indefinitely. They suggest that the increase in calcium may render the tissues less permeable to the nitrogenous toxins present in parathyroidectomized dogs.

Dragstedt, Phillips and Sudan (36) in experimenting with animals that had been kept on a milk diet following parathyroidectomy would be thrown into tetany by muscular activity, meat diet, constipation, high temperature, sexual excitement and injection. They (37) found, also, that parathyroidectomized dogs were much more susceptible to guanidin than were normal dogs. Normal dogs showed marked depression with doses up to .1 gram per kilo, while .05 gram per kilo would prostrate parathyroidectomized dogs. However, there was no tetany in any of the dogs. They would sneeze, tremble, cough and gasp, but there was no spasticity.

Dragstedt and Peacock (38) found that complete extirpation of the thyroids and parathyroids in dogs has no depressant effect on gastric secretion if the animal is kept from tetany by dietary control.
Collip (39) stated that in his opinion guanidin intoxication bears no relationship to parathyroid tetany. He noted conclusions of Paton and others that the two states are the same, but disagreed on the grounds that Nelken (25) and Salveson (34) could not confirm the statements of Watanabe (28) and others that the calcium in the blood decreases following guanidin injection. Collip observed guanidin intoxication in dogs coincident with parathyroid hormone overdosage.

Collip and Clark (40) found that during parathyroid tetany the non-protein nitrogen and urea in the blood remained nearly level, these two substances rose markedly in concentration following guanidin injection.

Greenwald (15) was unable to demonstrate a toxin in the blood of parathyroidectomized dogs.
While a great volume of work has been done on the parathyroid function, and the resulting tetany, there is still contention as to the facts brought out.

Paton and Findlay (1) and the men working in their laboratories maintain that parathyroid tetany and guanidin intoxication are identical. On the other hand, Collip (40), Dragstedt, (37) and others disagree. While there is a world of literature dealing with many phases of these symptoms, there remains many things that are by no means certain. Koch (7) found an increase in guanidin in the urine of parathyroidectomized dogs, but Greenwald (15) was unable to do so. Major and Stephenson (5) found a pressor substance in the urine of patients suffering from hypertension and intimated that these substances were guanidin or guanidin-like substances.

Burns (30) attempted to simulate the two sets of symptoms by his work on nitrogen metabolism. From his results he concluded that the two were the same.

Collip (39) found a marked increase in non-protein nitrogen in the blood of animals after guanidin injection. From that it would seem likely there would be an increase
in nitrogen in the urine. Greenwald (15) found this to be the case in tetany following parathyroidectomy. Burns (30) found an increase of total nitrogen excretion after parathyroidectomy and after guanidin injection. It seemed feasible that if the two conditions were the same, they by a long continued injection of guanidin, there should appear symptoms of tetany if normal dogs were used.

Burns (30) and others have noted an increase in metabolism following guanidin injection and this increase was borne out in our experiments. Nearly every worker gives the usual phenomena that follow guanidin injection in normal animals. Retching, attempts to vomit, defecation, gasping and general discomfort were the usual symptoms noted. Our animals would usually sneeze or cough before we had completed the injection.

In the light of the present evidence and lack of other, it seemed wise to determine the urine output over a long period of time and measure the total nitrogen excreted, and by using suitable controls, to determine if there is an actual increase or merely an increase due to the total nitrogen injected.

It was thought that since guanidin causes a marked rise in blood pressure, that it would be worth while to
study its effect on the kidney both as to urine output, and kidney volume. So far as we can learn there has not been a great deal of work done along this line. Our experimental results will follow.

In none of the animals used were any symptoms of tetany observed from the guanidin injection, though doses of .06 - .08 gram per kilo. was administered daily for ten to fourteen days consecutively.
PROCEDURE

Dogs were used. The animals selected were taken from the kennels, weighed and placed in metabolism cages. After a few days on a bread and milk diet, the urine was measured and tests for total nitrogen were run by the Macro Kjeldahl method. The same sample was run different times until the method was learned and results from two tests on the same sample would not vary more than 2%. It was thought that calculations this close would give accurate results, and if any error was present it would be the same on all samples.

The animals were left in the cage over the Christmas vacation and no urine was measured. Beginning January 5, 1927, tests were run daily on two or more of the dogs. On January 11, 1927, the animals were placed on standard diet consisting of 100 grams fresh beef heart (ground), 2 grams salt, 10 grams Kaolin, 15 grams cooked starch, 20 grams lard and enough water to make up to 200 grams when cooked. This was then mixed with 400 c.c. tap water and fed daily. Only during part of the experiment was water placed in the cages.

The animals remained in very good condition, though
they all lost weight. In the last month of the experiment the ration was increased one-third.

Thymol was used as a preservative for the urine being placed in the collecting bottles. The kaolin rendered the feces very hard so the cages were dry. They were thoroughly scrubbed two or three times a week, and glass wool was used to prevent any sediment from entering the bottles.

Several experiments were tried such as feeding the diet and giving the animals water ad libitum. The animals under those conditions would drink very little water, but the excretion of nitrogen remained somewhere near constant though the urine volume decreased to one-fourth.

Injections of guanidin were made by using 10% methyl guanidin sulphate freshly made up in .9% sodium chloride and warmed. The injection was made aseptically, using alcohol as the antiseptic. Only in one case did infection appear and that was in a dog that was injected subcutaneously and died from injection. The specific gravity was also measured but since it varied almost exactly with the water intake, graphs of it were not shown. The animals were kept on standard diet from January 11, 1927 to May 3-5, 1927, at which time complete parathyroidectomy was performed.
Dog IA had died March 27, 1927 at a period when subcutaneous injections were being given. Dog II died during the operation, and Dog III lived one day and Dog IB lived for about eight days, being in tetany nearly all of the time, excreting no urine except mixed with feces so it was impossible to analyze that. The glands were fixed and stained and will be discussed later.

Histologically, the parathyroids removed from these animals showed very little change from the normal. There was a slight hyperplasia of the adenomatous cells, but not enough to prove an actual hypertrophy. It is very doubtful that even continued injection of these drugs used would cause an absolute hypertrophy.

Since writing the early part of this paper the writer has had a personal communication with Weber (42) who is working out a method of recovering guanidin from the body fluids. His method will be published in the near future. He found however, that guanidin injected into the body of either man or lower animal, is rather quickly excreted, whether taken by mouth or hypodermically. He has been able to recover about 92% from a solution of known strength with his method. With this definite percentage, he can compute the amount in the various body fluids. He has found also that in various known kidney
lesions, there is a retention of guanidin and now in the Bell Memorial Hospital, Kansas City, Kansas, guanidin is being used as a kidney function test along with creatinine tests.
PROTOCOL.

Dog TA
Weight 12 kilo.
Male.

<table>
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<tr>
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<tbody>
<tr>
<td>Dec. 5-11</td>
<td>Not on standard diet</td>
<td>341</td>
<td>3.07</td>
</tr>
<tr>
<td>Dec. 11-17</td>
<td>Not on standard diet</td>
<td>433</td>
<td>3.07</td>
</tr>
<tr>
<td>Dec. 17-Jan. 6</td>
<td>Holiday. Dogs kept on bread and milk.</td>
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<td></td>
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<td>486</td>
<td>3.14</td>
</tr>
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<td>Jan. 15-23</td>
<td>Dogs on standard diet. Injected during period with 4.5 gm. guanidin sulphate</td>
<td>415</td>
<td>3.90</td>
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<td>Jan. 23-Feb. 15</td>
<td>Urine volume taken.</td>
<td>421</td>
<td>Not run</td>
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<td>Feb. 16-23</td>
<td>Food given dry. Water in cage.</td>
<td>108</td>
<td>1.86</td>
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<tr>
<td>Feb. 24-Mar. 12</td>
<td>Water in cage, 10 gms guanidin injected.</td>
<td>151</td>
<td>2.29</td>
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<td>Mar. 13-17</td>
<td>Food and 400 c.c. water. No injection.</td>
<td>417</td>
<td>2.82</td>
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<td>Mar. 18-24</td>
<td>Food ration raised one-third. Dog injected 4.5 gms. guanidin subcutaneously</td>
<td>501</td>
<td>3.3</td>
</tr>
<tr>
<td>Mar. 25-27</td>
<td>Dog found dead in cage. Thyroids removed and sectioned.</td>
<td>530</td>
<td>3.56</td>
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Dog IB  
Weight 11 kilo.  
Male.

<table>
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<tr>
<th>Date</th>
<th>Remarks</th>
<th>Av. Nitrogen Vol. in grams.</th>
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<td>Apr. 17-19</td>
<td>Fed in cage on standard diet.</td>
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<td>Apr. 19-23</td>
<td>Normal on standard diet</td>
<td>451</td>
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<tr>
<td>Apr. 24-30</td>
<td>Injected 3.9 gm. guanidin</td>
<td>387</td>
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<tr>
<td>May 1-3</td>
<td>Injected .65 gm. guanidin</td>
<td>468</td>
</tr>
<tr>
<td>May 3</td>
<td>Operated. Died.</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Remarks</td>
<td>Av. Nitrogen Vol. in grams</td>
</tr>
<tr>
<td>------------</td>
<td>------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Dec. 5-11</td>
<td>Not on standard diet</td>
<td>407 2.03</td>
</tr>
<tr>
<td>Dec. 11-17</td>
<td>Not on standard diet</td>
<td>427 3.07</td>
</tr>
<tr>
<td>Dec. 18-Jan 6</td>
<td>Fed on bread and milk</td>
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<td>Jan. 6-14</td>
<td>Not on standard diet</td>
<td>436 2.73</td>
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<td>Jan. 15-28</td>
<td>Fed on standard diet 4.5 gm. guanidin injected</td>
<td>413 3.85</td>
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<td>Jan. 23-Feb 15</td>
<td>Only urine taken, dogs on standard diet</td>
<td>465 --</td>
</tr>
<tr>
<td>Feb. 15-23</td>
<td>No water in food, water in cage</td>
<td>264 2.25</td>
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<td>Feb. 24-Mar. 12</td>
<td>Water in cage, 10 gm. guanidin injected.</td>
<td>181 2.52</td>
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<td>Mar. 13-17</td>
<td>Food and 400 c.c. water</td>
<td>452 2.94</td>
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<td>Mar. 18-24</td>
<td>Food ration raised one-third, dog injected 4.5 gm. guanidin subcutaneously.</td>
<td>536 3.21</td>
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<tr>
<td>Mar. 25-Apr. 2</td>
<td>Dog injected with 2.25 gm. guanidin subcutaneously.</td>
<td>458 3.04</td>
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<td>Apr. 3-14</td>
<td>Dog injected intravenously 6 gm. guanidin</td>
<td>460 3.20</td>
</tr>
<tr>
<td>Apr. 15-23</td>
<td>Injected 4.5 gm. guanidin 601 3.61</td>
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<tr>
<td>Apr 23-May 3</td>
<td>5.25 gm. guanidin intravenously. Died from ether.</td>
<td>487 320</td>
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Dog III  
Weight 16 kilo.  
Male.

<table>
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<tr>
<th>Date</th>
<th>Remarks</th>
<th>Av. Nitrogen vol. in grams</th>
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</thead>
<tbody>
<tr>
<td>Dec. 5-11</td>
<td>Not on standard diet</td>
<td>323 3.7</td>
</tr>
<tr>
<td>Dec. 11-17</td>
<td>Not on standard diet</td>
<td>500 3.7</td>
</tr>
<tr>
<td>Dec. 17-Jan.6</td>
<td>Holiday. Dogs kept on bread and milk</td>
<td>490 3.65</td>
</tr>
<tr>
<td>Jan. 6-14</td>
<td>Dogs on standard diet</td>
<td>398 2.8</td>
</tr>
<tr>
<td>Jan. 15-Feb.15</td>
<td>Only urine taken. Dog on standard diet</td>
<td>472 -</td>
</tr>
<tr>
<td>Feb. 16-23</td>
<td>Water in cage. Dog on standard diet</td>
<td>146 2.64</td>
</tr>
<tr>
<td>Feb. 24-Mar.12</td>
<td>Water in cage.</td>
<td>123 2.04</td>
</tr>
<tr>
<td>Mar. 13-17</td>
<td>400 c.c. water added to feed</td>
<td>482 2.45</td>
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<tr>
<td>Mar. 18-24</td>
<td>Food ration raised one-third</td>
<td>515 2.65</td>
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<td>Mar. 25-Apr.2</td>
<td>Standard diet</td>
<td>540 2.65</td>
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<tr>
<td>Apr. 3-14</td>
<td>Standard diet</td>
<td>510 2.80</td>
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<td>Apr. 15-23</td>
<td>Standard diet</td>
<td>544 2.45</td>
</tr>
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<td>Apr. 24-May5</td>
<td>Standard diet</td>
<td>535 2.67</td>
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<tr>
<td>May 5</td>
<td>Thyroparathyroidectomy.</td>
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<tr>
<td>May 5-6</td>
<td>Standard diet</td>
<td>360 3.78</td>
</tr>
<tr>
<td>May 6</td>
<td>Dog died</td>
<td></td>
</tr>
</tbody>
</table>
Graph of Nitrogen Excretion

Gas. of Nitrogen excreted daily.

- 4 gm.
- 3 gm.
- 2 gm.
- 1 gm.
- 0 gm.

Dog I - Red
Dog II - Black
Dog III - Purple

* - dog died
Explanation of Graph on Opposite Page.

The graphical results are shown on the Opposite page. Dogs IA and II, were injected at intervals with guanidin and at the same time Dog III was injected with physiological salt solution. The graphs show the total nitrogen excretion in grams over a period of approximately four months. It will be noticed that on the usual diet, Dog III excreted normally more nitrogen than Dogs IA and II, but on a standard diet it ran very nearly parallel.

On January 14th, Dogs IA and II were injected with .06 gm. per kilo. of methyl guanidin sulphate, and the injection repeated each day for thirteen days. It will be seen that the total nitrogen excretion rapidly rose while the control continued to fall.

On February 15th, the food was given without being mixed with water, but water was placed in the cages. The dogs passed very little urine, but under these conditions both IA and II showed a sharp increase in nitrogen output with little or no increase in urine output following injection of guanidin beginning February 23rd.
On March 18th, the food ration was raised 33-1/3% and all the dogs showed an increase in nitrogen out-put with a very slight increase in urine volume.

On April 2nd, subcutaneous injections were started and following this there was a rise in the nitrogen excretion but not so marked.

The three graphs run closely parallel except periods when the two dogs were injected and then there is a wide range of difference.
Amount of urine excreted daily.

- Dog IA Black
- Dog II Purple
- Dog III Red

* Dog died
Explanation of Graph on Opposite Page.

This chart shows how very closely the urine volume of the three dogs was parallel under similar conditions.

Exactly the same amount of food was given to the three animals and the same amount of water except from February 15th to March 12th, when water was placed in the cages.

It will be seen that while the total nitrogen excretion could be changed by the injection of guanidin there was little or no change in the total amount of urine passed over a long period of time.

Guanidin was injected at intervals beginning January 15th, March 12th, March 18th, March 25th and April 3rd. The urine volume varied almost exactly with the water intake.
DISCUSSION OF RESULTS.

From the above results, it would not seem that long continued injection would cause such marked increase in nitrogen excretion as a single dose would. Burns (30) found one dose of .7 gram guanidin to cause an increase of 4.7 grams of nitrogen, 6.4 grams guanidin to give a rise of 2.1, .9 gram a rise of 1.5 and 1.2 grams to give a rise of .1, all the above amounts to be over and above the nitrogen contained in the guanidin. His results agree with those obtained by us in the main. However, after several weeks of injection, there was not such a pronounced increase as at first. This pointed to a possible hypertrophy of the parathyroids and hence those glands were sectioned.

The urine volumes ran closely parallel except when water was placed in the cages. It will be noticed that in the Chart I (Nitrogen) Dog III had the highest rate of excretion and this dropped after placing it on standard diet while in the same period the nitrogen excretion rose in Dog II. Since these dogs came from stray cur dogs, the diet might have been different previous to this.

The animals when injected and for six to eight
hours after, were markedly restless and uncomfortable. They coughed, retched, sneezed and became popeyed, but usually salivated. The animals in no way showed any tetany or respiratory difficulty, save the choking at first. During the latter part of the experiment, Dog II was affected very little by a dose of .45 gram of guanidin, since it would eat after the injection.

Dog II, however, was very sensitive to guanidin and became very sick, and depressed. It excreted normally 4.25 grams nitrogen per day on standard diet, but this rose to 5.41 grams after the injection of .06 gram per kilo, and fell the last few days to 4.60 though the injections were kept up.

Easu working in this laboratory determined the calcium and phosphorus in the urine and feces of these animals. This will be given in a separate paper.

Since no symptoms of tetany were observed and since no noticable increase of urine was observed, it seemed probably that guanidin per se. is not a diuretic and that guanidin poisoning is not identical with tetany. In the latter part of this paper will be seen charts to show the graphical effects of guanidin injection on kidney function and urine flow on anaesthetized animals. De Waele and Bulcke (20) state that
guanidin intravenously has the same localization as pituitrin and adrenalin. Working on the kidney volume, it is shown that guanidin does not cause the characteristic constriction of the kidney as observed by Isenberger (41) and others. Another interesting thing observed was, viz., after the injection of guanidin, pituitrin caused not a rise in blood pressure but a fall.

Since Dog III was larger than Dogs IA and II, it excreted more nitrogen in the same unit of time before injections were started. However, during the 84 day period in which urine and nitrogen were measured, and in which the three dogs got exactly the same food and water, Dog IA excreted 18.87 grams more nitrogen than did Dog III, and Dog II excreted 31.86 grams more. Deducting the 6.3 grams of nitrogen as was contained in the guanidin, a total increase of 12.5 grams and 25.58 grams in the two dogs was observed. The total volume of urine too was greater in both dogs that were injected for the 84 day period, Dog IA excreting 756 c.c. more than control Dog III and Dog II excreting 2150 more than control Dog III. Since the dogs were not injected each day but series of injections were made, the period after each group of
injections finds a decrease in the nitrogen of the urine. The accompanying charts show the curves during the different series.
The above graph shows the characteristic rise in blood pressure that follows the injection of methyl guanidin sulphate. The kidney volume does not show the contraction that it does following the injection of pituitrin.

With a cannula in both ureters, the urine drops were counted and this graph shows the increase in drops. This drug is not an active diuretic as was shown by the urine volume over a long period of time. The initial increase was very constant, however.
This graph shows also the characteristic rise that follows the injection of rather a large dose of guanidin. Also, the customary slowing of the heart that is noted in injections of adrenalin is shown here. The rise in blood pressure is much longer sustained with guanidin that it is with adrenalin.

The initial increase in kidney activity is shown here by the increased urine flow.
This chart shows the characteristic rise in blood pressure following the injection of methyl guanidin sulphate.

The increase in urine flow is also shown as is the slight variation in the kidney volume.
This chart shows the effect of pituitrin on the blood pressure, and the urine flow. It will be noticed that this effect is obtained before guanidin is injected. There is a prolonged rise in pressure and a contraction of the kidney with no increase in the urine flow. In the next chart will be seen the effects after guanidin has been injected.
This chart shows the effects on the blood pressure and kidney volume of pituitrin following an injection of guanidin. Guanidin produces a prolonged rise in pressure that is reduced by pituitrin. The effect on the kidney volume is greater than with pituitrin alone. The urine is suppressed by the pituitrin. This same phenomenon was observed many times and this sort of graph was obtained.
This chart shows the effects of liver extract on the blood pressure. The high blood pressure was brought on by the injection of a small amount of guanidin (75mg.) According to the directions, the amount of liver extract used should neutralize this artificial hypertension.

It had been suggested that it was the preservative in the extract that had the depressor action. Accordingly, tricresol was injected, but had very little effect. While the liver extract did have a depressor action, the pressure quickly returned to the initial reading. This was tried in twelve cases and similar tracings were observed.
CONCLUSIONS

1. Continued injection of methyl guanidin sulphate produces an increase in protein metabolism over and above the nitrogen contained in the guanidin.

2. The nitrogen metabolism returns to normal shortly after the injections are stopped.

3. Continued injections of rather large doses of guanidin produce no symptoms of tetany.

4. Guanidin produces a temporary increase in urine secretion but over long periods of time, the flow is rather constant.

5. There was no evidence of accumulation of the guanidin in the animals after prolonged injections, as shown by normal excretion.

6. Liver extract when injected, lowered temporarily the hypertension produced by the guanidin, but the effects were only transient.

7. Pituitrin, when injected after guanidin, produces a fall in blood pressure.
BIBLIOGRAPHY

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