A STUDY OF THE EFFECTS OF GUANIDINE COMPOUNDS ON THE THYROID GLAND OF THE RAT.

by

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INDEX.

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INTRODUCTION.

The role of the thyroid gland in the maintainance of body immunity against bacterial toxins and other poisons has been studied for many years. As early as 1898 Roger and Garnier (1) reported that pathologic changes were produced in the thyroid of patients with acute infectious diseases, and Halsted (2) found that infected wounds in dogs were apt to produce a hyperplasia in that gland. Marine and Lenhart (3) observed that the microscopic picture of the thyroid gland removed from patients dead from acute infection occasionally cannot be distinguished from the type of hyperplasia seen in exophthalmic goiter. Billings (4) has called attention to the relation of focal infection to thyrotoxicosis; Evans, Middleton, and Smith (5) believe some types of goitre may be attributed to focal infections; and Beck (6) considers that coincidence cannot explain the frequent association of foci of infection with thyroid deficiency.

Burget (7) observed hyperplasia and loss of colloid of the thyroid gland in rats kept under unhygienic conditions. Jones and Rush (8) produced hyperplasia, hypertrophy, and loss of colloid of the thyroid of rabbits after intraperitoneal administration of a sterile saline extract of rabbit feces. The degree of hyperplasia was approximately parallel

to the amount of fecal extract given. The response, they stated, was a physiological increase in the gland, not a direct detoxicating function.

McCarrison (9) holds that the toxic effects of micro-organisms exert a very prominent influence in the production of simple goitre, and cites (10) an instance in his experiences in India in which an epidemic of goitre was eradicated by changing the water supply, whose iodine content was originally high, to that with a low iodine content. He remarks that "it (the thyroid) exercises a protective antitoxic and immunizing action, defending the body not only against the toxic products of its own metabolism, but against invasion by disease-producing microorganisms and injury by their products." Pickworth (11) found hyperplasia, loss of colloid with desquamation and an abnormally low percentage of iodine in the thyroid gland of persons dead from acute infectious diseases. He suggests that thyroxine, by virtue of its imine group, can combine with amine groups, and in this way function in the metabolism of waste tissue products and toxic amines. Methylization of the imine group removes the physiologic properties of thyroxin.

Cole and Womack (12 and 13) produced in dogs intestinal obstruction, peritonitis, empyemia, and acute cutaneous infections. The thyroid glands of the animals dying from these

infections and toxemias, in nearly all cases, showed hyperplastic changes accompanied by loss of colloid and desquamation of the epithelium of the acini. These changes could be prevented by the administration of Lugol's solution (14).. Womack, Cole, and Heideman (15) demonstrated that during the acute cutaneous infectious processes in dogs, the basal metabolic rate rises sharply. An almost constant correlation was found to exist between the basal metabolic rate and the severity of the infection as judged by the physical condition of the animal and the extent of the inflammatory process. The rise in metabolic rate on the first and second day, following the injection of bacteria and toxin, was usually entirely out of proportion to the rise in temperature, and frequently there occurred a basal metabolic rate 30% to 40% above normal without a rise in temperature. Large doses of histamine and glycine produced alterations in the thyroid gland similar to those seen in infections. These substances also caused an increased basal metabolic rate, but no fever. The authors suggest that possibly the demands made by other organs in the body on the thyroid for its catalytic secretion during periods of excessive strain are sufficient to alter the basal metabolism. This assembled data indicates that the thyroid takes an active part in the resistance of the body to certain infectious processes and certain toxins.

The protein molecule contains a few groupings known to possess toxic properties and guanidine is one of these groupings. Paton (16) states that guanidine may be produced from the cholin of lecithin of the liver. Pathologically the compounds of guanidine have considerable interest from many points of view. Koch (17) found guanidine compounds in the urine of parathyroidectomized dogs, and Burns and Sharp (18) reported increased amounts of the base in the blood and urine of similarly treated dogs, but Greenwald (19) reported that no guanidine was excreted in the urine of parathyroidectomized dogs. Paton and Findlay (20) asserted that the symtoms of tetania parathyreopriva are identical with those of guanidine intoxication, but this statement has since been denied (27 and 28) Methyl guanidine has been isolated from the feces and urine of children with idiopathic tetany (21). Major (22) believes there is a possible relationship between guanidine metabolism and hypertension in man. Stoland (23) found that the tonus of smooth muscle was increased by guanidine. According to Heyde (24 and 25), fairly large quantities of methylguanidine are reported to occur in the urine of animals killed by burning or by anaphylactic shock. Burns (26) observed that methylguanidine protects sensitized animals from toxic doses of antigenic protein, indicating a desensitizing action; and to the contrary it has been suggested (24 and 25) that the poison producing

protein shock may be methylguanidine formed by the toxicogenic destruction of protein.

Because of the widespread interest in guanidine and methylguanidine, their toxicity, and their possible origin from substances occuring naturally in the animal body, it seemed that a study of their relationship, if any, to the thyroid gland would be desirable. It has already been indicated that this gland takes an active part in the resistance of the animal body against certain toxins.

METHOD.

The white rat was selected as the experimental animal to use for the study of the effects of guanidine and methylguanidine sulfate on the thyroid gland. Rats of approximately the same age were used in each series. They were kept in cages in an iodine-free room where the temperature was maintained constant (65°-75°F.) throughout the course of an experiment. The cages were eually illuminated by sun-light, and were cleaned three times each week. The rats received a daily diet of bread soaked in milk, and once each week lettuce or carrots were given. Water was supplied freely. As a criterion of the physical condition of the animals, a daily record was made of the weight of each.

Guanidine carbonate (or hydrochloride) and methylguanidine sulfate were dissolved in a 0.9% solution of sodium chloride in water, the saline having been previously sterilized in an autoclave. A fresh solution of each compound was prepared daily. Administration was made in all cases by injection subcutaneously in the lower ventro-lateral abdominal region. Aseptic conditions were maintained, and the site of injection was sterilized by the application of ethyl alcohol.

In each of the three series the rats were divided into three groups of about equal size. One group received methyl guanidine sulfate, another group received guanidine carbonate

(or hydrochloride) and the third group was kept as control animals, receiving injections of physiologic saline containing no guanidine. The amount of guanidine carbonate and methylguanidine sulfate was so adjusted that each group respectively received equivalent amounts of guanidine base per kilogram of body weight regardless of the absolute amount the compound used. Furthermore, the concentration of the guanidine salts in their solutions was so adjusted that each group received not only equivalent weights of guanidine base per kilogram of body weight, but also equal volumes of solution per kilogram of body weight. The control animals received this same volume of saline (containing no guanidine).

Guanidine carbonate, although slightly soluble, is irritant because of its alkalinity. When concentrated solutions of the non-methylated guanidine salt were desired, the hydrochloride was used. This was formed by adding to a measured amount of guanidine carbonate, just enough (determined by calculation) hydrochloric acid to completely replace the carbonate radical by chloride. The resulting solution was only slightly irritant. From the investigation of Alles (29) it may be assumed that the physiologic action of guanidine carbonate and guanidine hydrochloride are not unlike.

At the end of the period of guanidine administration, the rats were anesthetized with chloroform and bled to death.

The right thyroid gland and other tissues to be examined, were immediately removed and placed in a fixative solution consisting of 10% formaldehyde in 80% ethyl alcohol to each 100 c.c. of which had been added 1.5 c.c. of glacial acetic acid. However, the tissues from Series II were fixed in formalin-Zenker. The tissues were dehydrated in alcohol, cleared in xylol, infiltrated with and imbedded in paraffin, and sectioned 7.5 micra thick. Complete serial sections were made of each thyroid gland. The ordinary haematoxylin and eosin method was used for staining.

The left thyroid of each rat was placed in a moist weighing bottle, weighed within five minutes after its removal, then dried in an oven at a temperature of 55°C. and finally the weight of the dry gland was determined. The dessicated glands were used for the determination of their iodine content. Due to the small size, the amount of iodine in a single gland could not be determined with the methods available. Therefore the iodine determination was done on a group of glands, the group being composed, in each series, of the thyroids of rats which had received like treatment; i.e., in Series I, a single determination was made of the iodine content of the thyroids of that group of rats receiving guanidine carbonate. Another determination was made of the iodine content of the thyroids of that group of rats

which had received only saline, etc.

The amount of iodine in each group of thyroids was too small to be determined satisfactorily by titration methods, and the combustion apparatus for McClendon's (30) colorimetric procedure was not available. Hence, with some modifications, the alkalie-ashing method of Pickworth (31) was substituted for McClendon's oxygen combustion chamber, and the latter's process for extracting sodium iodide from the ash was also somewhat changed. The following combination and modification of these two methods was used in this work for the determination of iodine in the thyroid glands: The desiccated thyroids were placed in a nickel crucible of about 150 c.c. capacity and equipped with a cover containing a 1/8 inch hole. They were then finely pulverized with the smoothed end of a glass rod. 5 c.c. of a 50% solution of sodium hydroxide was added, and mixed with the thyroids by carefully rotating the crucible. It now was covered, and slowly heated to dryness; then heated in an oven considerably below red heat but hot enough to yield a clear fusion product in about one hour. The crucible was next heated in a furnace by a large free flame so that crucible and lid were rapidly raised to a dull red heat, which was maintained one-half to one minute, but not longer.

The crucible was then allowed to cool, about 35 c.c. of

water added, and placed on a hot-plate for about one-half hour, when gentle stirring brought the whole into solution. The solution was now neutralized slowly with phosphoric acid and again rendered distinctly alkaline with sodium hydroxide. The crucible was then placed on a hot-plate until most of the water had evaporated, leaving a mass of crystals mixed with water. From this mass the iodine (and some sodium hydroxide) was extracted by adding three successive times 30 c.c. of aldehyde-free 95% ethyl alcohol, and heating just to the boiling-point. The alcohol was removed by decantation, and transferred to a small Erlenmeyer flask.

(The aldehyde-free alcohol was prepared in the following manner (32) : Dissolve 1.5 grams of silver nitrate in 3 c.c. of water, and add to one liter of 95% ethyl alcohol in a stoppered glass cylinder, shake, and let stand for fifteen minutes. Dissolve three grams potassium hydroxide in 10-15 c.c. warm alcohol and add slowly to glass cylinder but do not shake. Let it stand over night or until the precipitate of silver oxide has completely settled. Filter and distill.)

The alcoholic extract was evaporated to dryness, taken up in about 2 c.c. of water, and the solution neutralized with phosphoric acid (to which one-fifth volume of one-tenth normal sulfurous acid had been added), using an indicator paper made by drying an alcoholic solution of methyl-orange

on ash-free filter paper. The solution was heated to boiling to expel carbon dioxide and sulfur dioxide and cooled. It (and subsequent washings) was then transferred to a 12 c.c. separatory funnel, and the volume made up to 10 c.c. One c.c. of purified carbon tetrachloride and one milligram of sodium nitrite was then added, and the separatory funnel shaken 100 times; (during the shaking 85/95 of the iodine should pass into the carbon tetrachloride, coloring it pink, so that by multiplying the yield by 1.118 the total iodine in the sample may be calculated). The carbon tetrachloride was cloudy with water droplets, so it was run into a 1 c.c. glass-stoppered bottle, centrifuged, then transferred to the left cup of a Bausch and Lomb micro-colorimeter and set at 20 millimeters. Carbon tetrachloride containing pure iodine of the concentration 0.01 milligram per c.c. was placed in the right cup and a color match made. Ten readings were made and their averages taken. The reading in millimeters divided by 2000 and multiplied by 1.118 gave the yield of milligrams of iodine.

Check determinations were made with known amounts of iodine. The errors by this method were 2%-3%.

Technical carbon tetrachloride used in the analysis was purified by the procedure described by McClendon (33): "Add bromine to the carbon tetrachloride, let stand in the sun a week, wash with sodium hydroxide solution, then tap water,

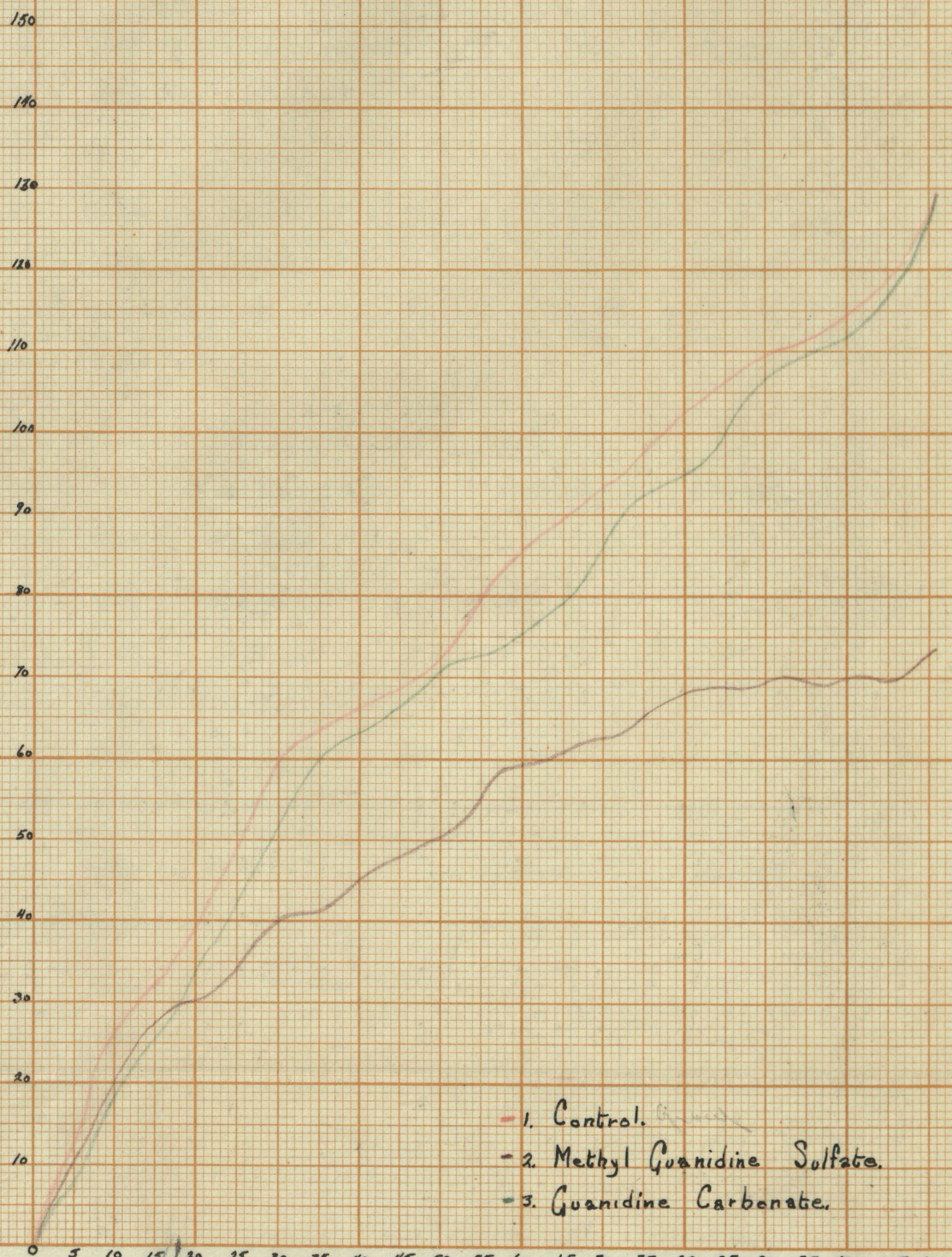
then distilled water, filter thru paper, dry by filtering through plaster of Paris, and then distill, rejecting the first part of the distillate, and any part above 70°C."

EXPERIMENTAL.

The effects of small doses of methyl guanidine sulfate and guanidine carbonate over a long period of time was first determined. Sixteen rats about three months of age (number 1 to number 16) were used in this experiment. (Series I). Rats 1 to 6, inclusive, received the methyl derivative; rats 7 to 12 were injected with a solution of guanidine carbonate, and rats 13 to 16 were the control group, receiving only physiologic saline. The animals were weighed daily from March 11 to March 28 to determine their health. Daily injections were begun on March 29 and continued until June 29, a period of 93 days. The rats were killed on the last day injections were made. The amount of methyl guanidine sulfate administered was 0.020 gram per kilogram of body weight, and the solution contained the salt in a concentration of 0.005 gram per c.c. The dosage of guanidine carbonate was 0.015 gram per kilogram of body weight and the solution contained 0.00375 gram of the dry salt per c.c.

Evidence that the dosage was large enough to produce some physiologic effects is seen in the growth curves, Figure 1. The rate of growth of the control group is much in advance of that group receiving methyl guanidine sulfate, and somewhat greater than the rate of those rats receiving guanidine carbonate. These differences are no doubt due to the toxicity

SERIES I.



Injections begun.

Figure 1.

SERIES I.

Rat	Sex	Weight in grams	Am't of Guanidine Received in ninety three days	Appearance of Thyroid	Appearance of Parathyroid.
1	M*	-	-	Animal Accidently killed	
2	M	395	0.615 gram	Normal	Normal
3	M	276	0.423 "	"	"
4	M	256	0.365 "	"	"
5	M	239	0.340 "	"	
6	M	235	0.350 "	"	Normal
			Am't of Guanidine Carbonate received in ninety three days		
7	M	340	0.293 gram	Normal	Normal
8	M	305	0.240 "	"	"
9	M	283	0.244 "	"	"
10	M	345	0.394 "	"	"
11	M	360	0.360 "	"	"
12	M	290	0.281 "	"	"
13	M	260	-	Normal	Normal
14	M	280	-	"	"
15	M	304	-	"	"
16	M	290	-	"	"

*M - Male

Table 2

SERIES I.

Rat	Wt. of Fresh Gland (gram)	Haitai Computed Weight	Water Content	Guanidine Compound Administered	Actual Wt. Divided by Computed Wt. (Average)
2	0.0248	0.02747	75%		
3	0.0202	0.02069	74.3%	Methyl	
4	0.0120	0.01951	72.7%	Guanidine	
5	0.0166	0.0185	65.2%	Sulphate	
6	0.0110	0.01875	63.6%	(Small Doses)	
Av.	0.0158	0.02088	70.2%		0.756
7	0.0162	0.0249	68.9%		
8	0.0176	0.0224	74,6%	Guanidine	
9	0.0151	0.0211	66.3%	Carbonate	
10	0.0111	0.0246	58.6%	(Small Doses)	
11	0.0178	0.0255	70.8%		
12	0.0186	0.0215	71.5%		
Av.	0.0165	0.0233	68.5%		0.690
13	0.0129	0.0197	64.3%		
14	0.0185	0.0214	74.1%	Control	
15	0.0134	0.0199	63.4%	Group	
16	0.0221	0;0215	73.7%		
Av.	0.0168	0.0207	63.9%		0.811

Table 3

SERIES I.

Rat	Percent of Iodine in Thyroids (Dry)	Milligrams Iodine per Kilogram Body Weight
2,3,4,5 & 6 (Methyl Guanidine Sulphate)	0.069%	0.0115
7,8,9,10,11 & 12 (Guanidine Carbonate)	0.066%	0.0104
13,14,15, & 16 (Controls)	0.067%	0.0122

Table 3 (Cont.)

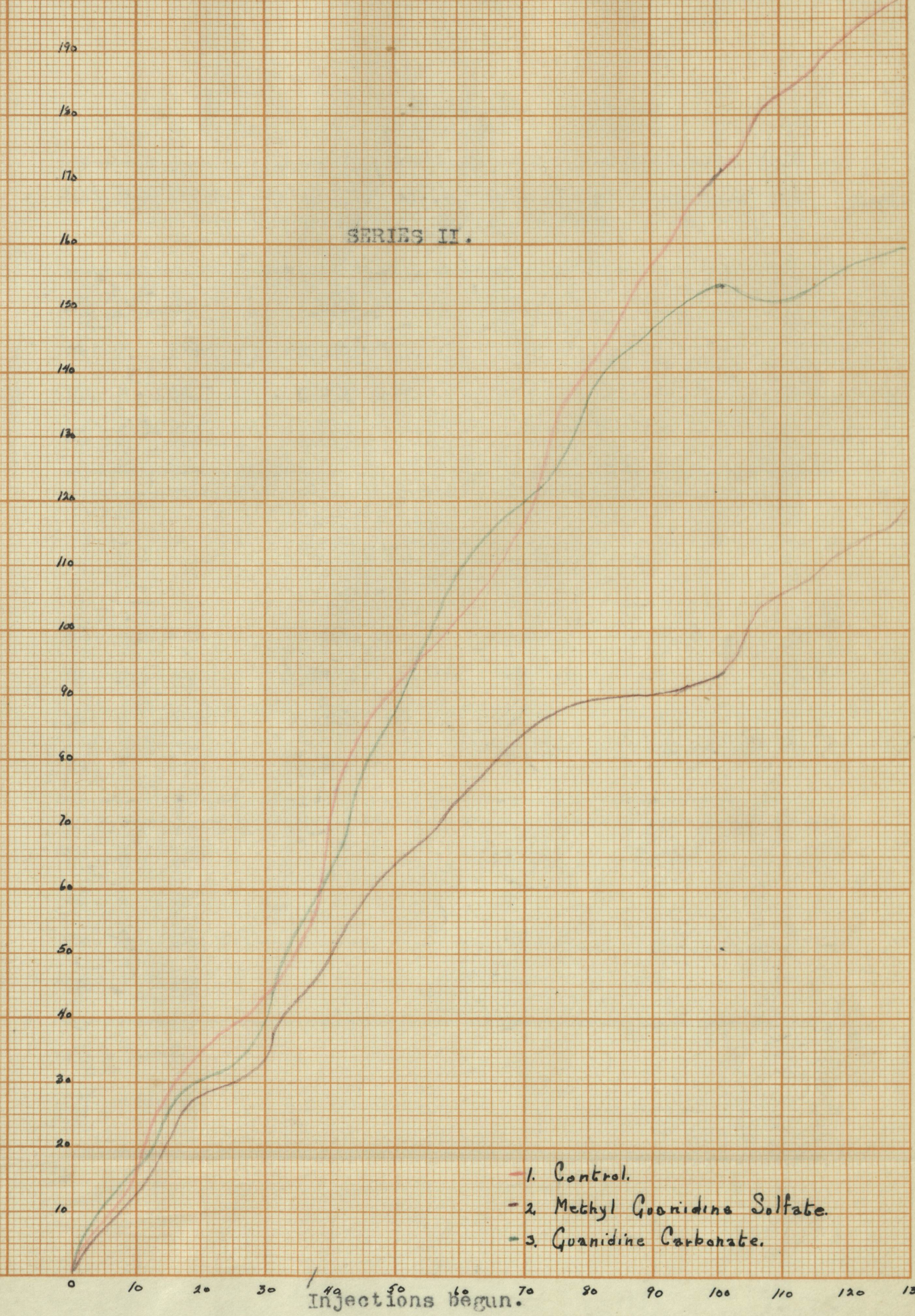
of guanidine.

The microscopical findings in the thyroids and parathyroids of the rats of Series I are summarized in Table 2. In the thyroid glands the alveolar epithelium in all rats was cuboidal with a few low cuboidal and some high cuboidal cells. The peripheral alveoli were large and had a low epithelium. The more centrally placed vesicles were smaller and regular in shape. The colloid was well stained, retracted (especially in the larger peripheral alveoli), and highly vacuolated, particularly in the smaller vesicles. The parathyroid glands appeared normal. The cells were principally dark chief cells. They were arranged in irregular groups and cords. No colloid was present; there were no degenerative changes, and no evidence of mitotic proliferation.

The weight of the thyroid gland of each rat was calculated from the body weight by the formula of Haitai (34), and the average of each group compared with the average actual weight. (Table 3) In this series, the computed values indicate that the glands of the treated rats were smaller, in respect to body weight, than were those of the control group. There was no significant difference in the percentage of iodine in the glands of each of the three groups of Series I (Table 3), but the dry thyroids of the control animals contained more iodine per kilogram of body weight, than did the treated rats.

Series II contained twelve rats divided into three groups of equal size. The rats were weighed daily from March 31 to May 7, a period of thirty-eight days, and injections were begun on May 8 and continued to August 16, a period of one hundred and one days, when they were killed. Rats 19 and 20 received daily injections of 0.020 grams of methyl guanidine sulphate per kilogram of body weight throughout the period. Rats 17 and 18 received daily this same amount of methyl guanidine sulphate until August 2, when larger injections were commenced with the intention of producing intoxication symptoms. The dosage was increased from day to day until August 16 when the rats were killed. The amounts administered expressed in grams per kilogram of body weight: Aug. 2 and 3, 0.02 gram; Aug. 4-7, 0.10 gram; Aug. 8-10, 0.20 gram; Aug. 11-13, 0.40 gram; Aug. 14, 0.70 gram, Aug. 15, 1.0 gram; and Aug. 16, 1.20 grams. Symptoms of intoxication first appeared on August 13 and increased in intensity until August 16, when the animals seemed to be at the point of death. The severe symptoms were muscular weakness, prostration, occasional clonic convulsions, diarrhea, anorexia, and loss of body weight.

Rats 23 and 24 received daily injections of 0.015 gram guanidine carbonate per kilogram of body weight throughout the course of the experiment. Rats 21 and 22 received a like amount until August 2 when administration of larger amounts


SERIES II.
190
180
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90
100
110
120
130
Injections begun.
1. Control.
2. Methyl Guanidine Sulfate.
3. Guanidine Carbonate.

Figure 4.

SERIES II.

Rat	Sex	Weight in Grams at Death	Am't of Methyl G.Sulphate Received in 101 days.	Appearance of Thyroid	Appearance of Parathyroid
17	F*	160	1.122 grams	Normal	Normal
18	F	170	1.127 "	",	"
19	F	201	0.365 "	"	"
20	F	207	0.330 "	"	"
			Am't of Guan. Carbonate Received in 101 days.		
21	M**	219	0.732 gram	Normal	Normal
22	F	165	0.665 "	"	"
23	M	282	0.338 "	"	"
24	M	300	0.383 "	"	"
25	M	259	-	Normal	Normal
26	M	267	-	"	"
27	M	281	-	"	"
28	M	290	-	"	"

*F - Female

**M - Male

Table 5.

SERIES II

Rat	Wt. of Fresh Gland (gram)	Haitai Computed Weight (gram)	Water Content	Guanidine Compound Administered	Actual Wt. Divided by Computed Wt.
17	0.0076	0.0089	53.6%	Methyl Guanidine Sulphate	
18	0.0119	0.0146	68.9%		
21	0.0087	0.0173	57.5%	Guanidine Hydrochloride	
22	0.0098	0.0170	63.6%		
Av.	0.0095	0.0144	60.9%		0.66
19	0.0120	0.0162	64.2%	Methyl Guanidine Sulphate	
20	0.0102	0.0153	61.8%		
23	0.0112	0.0210	63.9%	Guanidine Carbonate	
24	0.0112	0.0221	62.5%		
Av.	0.0112	0.01865	63.1%		0.60
25	0.0097	0.0197	58.8%		
26	0.0106	0.0201	60.4%	Control Group	
27	0.0103	0.0210	62.2%		
28	0.0110	0.0224	60.9%		
Av.	0.0104	0.0208	60.6%		0.50

Rat	Percent of Iodine in Thyroids (Dry)	Milligrams Iodine per Kilogram Body Weight
17,18,21 & 22 (large doses)	0.069%	0.0144
19,20,23 & 24 (small doses)	0.071%	0.0120
25,26,27 & 28 (controls)	0.068%	0.0110

Table 6

of guanidine was begun. The amounts administered (weighed as guanidine carbonate but injected as the hydrochloride) expressed in grams of guanidine carbonate per kilogram of body weight were; Aug. 2 and 3, 0.045 gram; Aug. 4-7, 0.09 gram; Aug. 8-10, 0.180 grams; and Aug. 11-16, 0.280 Grams. The acute symptoms were like those produced in Rats 17 and 18 by methyl guanidine, but appeared earlier (August 11) and were continued although smaller amounts of guanidine base were injected into Rats 21 and 22 than into Rats 17 and 18. The greater toxicity of the non-methylated compound may be due to a slower rate of excretion in comparison to the methyl derivative. This difference in excretion rate has been observed in rabbits by C.J.Weber. Alles (29) reported an equal toxicity of the two compounds in the white rat.

The rate of growth of each of the three groups of Series II is shown in Figure 4. It is essentially the same as Figure 1, in which the growth rate of the control rats exceeded that of the treated groups.

Table 5 epitomizes the microscopic findings of the thyroid and parathyroid glands. The appearance of the former gland in all rats of this series was very similar to that of the rats of Series I. There was, however, a lesser degree of vacuolation and retraction of the colloid. The microscopic picture, again, was that of a typical resting-stage

gland, and the large doses of guanidine had produced no observable nor significant change in its structure. The parathyroid cells were normal in arrangement and appearance, being in irregular anastomosing cords and groups, and consisting mainly of chief cells. There was no evidence of hyperplasia in the glands.

Microscopic observations on other tissues were made. The hypophysis of each of the twelve rats of Series II appeared to be normal. There was some (not marked) congestion in the suprarenals of those rats which had received large doses of guanidine. No changes were observed in the pancreas. In the spleens of those animals into which had been injected large amounts of guanidine there was apparently an increase in the number of splenic macrophages over the number in the spleens of the rats which were untreated or had received only small doses of guanidine. This suggests an exaggerated erythrocyte destruction. The liver cells of all these rats showed some fatty changes, but they were slightly greater in the livers of the rats which had developed symptoms of guanidine poisoning. The cells of the convoluted tubules of the kidneys of all the rats showed some bursting of the cellular membrane, and there was coarsely granular, eosin-staining material in the lumina of the tubules. This condition may have been a fixation artefact.

The ratio of the actual weight to the computed weight of the thyroids (Table 6) of the rats of Series II indicates that the treated rats had heavier thyroids than untreated, the difference being most marked in the case of those which had been severly poisoned (Rats 17,18,21,and 22). In Series I the control group had heavier thyroids than the treated animals. The percent of iodine in the dry thyroids was about the same for the three groups of rats of Series II. However, the milligrams of iodine per kilogram of body weight was least in the control group and greatest in the group which had received large amounts of guanidine. In Series I, the control group had more iodine per kilogram of body weight than did the treated rats.

The effect, if any, of large doses of guanidine compounds on the thyroid, parathyroid, liver, and kidney of the white rat was studied in eighteen rats of Series III. They were weighed daily for eight days (November 18 - 25) before administration of guanidine was commenced. Injections were first made November 25 and were continued until December 3, a period of eight days. A daily record of the weight of each rat was made during that time. Rats 29 - 34 and also 44 received once daily 0.400 gram of methyl guanidine sulphate per kilogram of body weight. A 10% solution was used. Rats 35 - 41 received once daily 0.300 gram of guanidine carbonate per

SERIES III

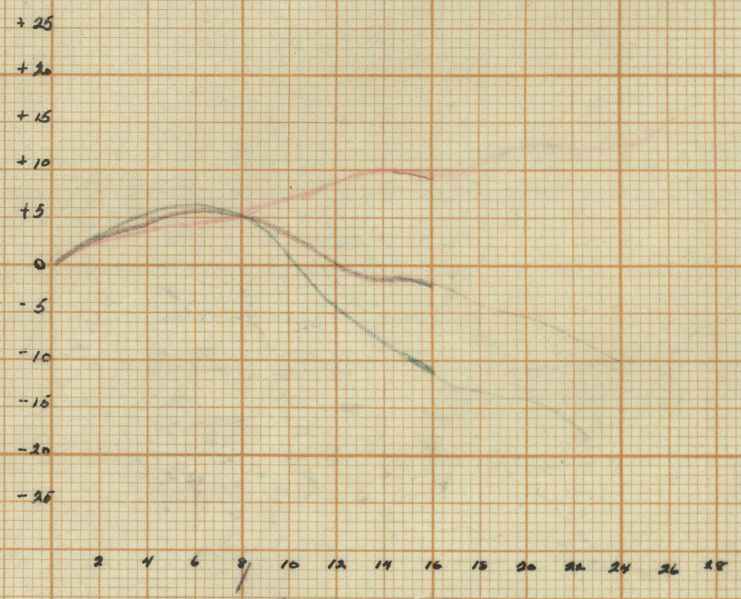

Figure 7.

SERIES III

Rat	Sex	Wt. in Grams at Death	Amount of M.G.S. Received in 8 days	Appearance of Thyroid.	Appearance of Parathyroid.
29	F*	192	0.640 gram	Slightly Active	Normal
30	F	157	0.560 "	"	"
31	F	223	0.720 "	"	"
32	F	203	0.640 "	"	"
33	M**	240	0.720 "	"	"
34	M	168	0.560 "	"	"
44	M	150	0.560 "	"	"
			Am't of Guanidine Hydrochloride Received in 8 days		
35	F	223	0.540 gram	Slightly Active	Normal
36	F	150	0.390 "	"	"
37	F	212	0.600 "	"	"
38	F	212	0.540 "	"	"
39	F	148	0.270 "	"	"
40	M	250	0.450 "	"	"
41	M	203	0.450 "	"	"
42	F	250	-	Slightly Active	Normal
43	F	203	-	"	"
45	M	387	-	"	"
46	M	238	-	"	"

*F - Female
**M - Male

Table 8

SERIES III.

Rat	Wt. of Fresh Gland (gram)	Haitai Computed Wt.(gram)	Water Content	Guanidine Comp'd Administered	Actual Wt. divided by Computed Wt.
29	0.0096	0.0156	80.3%	Methyl	
30	0.0169	0.0126	88.1%	Guanidine	
31	0.0258	0.0175	84.9%	Sulphate	
32	0.0163	0.0163	87.7%	(large	
33	0.0144	0.0185	81.3%	doses)	
34	0.0060	0.0142	70.7%		
44	0.0126	0.0125	80.2%		
Av.	0.0149	0.0153	81.9%		0.97
35	0.0093	0.0175	84.3%		
36	0.0071	0.0125	73.3%		
37	0.0199	0.0168	91.0%	Guanidine	
38	0.0146	0.0168	81.5%	Hydrochloride	
39	0.0081	0.0128	76.6%	(Large doses)	
40	0.0167	0.0192	91.6%		
41	0.0128	0.0163	86.8%		
Av.	0.01264	0.01598	83.6%		0.79
42	0.0081	0.0192	83.4%		
43	0.0084	0.0163	80.2%	Control	
45	0.0221	0.0265	80.6%	Group.	
46	0.0140	0.0184	79.3%		
Av.	0.01315	0.0201	80.9%		0.65

Table 9

SERIES III

Rat	Percentage of Iodine in Thyroids (Dry)	Milligrams Iodine per Kilogram of Body Weight.
29,30,31,32,33,34, &44	0.087%	0.0112
35,36,37,38,39,40 & 41	0.088%	0.0084
42,43,45, & 46	0.086%	0.0100

Table 9 (Cont)

kilogram of body weight. A 7.5% solution was used. The carbonate was converted to the hydrochloride before injection. Rats 42,43,45, and 46 were control animals and received only saline injections.

The rapid loss of weight of the treated rats is shown in the growth curve of Figure 7. The amounts of guanidine administered were sufficient to produce profound symptoms of guanidine poisoning, these symptoms being the same as in Series II. As in Series II, the rats receiving guanidine hydrochloride developed signs of intoxication earlier than those receiving methyl guanidine sulphate, and the symptoms were more severe. Those rats which appeared to be at the point of death on December 2 were killed then, and the others on December 3. All those killed December 2 were receiving guanidine hydrochloride.

The microscopic appearance of the thyroid and parathyroid glands of Series III is given briefly in Table 8. The thyroid gland in the case of each rat had low columnar to columnar alveolar epithelium. The cells were larger than those in the glands of the previous two series. In all the thyroids of this series the colloid stained well, was somewhat retracted, and usually vacuolated especially at the periphery. Some vesicles in almost every gland were irregular in shape, one of its walls bulging into the lumen. The glands appeared to be in a slightly active stage, but no marked nor significant difference in the

appearance of the glands of each of the three groups of Series III was observed. The thyroid glands from treated and untreated animals were the same in structure. The parathyroids appeared to be normal.

The compilation of data in Table 9 indicates that the thyroid glands of treated rats are heavier in reference to body weight than those of the controls; the rats which had received methyl guanidine sulphate having the heaviest gland in respect to body weight. This is in agreement with the observations made on the rats of Series II (Table 6), but differs from Series I (Table 3). There was little variation in the percentage of iodine in the treated and control rats, but those rats which had received methyl guanidine sulphate had more milligrams of iodine per kilogram of body weight than did the other two groups. Those rats which had received guanidine hydrochloride had the least amount, even less than the control group.

The kidneys of the rats of this group showed some swelling of the cells of the convoluted tubules, and sometimes rupture of the cellular membrane. Granular, eosin-staining material was commonly present in lumina of the tubules. This condition was more marked in the treated animals than in the controls. The livers of the rats of this series showed fatty changes, which were most pronounced in the treated animals.

Elkourie and Larson (35) observed marked congestion of

the viscera of animals dead from guanidine hydrochloride poisoning. They reported great fatty degeneration of the liver, simple necrosis of tubules of the kidney with nuclear changes, and simple necrosis in the adrenal. In the rats of Series II and III which had been poisoned severely with guanidine, there did occur some of the same changes described by these authors, but they were also present in the control animals. The changes described in the livers and kidneys of the rats of Series II and III, were, however, more pronounced in the treated than in the control group. This agrees with the observations of Elkourie and Larson. The latter could find no histiological changes in the parathyroids of animals dying of guanidine intoxication which is in agreement with the observations on the parathyroids of the rats of this investigation. Susman (36) reported microscopical changes in the parathyroids of rabbits following the injection of guanidine. He observed hypertrophy and hyperplasia, which he interpreted to be functional changes. No hypertrophy nor hyperplasia was present in the parathyroids of the rats of this study in any case, treated or untreated, although the dosage of guanidine in some instances was great enough to cause death.

CONCLUSIONS.

1. Methyl guanidine sulphate and guanidine carbonate in small daily doses over a long period of time (90 - 100 days) causes no structural changes in the thyroid and parathyroid glands of the white rat. Neither does there occur a marked change in the percentage of iodine in the dry thyroid, nor a consistent variation from normal in the amount of iodine per kilogram of body weight. No significant changes in the water content of the thyroid are produced. Growth (body weight) is somewhat inhibited.
2. Methyl guanidine sulphate and guanidine hydrochloride in doses large enough to produce severe symptoms of intoxication cause no structural changes in the thyroid and parathyroid glands of the white rat. The percentage of iodine in the thyroid is not altered, and the amount of iodine per kilogram of body weight shows no consistent variations from the normal. The water content of the thyroid is not markedly altered. There may perhaps be an increase in the weight of the gland accompanying the guanidine administration. There may be produced some fatty changes in the liver, and swelling of the cells of the convoluted tubules of the kidney with bursting of the cellular membrane. Loss of body weight occurs.

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