CXVI. GUANIDINE STUDIES.

II. DISTRIBUTION OF GUANIDINES IN ACUTE GUANIDINE AND PARATHYROPRIVIA TETANIES.

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INTRODUCTION.

ALTHOUGH guanidines can be recovered from the urine of animals after injections of guanidine salts, recoveries are not complete, especially with large doses [Burns, 1916]. This loss of guanidine could follow either the retention of guanidine as such or the chemical alteration of the guanidine by the body. The prolonged twitchings of voluntary muscle after the injection of even small quantities of guanidine salts, or after parathyroidectomy, suggest the retention of active guanidine, although they may equally well be the result of some unadjusted disturbance merely initiated by the guanidine in the case of the guanidine injections, or by guanidine or some other substance in the case of parathyroidectomy. Accordingly determinations of the guanidine of five different tissues from normal and injected rabbits have been made.

EXPERIMENTAL.

A modification of the extraction method previously described for invertebrate tissue [Ellis, 1928] was used for the separation of the guanidines which subsequently were reclaimed by adsorption with blood-charcoal in an alkaline medium, and determined colorimetrically by the methods of Marston [1924] and Weber [1927].

The tissues were taken from the animal as quickly as possible and weighed quantities placed directly in 5 to 10 volumes of alcohol, and immediately finely minced. After 18 hours the alcoholic mixture was extracted in a Soxhlet apparatus for several hours over a water-bath. To avoid adsorption no filter-paper thimbles or retainers were used. The alcoholic extract was evaporated to dryness under reduced pressure at about 90°, the residue taken up in a small quantity of warm water, diluted to 100 cc. with water after cooling, and protein was precipitated by adding 10 cc. of 2N/3 sulphuric acid and 7 cc. of 10 % sodium tungstate solution. After an hour the whole was filtered, approximately 1 g. of blood-charcoal (purified by acid) added to the filtrate, and the

mixture again filtered after about 5 minutes. A measured quantity (as large as possible) of this filtrate was made barely alkaline to litmus with 10 % sodium hydroxide and approximately 1 g. of blood-charcoal added for the adsorption of the guanidines. After 5 minutes the fluid was filtered off and discarded. The filter-paper and charcoal were drained quite free from fluid, and placed in the flask which had contained the alkaline mixture before it was filtered (to avoid loss of guanidines on the inside of the flask), together with 25 cc. of absolute alcohol. Sufficient hydrochloric acid (a few drops) was added to make the mixture distinctly acid to litmus, and the flask, securely stoppered, set aside for 18 to 24 hours. The charcoal was then filtered off, a measured quantity of the filtrate evaporated to dryness at 90°, and the residue taken up in water. Aliquot parts of this solution were used for the determination of guanidine [Marston, 1924; Weber, 1927], and of total and preformed creatinine [Folin, 1922]. By means of these two creatinine readings the proper corrections of the guanidine determinations were made [Weber, 1927; Major and Weber, 1927].

Total and preformed creatinine determinations were also made on a portion of the first acid filtrate after the removal of the proteins before charcoal was added, in order to obtain the creatine values for the entire sample. Blood-sugar values were secured from samples of fresh blood by the method of Hagedorn and Jensen [1923].

Young adult male rabbits were used in these tests, as the rabbit is readily poisoned by guanidine. Two sets of samples were taken; the first consisted of blood samples only, drawn from the ear vein of each rabbit after a 24-hour fast. From 5 to 7 cc. of blood were taken from each rabbit and the analyses of these samples are listed as "normals" in Table I. After taking the first blood samples the animals were fed for 4 days. No food was given on the 5th day, and the injections were made during the morning of the 6th day.

The blood samples after injection averaged about 18 cc. In addition to blood, the entire liver, without the gall-bladder, both kidneys, the entire brain, and a sample of voluntary muscle from the legs averaging 65 g. were preserved. Muscle was taken because of its obvious part in the tetany, liver and kidney because of their excretory functions, and the brain because it is an inactive tissue from a metabolic standpoint and because of its possible connection with the muscular tetany.

DISCUSSION.

The blood-guanidine values together with the readings for blood-sugar and blood-creatine have been assembled in Table I. The "normal" blood-guanidines of the rabbits varied from 0.086 mg. to 0.430 mg. per 100 cc. of blood, the average being 0.211 mg. The average of the rabbit guanidine values is slightly higher than those for human blood-guanidine [Major and Weber, 1927]. The blood-guanidines of the animals injected with guanidine or phenol lie within the limits of the normals, but their average falls below the general average, 0.176 mg. per 100 cc., of the normal and injected animals.

It is evident that high blood-guanidine at the time is not a requisite of acute tetany, for the guanidine-poisoned animals were in violent tetany when the blood samples were taken. There is however the possibility that the blood-guanidine may have risen in the injected rabbits at some time previous to the taking of the final blood sample, for Major and Weber [1927] report a rise in blood-guanidine from 0.108 to 0.530 mg. per 100 cc. in a dog during the first hour following the injection of 200 mg. of methylguanidine sulphate. The low blood-guanidine values in the acute stage of guanidine tetany suggest that the guanidine content of the blood may depend upon some part or parts of the body concerned with the elimination from the blood, or the destruction, of guanidine, rather than the actual amount present in the body or the stage of

Table I.

Rabbit no.	Treatment	Mg. per 100 cc. blood		Creatinine		
		Guanidine base	Sugar	total	preformed	Creatine
2	Normal	0.170	121	3.53	1.06	2.86
6	Normal	0.105	106	3.71	1.21	2.90
4	Normal	0.430	106	4.05	1.27	3.22
	5 cc. saline	0.200	126	3.86	1.01	3.51
5	Normal	0.086	87	3.94	1.43	2.90
	0.1 g. guan. hydrochlor.	0.087	31	4.73	1.69	3.52
3	Normal	0.380	84	3.84	2.01	2.12
	0.5 g. guan. hydrochlor.	0.097	39	2.61	1.30	1.51
1	Normal	0.108	103	4.02	1.52	4.00
	1 g. phenol	0.102	105	4.87	1.11	4.36
Average normal rabbits		0.211	104	3.85	1.33	3.07
Average guanidine rabbits		0.092	35	3.67	1.49	2.51
Phenol rabbit		0.102	105	4.87	1.11	4.36

Rabbits 2 and 6, controls. Blood samples taken from ears after 24-hour fast. No injections made.

Rabbit 4, control. Blood sample taken from ear after 24-hour fast; 4 days' full feeding; 5th day no food; 6th day, 10 a.m. injected 5 cc. of physiological saline, 2.30 p.m. animal killed by blow on head, samples taken immediately.

Rabbit 5, guanidine. Blood sample taken from ear after 24-hour fast; 4 days' full feeding; 5th day no food; 6th day, 10.10 a.m. injected 100 mg. guanidine hydrochloride (Merck) in 5 cc. physiological saline, little effect during 1st hour, tetany tremors began about 11.30 a.m. and increased steadily in violence and duration until 1 p.m. when respiration ceased, animal by this time in rigid tetany, decapitated immediately after cessation of respiration and a large sample of blood taken, heart still fibrillating when thoracic cavity was opened, muscles so rigid that they tore when attempt was made to bend leg, tissue samples taken at once.

Rabbit 3, guanidine. Blood sample from ear after 24-hour fast; 4 days' full feeding; 5th day no food; 6th day, 10.15 a.m. injected 500 mg. guanidine hydrochloride (Merck) in 5 cc. physiological saline, showed a little uneasiness after about 15 minutes, but became quiet again save for a slight twitching of ears, about 11 a.m. became restless again, suffering two short tetany spasms, at 11.30 a.m. gave one quick jerk, muscles became rigid, legs stiffened and were extended, and respiration ceased, decapitated immediately and a large blood sample collected, heart still fibrillating slightly when thoracic cavity was opened, muscles not so rigid as those in No. 5, tissue samples taken at once.

Rabbit 1, phenol control. Blood sample from the ear after 24-hour fast; 4 days' full feeding; 5th day no food; 6th day, 10.5 a.m. injected 100 mg. phenol in 2 cc. physiological saline, 11 a.m. animal showed slight tremor, 2 p.m. slight tremor, injected 400 mg. phenol in 8 cc. physiological saline, 2.30 p.m. tremor increased to tetany, injected 500 mg. phenol in 10 cc. physiological saline, 3.30 p.m. tetany spasms frequent and violent, 4.45 p.m. animal lying prostrate, respiration feeble, tetany violent, decapitated and a large blood sample collected, other tissue samples taken at once

muscular tetany. This view is strengthened by the observations of Major and Weber [1927], who report that fluctuations in the blood-guanidine (from 0.140 to 0.350 mg. per 100 cc. and back again in the course of an hour) are not uncommon in dogs which have been injected with methylguanidine sulphate. If the actual value of the blood-guanidine be correlated with some factor other than that of muscular tetany alone, the lack of agreement in the guanidine values reported from parathyroidectomised animals by various writers can be readily understood.

The phenol rabbit, a tetany control against the guanidine rabbits, showed little change in blood-guanidine despite severe and prolonged tetany.

The guanidine rabbits developed marked guanidine hypoglycaemia, but neither the blood-creatine nor the blood-creatinine showed any definite correlation with guanidine poisoning.

The guanidine and creatine contents of the tissues are given in Tables II and III. The muscle-guanidines of the tetany animals were low, both actually and relatively, being but 12 % and 24 % of the muscle-guanidine of the normal rabbit. These muscle-guanidine values of the tetany rabbits were of the same magnitude as the blood-guanidine values, although actually the muscle-guanidine in each tetany animal was higher than the blood-guanidine taken at the same time, so that as far as equilibrium is concerned there could have been a movement of guanidine from the muscle tissue into the blood. This again suggests that the actual blood-guanidine value depends upon other factors than the guanidine content of the muscle alone. The presence of a large amount of guanidine in the muscles is evidently not essential to the maintenance of guanidine tetany.

Table II.

Guanidine and creatine: mg. per 100 g. tissue.

Rabbit no.	Treatment	Muscle.	Guanidine	Creatine
4	5 cc. saline		0.999	311.61
5	0.1 g. guan. hydrochlor.		0.124	202.10
3	0.5 g. guan. hydrochlor.		0.145	387.04
1	1 g. phenol		0.241	332.73
		Liver.		
4	5 cc. saline		0.625	16.50
5	0.1 g. guan. hydrochlor.		3.700	13.79
3	0.5 g. guan. hydrochlor.		6.340	12.44
1	1 g. phenol		0.161	12.40
		Kidney.		
4	5 cc. saline		Trace?	81.24
5	0.1 g. guan. hydrochlor.		0.852	102.10
3	0.5 g. guan. hydrochlor.		4.061	243.38
1	1 g. phenol		0.438	206.55
		Brain.		
4	5 cc. saline		Trace?	52.17
5	0.1 g. guan. hydrochlor.		0.600	11.28
3	0.5 g. guan. hydrochlor.		1.781	26.19
1	1 g. phenol		Trace?	39.31

The livers of the guanidine tetany rabbits were very rich in guanidine as compared with the livers from either the control or the "phenol control" rabbits, which suggests that the liver is concerned with the excretion or destruction of guanidines, or possibly both. The guanidine recoveries from the kidneys of the guanidine tetany rabbits were also high, both actually and relatively; this is not surprising however as guanidine is excreted as such in the urine.

The amounts of guanidine present in the brains of the normal and "phenol control" animals were too small to be determined, it being doubtful whether there was any guanidine in the brain tissue of these animals which could not be accounted for by the traces of blood which were unavoidably present, but the quantities of guanidine present in the brains of the guanidine tetany animals were large and, indeed, many times greater than either the muscle- or blood-guanidines of these same animals. The finding of guanidine in the brain is of interest in connection with the maintenance of the prolonged tetanics of guanidine poisoning, and points to an affinity of the brain tissue, and possibly of other nervous tissue, for guanidine. In view of the experiments of Fühner [1907], Meighan [1917], and Grant [1920], all of which show that guanidine acts on the nerve endings, the continued guanidine tetanics of muscles in which the actual guanidine content was low could be accounted for by the nerve endings taking up quantities of guanidine sufficiently large to produce marked activity in them, yet so small that as a part of the total guanidine of a large mass of muscle the guanidines contained in these nerve endings would make no appreciable difference in the determination of the muscle-guanidine.

Considering all the tetany animals together, both guanidine and phenol, there was a slight fall in liver-creatine, a more distinct fall in brain-creatine, and a very definite rise in kidney-creatine, associated with the tetany condition, whilst the muscle-creatine values varied about the normal. As the total creatine in the body is known to rise after some forms of muscular activity the high kidney creatine in these tetany animals may have been correlated with the severe tetanics which these animals experienced, although the high kidney creatine might also be interpreted as favouring the view advanced by Jaffé [1906] and Noël Paton [1925] that guanidine may be acetylated to form creatine. The urine was not analysed, so it is not known whether creatine, although found in considerable quantity in the kidneys, was actually being excreted in quantity by these guanidine-poisoned rabbits, and, as Jaffé [1906] did not find a rise in the creatine of the urine following the injection of methyl-guanidine, it is possible that the kidney-creatine found in these guanidine-tetany animals was not being excreted.

From the guanidine values for the tissues it is possible to make a rather rough estimate of the disposal of the guanidine injected. Taking the average body weight of the guanidine tetany rabbits as 1 kg., rabbit No. 5 received 10 mg. guanidine hydrochloride (5.2 mg. guanidine base) per 100 g.

tissue, and rabbit No. 3, 26 mg. guanidine base per 100 g. tissue. If the injected guanidine were uniformly distributed throughout the body, and not destroyed or removed, approximately those values should have been recovered. It is not at all probable that such a uniform distribution of any soluble substance throughout the body would occur, but even considering these values to be the minimum required to account for the guanidine actually injected they greatly exceed any value found by analysis. The results of the present analyses, therefore, seem to substantiate the findings of Gergens and Baumann [1876] and others, that a considerable portion of the guanidine injected is changed in the body. It must be added however that the presence of considerable quantities of guanidine in the livers of the guanidine-tetany animals suggests the possibility that guanidine may be excreted through the bile (discussed in a following paper). Sharpe [1920] found guanidines in the faeces of children suffering from tetany, and if these guanidines were excreted they may have reached the intestine in the bile.

Table III. *Parathyroidectomised cats.*

Distribution of guanidine in the tissues: mg. per 100 g. (or cc.).

Cat	Treatment	Blood	Muscle	Liver	Kidney	Brain	Urine	Bile
<i>A</i>	None after operation	Trace	2.155	0.281	3.623	1.704	—	—
<i>B</i>	Dextrose and adrenaline	0.071	0.552	Trace	1.943	0.826	2.941	—
<i>C</i>	Insulin	0.110	1.193	0.012	1.198	0.433	—	None

For comparison with the tissues from the guanidine tetany animals analyses of tissues from three parathyroidectomised cats have been given in Table III. Cat *A* had no special treatment after the operation, and was killed on the 4th day after the operation when definite parathyroprivia tetany had developed. In cat *B* the tetany was controlled for 5 days (see last paper of this series) with injections of dextrose and adrenaline. The injections were then discontinued and the animal killed 24 hours later, *i.e.* 6 days after the operation, when tetany had returned. Cat *C* was given 20 units of insulin shortly after the first appearance of parathyroprivia tetany and was killed about 12 hours later just as it was about to die in acute tetany.

Perhaps the most striking feature of these analyses of tissues from the parathyroprivia tetany animals is the relatively high guanidine content of the brain, and if, as pointed out in the discussion of the guanidine-tetany animals, the guanidine content of the brain is in any way indicative of the guanidine content of the other nervous tissues, these analyses offer evidence in favour of the contention of Noël Paton *et al.* [1916] that the tremors and jerkings of the muscles are due to the action of guanidines on the central nervous system. The guanidine values from the tissues of cat *A*, the untreated parathyroidectomised animal, are in general similar to those of the guanidine tetany animals, except that the muscle guanidine in cat *A* is much higher. As the injections received by the other two cats had altered the sugar level in these animals the guanidine values from the tissues of cats *B* and *C* are not

strictly comparable with those of the guanidine tetany animals, but in connection with the relation of sugar to guanidine action (see last paper of this series) the low guanidine contents of the livers of cats *B* and *C* should be noted.

SUMMARY.

1. Analyses of blood, voluntary muscle, liver, kidney and brain of rabbits in acute guanidine tetany and acute phenol tetany were made and compared with normals for guanidine and creatine content.

2. The guanidine contents of the livers, kidneys and brains of animals in acute guanidine tetany were high.

3. The blood-guanidines and muscle-guanidines of animals at the point of death with acute guanidine tetany were lower than the blood- and muscle-guanidines of the control, showing that neither high blood-guanidine nor high muscle-guanidine is required to maintain guanidine tetany.

4. The presence of relatively large amounts of guanidine in the brains of the guanidine tetany animals offers a possible explanation of the maintenance of the prolonged tetanies regardless of the actual guanidine content of either the muscles or the blood during guanidine tetany.

5. No storage of guanidine exceeding or even equalling the average tissue guanidine, predicted on the basis of simple distribution of the guanidine injected, was observed.

6. The guanidine content of the brain of the untreated cat in acute parathyroprivia tetany was relatively high.

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