THE EFFECTS OF METHYL GUANIDINE SALTS ON SOME OF AUTONOMIC NERVES OF THE DOG.

by

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SIGNIFICANCE OF GUANIDINE COMPOUNDS OF THE BODY.

In 1924 Sharpe (56) reported evidence that in the incubation of hen's eggs choline decreases at the same time that guanidine is increasing. From this he concluded that choline is a source of guanidine. Paton, 1925, (87) gave it as his opinion that guanidine is produced from the choline of lecithin in the liver.

Concerning the body tissues and fluids that normally contain guanidine, Paton reported its quantitative determination in muscles of normal and of parathyroidectomized dogs. His observations were confirmed by Henderson, 1918, (23). Greenwald, 1924, (21) after work on parathyroidectomized dogs, reported that no guanidine was excreted in the urine. This, however, was denied by Kuhnau, 1925-1926, who made determinations of guanidine in the urine of normal and of parathyroidectomized dogs, and of normal and thyroidectomized humans (30), (31), (32). It was also vigorously denied by Paton (49) in an article defending his own work. Major, Orr, and Weber (39) were able to find no change in the blood guanidine of dogs whose parathyroids had been removed. However, they did find clear cut increases of guanidine-like compounds in the blood of many persons suffering with hypertension (40). Minot and Cutler (42) in a series of noteworthy investigations found that in certain types of liver disease and in eclampsia the blood sugar level is low whereas the blood guanidine level is high. The same changes were present in the blood chemistry

of dogs during intoxication produced by carbon tetrachloride or by chloroform. Calcium medication gave prompt relief in some of the clinical cases showing high blood guanidine and low blood sugar concentrations.

On skeletal muscle and myoneural junction, guanidine compounds have been stated to have marked physiological and pharmacological effects. Meigan, 1917, (41) reported that less than one percent will produce twitching in frog muscle, but, strangely enough, that more than one percent is not effective. He also reported a curara-like action which warmth up to a certain point increases. Previous to this, 1916, Paton and Findlay had asserted that guanidine compounds have exactly the same effect upon the myoneurone as does parathyreopriva, i.e., they produce an increased electrical excitability of the muscle involving principally neural structures, but to some degree, post neural structures as well. The curara effect reported by Meighan was confirmed by Grant, 1920, (20) who also observed the effects of small concentrations (between 0.125% and 0.5%). Such concentrations, he said, cause increased excitability preceeding the curara effect. This confirmed, at least in part, the statements of Paton and Findley. Frank, Nothmann, and Guttmann, 1923, (16) presented evidence that the excitability of skeletal muscles to drugs like choline is increased by guanidine. That the creatine content of muscle is increased after guanidine injections in cats and dogs is affirmed by Wishart, 1920, (69). Stoland,

1926, (60) showed that guanidine increases the tone of smooth muscle.

A number of workers have observed changes in heart action following guanidine administration. Putzey and Swaen (51), and Burns and Watson (5), the former working on dogs, the latter on frogs, agree that moderate concentrations cause cardiac acceleration followed by marked slowing. No acceleration was reported by Sinalnicoff and Bovshik, 1916, (58), but retardation which could be partially offset by cutting vagus nerves was observed. This effect was obtained with dimethyl guanidine. Monomethyl guanidine was less effective. Guanidine was said to have no effect at all. Casilan (7) investigating the relative effectiveness of these three derivatives on the vagus nerve of the frog, found just the opposite. Guanidine was most effective; methyl guanidine was next in order of effectiveness; while dimethyl guanidine was least effective. Major (38) could detect no change in strength of heart beat after guanidine injections. Diastolic standstill was obtained by Rosenow (52) with isolated frog's heart.

Sinalnicoff and Bovshik found also that dimethyl guanidine (5mg. per kilo intravenously) caused a temporary fall of blood pressure followed by a long continued rise. They concluded that peripheral changes were responsible for the blood pressure effects. Guanidine, according to Maele and Bulke, 1926, (35) causes protein shock, but the fall of blood pressure is largely compensated for by vasoconstriction. Stoland (60) and Major

(37) report marked pressor effects. Major says this may not occur if injection is extremely slow. Smith, working in this laboratory, is investigating the effects of long continued administration of small amounts of guanidine compounds.

Reports on the effects of guanidine on blood coagulability are at variance. Exudation of plasma and incoagulability of blood results, according to Maele and Bulcka (35); no change in viscosity or coagulation time, according to Major (38). Some work done in this department by medical students showed that the clotting time following guanidine injections is very variable. In ninety percent of the cases the clotting time was reduced at some time during the experiment.(55).

Watanabe (65-66) and Collip (8) found that guanidine causes a reduction in blood sugar. Alles (1) thinks this reduction in blood sugar is due merely to the greater reflex irritability or the convulsions caused by the guanidine. Dublin and Corbitt (12) investigated a number of guanidine compounds and decided they were not related to insulin.

That blood phosphorus is increased after guanidine injections is attested by Watanabe, 1918, (67) and Nelken 1923, (44). However, these two do not agree as to the effect of guanidine upon blood calcium; Watanabe saying a small decrease occurs, Nelken that an increase results. Bayer, 1922, (2) says the decrease of blood calcium ions may be as great, following guanidine injection, as that

caused by parathyroidectomy.

Maele and Bulcke (35) assert that guanidine increases the metabolic rate of dogs and rabbits.

The violent nature of the symptoms characterizing guanidine tetany has called attention to the importance of ascertaining the exact effect of this drug upon the nervous system. Guanidine salts produce jerkings and tremors in decapod crustacea due to an action upon the central nervous system, according to Sharpe (56). Fuchs has observed symptoms of encephalitis in cats which have been injected with guanidine. Increased excitability of the nerves after small doses is reported by Frank, Stern, and Nothmann (15). The effect, they say, is upon the peripheral nerves and not upon the anterior horn cells, a conclusion that is somewhat at variance with that of Paton and Findlay (46). The latter believe that the chief effect is on the efferent neurones of the spinal arc. Sectioning of the afferent fibers does not reduce the amount of twitching. The cerebrum is not usually involved except in moderating the action of the lower reflex arcs. In severe cases, however, the cerebrum may become involved in causing epileptiform convulsions. The cerebellum is involved in causing extensor regidity and, in some cases, disturbances of equilibrium.

Several investigators have attributed the effects of guanidine compounds upon the heart to action of this drug upon the autonomic nerves supplying this organ. By

use of drugs to localize the effects of guanidine compounds, Burns and Watson (4) were led to conclude that these substances have a nicotine-like action upon ganglia, and an atropine-like action upon vagus endings. Burns (3) acting upon the assumption that much of the syndrome of anaphylactic shock is due to over activity of the vagus nerves, administered guanidine to shocked animals to prevent or relieve the symptoms, and secured a marked reduction in death rate. The primary cardiac acceleration in frogs following use of low concentrations of guanidine salts was ascribed by Burns and Watson (5) to the stimulation of accelerator nerves to the heart. The after slowing was thought to be due to vagus stimulation and reflex slowing following vasoconstriction. That guanidine acts like a parasympathetic drug is also affirmed by Frank, Stern and Nothmann (15); and by Frank, Nothmann, and Guttmann (16). Maele and Bulke (35) have observed brief vagal excitation in dogs and rabbits. Sileninkow (59) believes the effect upon rate and intensity of contraction of intestinal segments of cats is due to action upon Auerbach's plexus.

The relationship of guanidine compounds to parathyroid tetany has attracted much attention. Investigation of this question has followed two general lines: 1. The change in the amount of guanidine compounds in blood and urine following parathyreopriva; and 2. The similarity of symptoms and methods of alleviation between

parathyroid tetany and guanidine poisoning. Henderson (23) detected a loss of free guanidine and an increase of creatine in muscles following parathyroidectomy. The fall in total guanidine was especially pronounced. She interpreted her results as showing either an active loss of guanidine or a loss of the ability of the muscle to take it up after parathyreopriva. Kuhnau (30), (31), (32) asserts an abnormal amount of guanidine was present in the blood and urine of parathyroidectomized dogs. He interpreted this as favoring the guanidine theory of tetany, notwithstanding the fact that this increase did not appear until after the onset of tetany. A two hundred percent increase of blood guanidine was found after parathyroid removal by Paton and Sharp (48). On the other hand no guanidine was isolated from the urine of parathyroidectomized dogs by Greenwald (21). Major, Orr, and Weber (39) were also unable to detect any increase in the blood guanidine of dogs suffering from tetania parathyreopriva. That tetany in humans is accompanied by substantial increases of guanidine excretion is claimed by Kuhnau (30), and Natrass and Sharpe (43).

Watanabe (65) (66); Frank, Stern, and Nothmann (15); Klinger (28); Sabovie (53); Paton, Findlay, and Burns (45); and Paton and Findley (46) find similar conditions following guanidine poisoning as after parathyroidectomy, except that Klinger and Watanabe do not find that calcium will relieve the symptoms of guanidine poisoning. Ellis (14)

found that after parathyroid removal the concentration of guanidine in the liver, kidney, and brain increased as after guanidine injections. In muscle, however, he found that after parathyroid removal the guanidine concentration increased, after guanidine injections it fell. Elkourie and Larson (13) report that parathyroid tetany causes marked congestion of the viscera; guanidine poisoning does not do this. Fatty degeneration of the liver is present in guanidine poisoning but absent in tetany; Necrosis is present in both conditions. They conclude that the symptomatology of the two conditions is quite dissimilar. Fuchs (17) finding that calcium administration does not relieve guanidine poisoning, concludes that the two conditions are not identical.

Lands (33) 1928, found that the blood from parathyroidectomized dogs at time of onset or during tetany reduces the irritability of the vagus nerves of frogs and turtles much as guanidine compounds had already been shown to do. (Potter, 1927, 50) (Casilan, 1928, 7).

Some relationship between the parathyroid glands and body guanidine is suggested by the findings of Susman (62). The cells of the parathyroids contain numerous fat globules. An injection of guanidine nitrate causes depletion of the parathyroid globules within 18 hours. The normal appearance is restored within 48 hours. Repeated injections sufficient to keep a constant degree of hypertonicity in the muscles, causes hypertrophy and hyper-

plasia in the parathyroid cells as well as a marked increase in the acidophile cells, and a striking absence of the large globules. Guanidine injections, therefore, involve increased functioning of the parathyroids, as shown by the above enumerated changes.

Although the decrease of calcium ions usually is less after guanidine poisoning than after parathyroid removal, it may be as great, according to Bayer (2).

Attacking the problem from the standpoint of the results of giving parathyroid extract to combat guanidine, Major and Buikstra (36) report that parathyroid extract will lower blood pressure increased by guanidine. White and Cameron, however, say the effects of parathyroid extract are negligible (68). Collip (9) concludes from the results of his use of parathyroid extract that the facts favor McCallum's rather than Paton's theory.

Intra venous and oral administration of methyl guanidine salts has convinced Swingle and Nichols (63) that the effects are hardly comparable to symptoms of tetany. Comparison of urea and nonprotein nitrogen in parathyroidectomized dogs and in dogs injected with guanidine has led Collip and Clark (10) to the conclusion that the two conditions are dissimilar.

Little work seems to have been done with reference to the possibility of acid-base equilibrium disturbance by guandine, although Gyorgy and Vollmer (22) claim that

alkalosis is a prominent feature of guanidine poisoning. As would be expected if this were true, they find that acid administration is beneficial. Interesting in this connection, is the observation of Hummel (26), that an isolated gastrocnemius in Ringer's solution containing guanidine, loses its irritability much less rapidly if the reaction is alkaline and gelatine is present. Gelatine seems to make no difference in an acid reaction. They also found that starch absorbs guanidine much more rapidly in an alkaline medium.

Rosenow (52) sees no relationship between guanidine and anaphylactic shock since necessary dosage is greater than could result from parenterally introduced protein. Heyde (24) reported that methyl guanidine poisoning in guinea pigs and rabbits showed a marked similarity to anaphylactic shock. This, Fuhner (18) using rabbits and Loewitt (34) using guinea pigs, were not able to verify when using a synthetic methyl guanidine compound. Klinger (28) in 1921 observed that guanidine effects the respiration in cats. Effects of guanidine compounds upon the respiration of dogs has been frequently observed in this laboratory. Frank, Stern, and Nothmann (15); and Paton (47) believe that guanidine has the important physiological function of maintaining muscle tonus. The condition of the muscles, according to this conception, controls the production of guanidine from choline of lecithin in the liver. The secretion of the parathyroids regulates this

activity. Kamarrow (29) thinks of guanidine as a secretory hormone.

Not much appears to be certainly known concerning the fate of guanidine in the body. As previously stated, several workers have reported it in the urines of normal and of parathyroidectomized dogs and of normal persons as well as of those suffering from tetany. Its existence in the feces is also probable. In a case of idiopathic tetany, 305mg. were extracted in the urine, 17mg. in the feces (Mattrass and Sharpe) (43). Since very slow injections do not produce the usual pressor effects, Major (37) believes it is destroyed or neutralized. Collip and Clark (10) suggest the possibility that guanidine may be converted into urea; while Paton, basing his conclusion on the increase of muscle creatine following guanidine injections (47), presents the theory that guanidine is converted into creatine as a detoxication process.

1. Effects on the Vagus Nerve of the Dog.

Previous work in the department had shown that methyl guanidine salts have a profound effect upon the ability of the vagus nerve to produce cardiac inhibition in the frog and turtle. This work was undertaken to determine whether the same or similar conditions would be found to obtain in the dog. The experimental work was done on forty six dogs. The dogs were anesthetized with ether; a tracheal cannula was inserted; the carotid artery was dissected out and cannulated for blood pressure tracings.

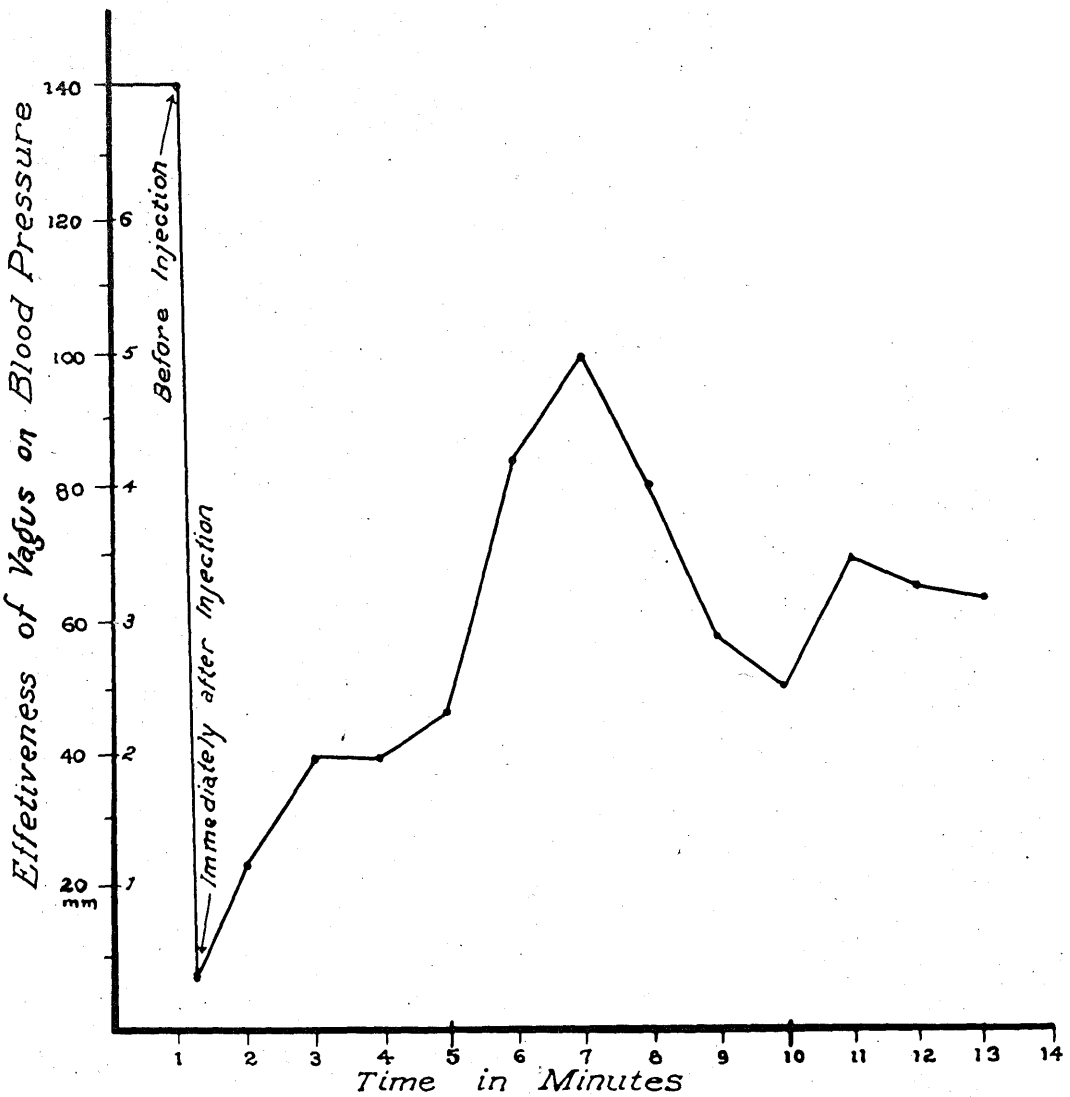
The vagus nerve was dissected out, supplied with a lifting ligature, and cut so that the peripheral end could be stimulated. In almost every case the left vagus was cut. However, in one or two cases where the left vagus was not active or for some other reason did not appear suitable, the right was used. Injections were made into the left femoral vein by means of a syringe.

In giving the anesthetic an ether bottle was used which was so constructed that the valves could be opened wide at the beginning of the experiment and left open, usually without change during the experiment. This largely eliminated changes in depth of anesthesia during the work.

Vagus stimulation was by means of the usual laboratory inductorium furnished by the Harvard Apparatus Company. Four dry cells averaging 20 amperes each were connected in series in the primary circuit. After the strength of stimulation required to give a moderate reduction in blood pressure was found, the secondary coil was fastened and left undisturbed during the rest of the experiment. This procedure was departed from in the case of Dog No. 42. With this dog the aim was to find the minimal effective stimulation at different times during the experiment rather than to keep the stimulation at a constant strength.

It was found by the work on these dogs that methyl guanidine has marked effects upon the vagus nerve of the

PLATE III



CURVE SHOWING VAGUS EFFECTIVENESS IN LOWERING BLOOD PRESSURE BEFORE AND AFTER AN INJECTION OF 0.06 GRAM METHYL GUANIDINE SULPHATE PER KILO.

dog. Small doses 0.025 gm. per kilo of body weight or less often cause an increased effectiveness in inhibiting the heart (as measured by reduction of blood pressure). Larger doses if injected slowly intravenously or subcutaneously often have the same effect.

Moderate doses of methyl guanidine sulphate (0.05-0.06 gm. kilo of body weight) usually cause a sharp decline of vagus effectiveness followed by a quick recovery to near normal effectiveness, which, in turn, is often followed by a secondary decrease. This is shown graphically in the curve shown in Fig. 1. This curve is a composite curve taken from the results obtained on a whole series of dogs. The curve shows the amount that the mean blood pressure could be depressed by stimulating the vagus nerve immediately before the injection of the drug (normal), and at various time intervals after the injections. It will be noted that immediately following the injection the irritability of the vagus is almost completely lost. This "atropine-like" action of methyl guanidine is much more lasting in frogs or turtles.

Large doses (0.1 gm kilo of body weight or above) usually cause profound depression of the vagus nerve often accompanied by cardiac irregularities of different kinds. Sometimes these large injections cause slowing of the heart and concomitant block of the vagus nerve. This action appears to be a combination of a pilocarpine-like effect upon vagus endings and at the same time a block somewhere along the vagus pathway, perhaps a nicotine-

like effect upon the ganglia. The slowing of the heart caused by these large doses can be quickly and completely removed by injections of atropine. More often these large doses of methyl guanidine cause cardiac slowing of the heart unaccompanied by complete block.

Large doses (o.1 gm. per kilo and above) are frequently lethal by causing respiratory arrest, the heart continuing to beat long after respiration has ceased.

Burns and Watson report that injections of calcium lactate restores the vagus to its normal activity after its effectiveness has been depressed by guanidine. Since the ability of guanidine salts to do this would appear to be quite significant with reference to the relationship of guanidine compounds to parathyroid tetany, a group of 8 dogs was used in an attempt to verify Burn's and Watson's findings. The results as observed during the experiments were not striking or conclusive. Indeed, the opinion of the workers was that no beneficial results followed the administration of the calcium solutions. However, when the results were compiled in the form of a table and compared with the results obtained on other dogs not treated with calcium, it appeared that the calcium did to a certain extent combat the methyl guanidine effect. The fact that we obtained much less striking results with calcium than did Burns and Watson, we ascribe mostly to the rather quick recovery which occurs in the vagus nerve of the mammal after guanidine injections. We imagine it was this quick spon-

taneous recovery which these workers attributed to the calcium.

We also tested the effect of physostigmine and of liver extract (Heparmone) in removing the methyl guanidine effects on the vagus nerve. Physostigmine was tried because the effects observed in the experiments on the vagus nerves appeared to resemble closely the effects produced by atropine and it appeared desirable to find out if this effect could be overcome in any degree by the action of physostigmine. Heparmone was tried because it was being used by others in an attempt to combat the pressor effects of guanidine-like compounds. Neither of these substances showed any tendency to neutralize the action of methyl guanidine upon the vagus nerve.

The effects of injections of methyl guanidine salts on the vagus nerve seems to be dependent upon several factors: the size of dose, the rapidity of introduction into the blood stream, and the previous condition of the vagus.

II. Effect of Methyl Guanidine on Salivary Secretion.

Since the guanidine salts we were using had been found to exert a profound influence upon the vagus nerve, it appeared desirable to ascertain whether or not this or similar effects could be demonstrated upon other autonomic nerves, especially parasympathetics.

Accordingly we ran a series of experiments upon twenty dogs to determine how the submaxillary gland and

more particularly how the chorda tympani nerve would be affected by injections of these substances. In the first part of the work on the submaxillary gland, the aim was to discover whether methyl guanidine would produce a block in the course of the chorda tympani fibers similar to the block produced in the cardiac inhibitory fibers of the vagus. We quickly discovered, however, that injections of methyl guanidine of 0.05 - 0.06 gm. kilo of body weight cause a considerable secretion of saliva by the gland. In most of these experiments the chorda tympani had been cut and supplied with a lifting ligature so the secretion that was obtained by intravenous injection of the drug was clearly caused by peripheral effects upon the nerve fibers or directly upon the gland. A dose of 0.1 gm. kilo of body weight was hit upon as best for the work on salivary secretion and adhered to throughout. Doses of this size produce a copious secretion of saliva. Since secretion by the gland is already occurring it is very difficult to determine, after methyl guanidine injections of the above mentioned dosage, whether the chorda tympani fibers are normally irritable to electrical stimulation or not. In many cases, however, we were able to demonstrate a reduced irritability on the part of the chorda tympani fibers to electrical stimulation. That the secretion of saliva that resulted from guanidine injections was actually due to stimulation of the parasympathetic fibers of the chorda tympani was shown by the fact that atropine injections would stop such salivary secretion almost instantaneously.

When the methyl guanidine injections were repeated several times it was possible to produce a complete and lasting block of the chorda tympani fibers to electrical stimulation. Two doses of 0.10gm. kilo were sufficient to cause a complete nerve block in some of the dogs; in other dogs as high as five doses were sometimes required to produce a complete block. Such large doses are, of course, extremely toxic and when doses of this magnitude are employed it is necessary to give the animal artificial respiration. We mentioned in the preceeding section that lethal doses kill by causing respiratory arrest. That the loss of irritability in these cases was not due to the fatigue produced by long continued stimulation of the chorda tympani, was shown by the control dogs in which there was no loss of irritability over a period of stimulation longer than any of those in which the drug was used.

Here again we tried the effect of calcium injections. The calcium in the form of calcium chloride was injected into the femoral vein after a complete chorda tympani block had been produced by repeated doses of the guanidine compound. And here again it was found impossible to remove the parasympathetic block by using calcium, even though the injections were continued over long periods of time and large amounts of calcium were injected.

The results of the methyl guanidine injections upon the secretory activity of the submaxillary gland are tabulated in Table No. 1. It will be noted that the injections

TABLE I

EFFECT OF METHYL GUANIDINE ON SUBMAXILLARY GLAND.

Dog No.	M.G.S. Dosage per kilo	Saliva per min. before inj.	Saliva Per min. after inj.	Effect of M.G.S.	Duration of effect.
47	00.1mgm.	0.75drop	3.5	Inc. salivation	During remainder of experiment
48	0.05" four doses			Almost complete block of chorda tym.	
49	0.1	0	7	Salivation	Stopped immediately by atropine.
50	0.10			Appeared to reduce effective ness of c. tympani	
51	0.1 two doses	0	4	Salivation. C.t. part ly blocked.	
52	Do.	1	8	Salivation.	Two small doses of atropin did not com pletely stop sec.
53	Do.	1 in several mins.	6	Salivation.	3mgm. atropin did not completely stop salivation.
54	0.1	Do.	1¼	Salivation.	Completely stopper by 5mgm. atropine.
55	0.1	3	17	Chorda t. partially blocked.	Twenty min.
56	0.1	0	5		
57	0.1 2 doses	1	4	Salivation.	Until death.
58	Do.			Chorda t. markedly depressed.	
59	0.1	0	4	Salivation.	Stopped at once by atropin. M.G.S. then ineffective.
60	0.1	0	18	Salivation.	Stopped by atropin.
61	0.1	0	1	3 drops.1 per min.	
62	0.1	0	2	Salivation.	Stopped at once by atropin.
63	0.1	0	1 1/3	Salivation.	2nd &3rd doses of M.G.S. caused block of c.t.
64	0.1	0	2	Salivation.	Do. Calcium did not restore irritability.
65	0.1	0	12	Salivation.	4 more doses caused c.t. block. Ca. could not restore irritability.
66	Control	---	---		Repeated stimulation did not cause irritability loss.

invariably caused a clear cut increase of salivary secretion.

III. Effect on Blood Flow Through the Submaxillary Gland.

To determine the effect of the drug used upon the blood flow through the submaxillary gland the vein draining the gland was dissected out, all the other branches of the external jugular vein were tied off, and then the main trunk of this vein was cut and cannulated in such a way that all the blood coming from the gland flowed from the cannula. In the later experiments heparine was injected into the animal to render the blood incoagulable. All the work was done under ether anesthesia.

Table No. II shows the results obtained upon blood flow through the submaxillary gland. In the first of these experiments no precaution was taken to maintain blood pressure at a constant level and, as the table shows, in these experiments a rather marked increase of blood flow through the gland invariably occurred. Since injections of the size we were employing cause a considerable and sustained rise of blood pressure, it was obviously impossible to say whether the increased blood flow was due wholly to this rise of blood pressure or whether vasomotor changes also played a part. To settle this point it was necessary to keep the blood pressure constant during the course of the experiment. This was done by means of the blood pressure equalized described by Jackson (27).

TABLE II

EFFECT ON BLOOD FLOW THROUGH SUBMAXILLARY GLAND

DOG No.	Dosage mgm./kilo	No. drops per min. before	No. drops per min. after	Blood pr. kept constant	Effect on bl. flow	Remarks;
67	0.10	6	22	No		Subsequent inj. of 5 mgm. atropin caused still greater increase in blood flow.
68	2doses 0.10			No	(?)	Clotting caused so much trouble impossible evaluate ex.
69	2doses 010	56	68-6	No	then	Decrease after 2nd dose
70	0.10	13	18-6½	No	then	*After atropine.
71	0.10*	102	Constant stream	No		* Preceed- ed by 7mgm. atropine
72	2 doses 0.10*	8	13	No		*Do. and by Heparine.
73	0.10*	28	34	No		*Proceeded by Heparine and atropin.
74	0.10	24	18	Yes		Heparine
75	0.10	12	11	Yes	slight	H.
76	2 doses 0.05	3	2-4½*	Yes	then	-after the 2nd dose.
77	0.10	13	4	Yes	-	Atropine given after 2nd dose. H.
78	0.1o	12	17	Yes	*	*Ergotoxine had been given previously but did not caus adrenalin reversal. H.
79	0.10	15	19-12	Yes	*then -	H.
80	0.10	56	57-130	Yes	-	H.
81	0.10	5⅓	4 1/3 5 2/3	Yes	-slight then	H.
82	0.10	0 5/6	0 6/7	Yes	-slight	H.

We found that when the blood pressure was kept constant there was no regularity as to the effect on blood flow. Sometimes a slight increase occurred; sometimes a marked increase. Sometimes a slight decrease occurred; sometimes a marked decrease. Sometimes the blood flow fluctuated about the normal. Insofar, then, as the effects of methyl guanidine on the parasympathetic vasodilator fibers of the chorda tympani are concerned, no clear cut results were ob tained. This appears to indicate either that the parasympathetic vasodilator fibers are not stimulated by the drug, or, what seems more probable, that both parasympathetic vasodilator and sympathetic vaso constrictor fibers are being stimulated to such a degree that neither constantly predominates. This conclusion appears all the more likely in view of the apparent stimulating action that this substance exerts upon the sympathetic fibers to the bronchioles.

IV. Effect on Pancreatic Secretion.

From the results obtained in the experiments on salivary secretion it was rather expected that increased pancreatic secretion could be demonstrated following methyl guanidine injections because of the stimulating effect of the drug on vagus fibers. Accordingly, another series of experiments was run on dogs to determine whether or not such secretion of pancreatic juice following injections of the drug could be demonstrated.

In the first few of these experiments the small upper

TABLE III.

EFFECT ON PANCREATIC SECRETION.

Dog No.	No. drops per min. before M.G.S.	Dosage of M.G.S./ Kilo.	Drops per min. after M.G.S.	Remarks:
83	0	0.10gm.	6*	This secretion stopped soon after giving 15mgm. atropin. *May have been due to kink in duct.
84	1/ 3 min.	0.10	1 drop in ½ hr.	Two drops were formed following a 2nd injection of HCl into intestine.
85	1per 4 min. Do.	0.10	After long interval 3 drops in 1½min.	Injection of pilocarpine gave a copious secretion of pan. juice. Later injection of HCL gave secretion at rate of 8 in 10 min. This was stopped at once by 0.1gm. M.G.S./ kilo.
86	1.2 Do.	0.10	3 drops in 3½ min.*	Secretion stopped almost at once. Could not be reinduced by HCl but could by pilocarpine.
87	2 3/5Do.	0.025	5/8 then 0	Secretion was reinduced pilocarpine. Subsequent doses of 0.025gm.M.G.S./ kilo slowed but did not stop the secretion.
88	4 Do.	0.012 0.012	3drops after I. One " II.	Reinduced by HCland stopped by 2 doses 0.012 gm.M.G.S./kilo each.Reinduced by HCl, 2doses of like amt. stopped it. 10mg. pilocarpine caused copious secretion.
89	9/13 Do.	0.012	Only 1 drop afterward.	Subsequent injs. of HCl did not reinduce secretion but pilocarpine did. M.G.S.Appeared to stop it.
90	2 Do.	0.0065	Quickly slowed to 1 every 4 min.	Difficult to reinducewith HCl. Started again by HCl it was quickly stopped with 0.087gm.MGS/Kilo. Started again it was again quickly stopped (0.1gm.MGS/kilo.
91	2½ Do.	0.0063 0.0063 0.0063 0.0063 0.0063	2 per min. 12/7min. 5/8 " 1/3 " 1/3 "	Afurther injection of HCl did not cause secretion An injection of 10mgm. pilocarpine did not cause secretion.
92	2½after pilocarpine & HCl. 3 after 2 mgm.pilocarpine.	0.1 0.1 0.1	1.2 1 2/7	After pilocarpinethe pancreatic secretion was slowed but not stopped by M.G.S.

pancreatic duct was freed enough to cannulate (care being taken to disturb the pancreas and especially its blood supply as little as possible). Later the larger, lower pancreatic duct was used. After cannulation a rubber tube was fitted to the cannula and led out through the wound so that the drops of pancreatic juice could be counted. In almost every case an initial secretion of pancreatic juice was induced by the introduction of 0.5% HCl into the lumen of the duodenum.

The results of this series of experiments are shown in Table No. 3. Rather to our surprise we found that instead of causing a secretion of pancreatic juice the intravenous injection of methyl guanidine had the opposite effect, i.e., it tended to stop any secretion which was already occurring. After the secretion had been stopped by the methyl guanidine it was usually rather difficult to reinduce it by the introduction of more HCl into the duodenum. Pilocarpine, however, would promptly and effectively reinduce the secretion of pancreatic juice. In one dog (No. 91) even pilocarpine failed to cause any further secretion.

It seems, at first glance, rather odd that this guanidine derivative which had been found to exert profound effects upon parasympathetic nerves elsewhere in the body, should here in the pancreas interfere more with the "secretin" mechanism of secretion than with the vagus mechanism. The explanation for this may be that since guanidine causes vasoconstriction (Maele and Bulcke, 35; Stoland, 60; and

Major, 37) and since the pancreas is said to be especially susceptible to changes of blood supply, this substance would tend to effect especially that mechanism that is most directly dependent upon the blood supply, i.e., the secretin mechanism. Since pilocarpine causes the pancreas to secrete even after rather large doses of the guanidine derivative it follows that this substance has not materially depressed the irritability of that part of the vagus terminations upon which pilocarpine exerts its effect. The actual part of the parasympathetic fibers stimulated by pilocarpine appears to be at or near the myoneural junction (Gaisboeck, 59).

On account of the relationship thought by the Glasgow school of physiologists to exist between the guanidines and parathyroid tetany (45), (46) it is interesting to note that Stoland (61) found a decreased pancreatic secretion following the removal of the parathyroids of dogs.

In one dog out of the group (No. 63) a flow of pancreatic juice did follow the injection of methyl guanidine into the femoral vein. This result is so completely at variance with all the other results obtained on this series of dogs that we are at a loss to explain it. Two possibilities suggest themselves, however: There might possibly have been a kink at the junction of duct and cannula, which prevented the flow of pancreatic juice that was being secreted by the pancreas. By some movement of duct or tube this kink might have become straightened out soon

after the guanidine was injected, allowing the pancreatic juice which had been dammed back, to escape. The other possibility is that for some reason the vagus fibers to the pancreas of this dog were unusually susceptible to the stimulating effect of methyl guanidine.

V. Effects on the Bronchioles.

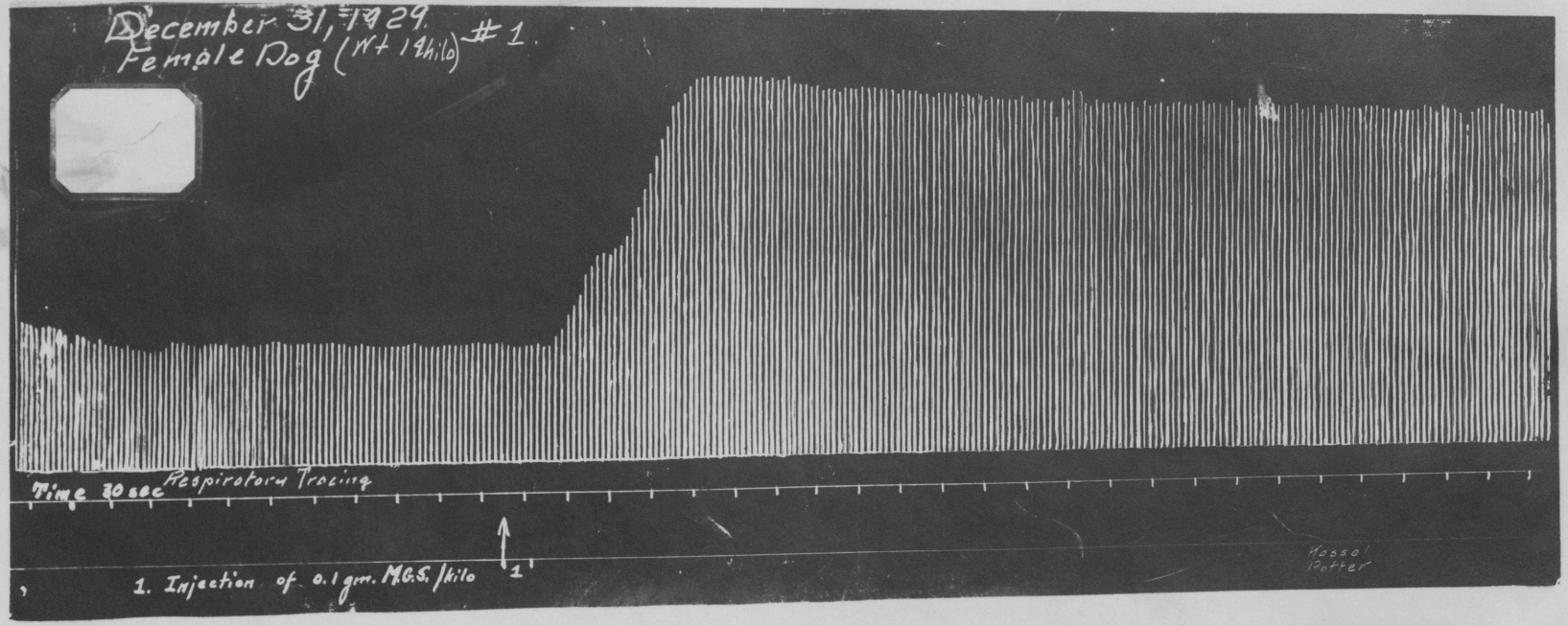
Another place where autonomic nerve effects can be tested readily is, of course, the bronchioles. In fact, the bronchioles appear to be exceptionally well adapted for testing the action of substances which, like the guanidines, seem to exert influences upon both sympathetic and parasympathetic nerves. We employed Jackson's method (27) of recording changes in the size of the bronchioles. This method incolves essentially the obtaining of a graphic record of the inflation of the lungs while they are being inflated artificially at a constant pressure. By this method dilation of the bronchioles is shown on the graph by greater excursions of the marker, since bronchio-dilation decreases the resistance offered the air in passing into the lungs, and, consequently, greater lung inflation results. At first we made use of a large tambour for recording the lung inflation, but we soon found that when the bronchioles dilated markedly, the rubber covering of the tambour became stretched to the limit of its elasticity and further dilation of the bronchioles would not be recorded accurately. We then substituted an instrument based on the principle of the spirometer for the tambour and found this recorder satisfactory in every respect.

TABLE IV.
EFFECT ON BRONCHIOLES.

Dog No.	Dose per kilo	Effect on bronchioles	Remarks:
93	0.025 CO_3 0.025 0.025	Dilation	This was given after a small dose of pilocarpine which showed no effect.
94	0.100 CO_3	Constriction for a short time.	No further effect could be produced by guanidine CO_3, atropine, or adrenaline.
95	0.100	Dilation (persistent)	Further doses guanidine carbonate gave no further dilation. A dose of physostigmine gave marked constriction.
96	0.025 0.025 0.025 0.025	Dilation.	Dilation began after second dose. Subsequent doses of glucose gave no result.
97	0.050 0.050	Dilation Constriction Dilation	Dilation began to come on after first dose. A transient constriction appeared after second dose followed immediately by further dilation. Atropine and adrenaline caused little or no increase.
98	0.025 0.025 0.025	Slight dilation.	This dilation becomes much more pronounced after administering 5 mgm. atropine.
99	0.050* 0.050	Dilation.	*Given after 5mgm. pilocarpine which caused a short transitory bronchioconstriction.
100	0.04 0.05 0.01	Dilation after dose.	The dilationwas not increased by atropine or adrenaline.
101	0.10	Dilation (Marked and lasting)	
102	0.10	Dilation (Marked)	
103	0.10	Dilation	
104	0.10	Dilation	
105	0.10	Dilation (Marked)	

A tabulation of the results of the experiments on the bronchioles appears in Table IV. In some of these experiments there was at times some evidence of parasympathetic stimulation following the methyl guanidine injections. The constriction that occurred in Dog 94 and in Dog 97 is indicative of parasympathetic stimulation. The much more promounced bronchio-dilation that occurred in Dog 98 following an injection of atropine also indicated that the parasympathetics were being stimulated and were resisting the dilation of the bronchioles.

However, by far the more striking effect was the bronchio-dilation that occurred at some time shortly following the methyl guanidine injection in every dog save one (Dog 94). This dilation, of course, was very strong evidence that the sympathetic endings in the bronchioles were being stimulated. To be sure, we recognize the possibility that the bronchio-dilation might be explained either as the result of sympathetic stimulation, parasympathetic block, or a combination of these two actions. We did not at the time believe that a block of the parasympathetics could explain the results we obtained, because sufficient chloroform had been injected into the vertebral artery to destroy the activity of the respiratory center, and we felt that if the respiratory center had been destroyed, then in all probability, the other medullary centers were also destroyed. With the medullary centers destroyed it seemed hardly possible that a flow of impulses could be


December 31, 1929.
Female Dog (Wt 14½ kilo) #1.
Time 30 sec Respiratory Tracing
1. Injection of 0.1 gm. M.G.S./kilo
1
Hasso
Potter

occurring over the parasympathetics. However, to make doubly certain that the effects we were obtaining were really due to sympathetic stimulation, we tried the effect of the injection of the guanidine derivative following an injection of atropine sufficiently large to paralize the parasympathetic endings. This was tried on three dogs (Dogs 106, 107, and 108) and in each case a well marked dilation followed the injection of the guanidine. Fig. 2 shows a graphic record of bronchio-dilation obtained in the case of one of the dogs of Table IV.

Discussion and Conclusion.

The results of our experiments show that methyl guanidine salts do exert powerful effects upon certain of the autonomic nerves. In the case of the vagus cardio-inhibitory fibers we were able to demonstrate clearly that a blocking effect can be produced. This block could be more easily established in frogs and turtles and was also more persistent in these animals, but could be clearly demonstrated also in dogs. This confirms the contention of Burns and Watson that guanidine has nicotine-like or atropine-like effects upon vagus endings (3) (4). That this action is a nicotine-like actionn ganglia rather than an atropine-like action on vagus endings, we are led to believe, since the block may be present at the same time that the vagus

endings are being stimulated. The fact that parasympathetic pathways may be blocked by methyl guanidine is further confirmed by the complete and persistent blocking of the chorda tympani which we were able to secure by the intravenous use of this drug. The primary cardiac acceleration in frogs following guanidine application, which Burns and Watson (5) ascribed to the stimulation of the accelerator nerves to the heart, is probably due chiefly to the vagus block and consequent interruption of the tonic vagus impulses to the heart. That sympathetic stimulation may play a part, we are very willing to admit, since we obtained definite evidence that methyl guanidine does stimulate sympathetic fibers elsewhere.

A blocking effect is not the only action of methyl guanidine upon the parasympathetic, however, for we obtained convincing evidence both in the vagus and in the chorda tympani experiments that a stimulating effect can be exerted. This stimulating effect on the vagus was shown by the marked slowing which sometimes followed the injection of methyl guanidine. In the case of the chorda tympani nerve, the stimulation was shown by the copious flow of saliva that resulted. In either case an injection of atropine always gave a complete and almost instantaneous cessation of the stimulating effect, indicating that the stimulation was via. the parasympathetic fibers and that the point where methyl guanidine was exerting its stimulating effect must be central to the seat of action of atropine. Maele and

Bulcke (35) report evidence of vagus stimulation by guanidine and Sileninkow (59) thought it stimulated Auerbach's plexus in the intestine, which some think of as corresponding to the post-ganglionic vagus fibers found elsewhere. We were not able to secure clear cut evidence of any methyl guanidine effect upon the vasodilator fibers of the chorda tympani nerve. Since, however, these fibers react differently to atropine than do the secretory fibers to the salivary glands, it may be that they also react differently to methyl guanidine salts.

From the results obtained on the bronchioles there appears to be no question that methyl guanidine also exerts a stimulating action upon sympathetic fibers. This supports the statement of Burns and Watson (5) that guanidine compounds stimulate the sympathetic fibers to the heart. A part of the vasoconstriction which causes such a marked rise in blood pressure following guanidine injections, may well be the result of guanidine stimulation of sympathetic vasoconstrictor fibers (Major, 37).

What the physiological significance of guanidine is seems very hard to determine. The report that guanidine compounds appear in large quantities in the urine of dogs following parathyroidectomy (30), (31), (32), (48); that the conditions following guanidine poisoning are similar to the conditions following parathyroid removal (45, 46); that the concentration of guanidine compounds in certain

viscera after guanidine injections is much the same as their concentrations in parathyreopriva (14); and that the blood of animals in parathyroid tetany will produce effects upon the vagus nerve similar to the effects produced by application of guanidine solutions (33), all appeared to indicate that guanidine compounds released into the blood stream in hypernormal amounts may be responsible for the syndrome of parathyroid tetany. That this is probably not the case, however, is strongly indicated by the following facts: Many good technicians have failed to verify the presence of increased amounts of guanidine in the blood and urine of parathyroidectomized dogs (21, 39). Calcium administration does not readily relieve guanidine poisoning (17, 23, 65, 66). The symptomatology of the two conditions (guanidine poisoning and parathyroid tetany) is quite dissimilar. It may be that a somewhat increased guanidine concentration in the blood of parathyroidectomized animals together with the increased irritability of the neural structures resulting from the low blood calcium level would involve the guanidine compounds in the production of the syndrome called parathyroid tetany. That the guanidine compounds are alone responsible for the tetany, or even that they are mainly responsible for it appears extremely unlikely, although they may have a part in its production.

Some have been inclined to assign to these substances the important function of maintaining muscle tonus (15, 47).

This theory we are inclined to question because of the probable toxicity of concentrations sufficient to affect tonus, and further, because the modern conception of tonus as being maintained by a tetanic contraction of small groups of muscle fibers acting successively does not seem to require the participation of any drug as potent as the guanidines.

We have shown that small concentrations of methyl guanidine in the blood stream actually increases the irritability of some of the parasympathetic fibers. Since these substances are normally present in very small concentrations (39) they may actually function in maintaining nerve irritability.

It would appear to us, however, that the most likely interpretation of the significance of the body (and especially of the blood) guanidines is that they are intermediary metabolites that arise from some substance like cholin, creatine, creatinine, or arginine and by diffusion gain entrance into the blood without having any real physiological significance there.

Summary.

1. Methyl guanidine salts have marked effects upon the vagus nerve of the dog. Small doses 0.025gm./kilo or less often cause an increased effectiveness in inhibiting the heart (as measured by reduction of blood pressure). Larger doses if injected slowly or subcutaneously often have the same effect.

2. Moderate doses of methyl guanidine sulfate (0.05 - 0.06gm./kilo of body weight) usually cause a sharp decrease of vagus effectiveness followed by a quick recovery to near normal effectiveness, which, in turn, is often followed by a secondary decrease.

3. Large doses (0.1gm./kilo of body weight or above) usually cause profound depression of vagus effectiveness often accompanied by cardiac irregularities of different kinds. Sometimes these large doses cause slowing of the heart and concomitant block of the vagus nerve. More often they cause cardiac slowing unaccompanied by complete vagus block.

4. Large doses (0.1gm/kilo of body weight and above) are frequently lethal by causing respiratory arrest. This arrest is not due to asphyxia caused by a spasm of the bronchial musculature.

5. Calcium lactate showed some value in counteracting the poisoning effect of methyl guanidine salts on the vagus nerve, but no apparent valve in counteracting the block produced in the chorda tympani secretory fibers to the submaxillary gland.

6. The effect of the injections of methyl guanidine salts on the vagus nerve seemed to be dependent upon several factors: size of dose, the rapidity of introduction into blood stream, and the previous condition of the vagus.

7. Moderate or large doses of methyl guanidine cause secretion of saliva by the submaxillary gland due to a stimulating effect exerted upon the secretory gland due to a stimulating effect exerted upon the secretory fibers of the parasympathetic chorda tympani nerve.

8. Very large doses of methyl guanidine produce a complete block of the chorda tympani secretory fibers.

9. Injections of methyl guanidine salts into the blood stream give no consistent effects upon the blood supply to the submaxillary gland.

10. The secretion of the pancreas is slowed or stopped by methyl guanidine injections.

11. Bronchio-dilation due to sympathetic stimulation follows methyl guanidine injections.

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