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The Action of Gramine.

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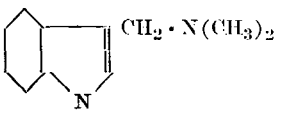
In the course of investigating Swedish barley, chemically and genetically, von Euler and Hellström[1] discovered a crystalline alkaloid, named gramine, in 2 chlorophyll-deficient mutants. Subsequently, Orechoff and Norkina[2] succeeded in isolating an alkaloid, called donaxine, from an Asiatic reed (botanically known as *Arundo donax* L.), which later proved to be identical with gramine.[3,4] Wieland and Hsing[5] showed that gramine conforms to the structure of 3-dimethylaminomethylindole, as follows:



Gramine was investigated pharmacologically by Supniewski and Serafenówna,[6] who showed that it caused a weak parasympathomimetic action. Raymond-Hamet[7] observed that small doses of gramine raised, but large doses lowered, the blood pressure in dogs.

In our laboratory, studies have been conducted with indole derivatives, both natural and synthetic. To explore this field further, gramine was tested pharmacologically in animals. Our sample was generously supplied by Dr. Richard H. F. Manske, National Research

1 von Euler, H., and Hellström, H., *Z. f. physiol. Chem.*, 1933, **217**, 23.

2 Orechoff, A., and Norkina, S., *Ber. d. deut. Chem. Gesellsch.*, 1936, **68B**, 436.

3 von Euler, H., Erdtman, H., and Hellström, H., *Ibid.*, 1936, **69**, 743.

4 Brandt, K., von Euler, H., Hellström, H., and Löfgren, N., *Z. f. physiol. Chem.*, 1935, **235**, 37.

5 Wieland, T., and Hsing, C. Y., *Liebig's Ann. d. Chem.*, 1936, **526**, 188.

6 Supniewski, J. W., and Serafinówna, M., *Bull. internat. Acad. polon. d. sc. et d. lett., Cl. méd.*, 1937, 479.

7 Raymond-Hamet, *C. R. Soc. de biol.*, 1937, **126**, 859; 1939, **130**, 1218.

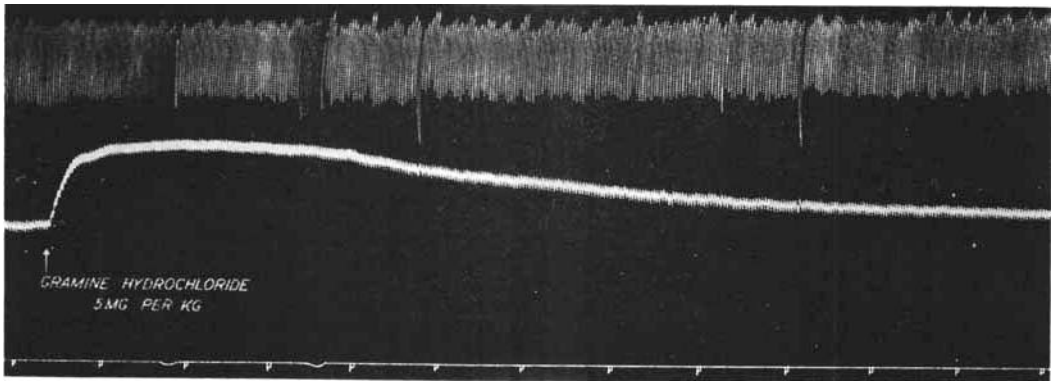


FIG. 1.
Action of Gramine on Blood Pressure and Respiration.
Cat, female, weighing 1.91 kg, was anesthetized by intraperitoneal injection of "Sodium Amytal" (Sodium Iso-amyl Ethyl Barbiturate, Lilly), 80 mg per kg. Records from above down are: respiration, carotid blood pressure, and baseline with time marks in minutes. Gramine HCl in 1% solution was injected rapidly into the femoral vein. The blood pressure rose from 100 to a maximum of 160 mm Hg, lasting for more than 12 minutes.

Council, Ottawa, Canada, and was originally isolated from *Arundo donax* L. The alkaloid was converted into the hydrochloride, in our laboratory, by dissolving the solid base in a solution of an equimolecular amount of hydrochloric acid. After evaporation under a fan, the residue was taken up in absolute ethanol. Upon the addition of anhydrous ether, the hydrochloride crystallized out with ease. The whole procedure must be carried out quickly, otherwise decomposition as evidenced by rapid discoloration takes place. This salt apparently had not been prepared previously. Gramine hydrochloride is freely soluble in water and melts at 190.5-191.0°C (corrected) with decomposition. Analysis for nitrogen satisfies the required composition. $C_{11}H_{14}N_2 \cdot HCl$ (Calculated: N 13.3%; found: N 13.1%). For pharmacologic experiments, fresh solutions were prepared each day.

1. *Action on Circulation.* In 6 anesthetized cats, 17 observations were made on the blood pressure and respiration following the intravenous injection of gramine HCl. Doses ranging from 1-20 mg per kg uniformly caused a sustained rise of arterial blood pressure, an example of which is shown in Fig. 1. No changes in respiratory amplitude took place. Amounts of 30-40 mg lowered the blood pressure, followed by a secondary rise. There was a decrease in amplitude of respiration immediately after injection with prompt return to normal. This change was most likely secondary to the initial fall of blood pressure.

It was also noted that epinephrine following the larger doses of gramine HCl, 30-40 mg per kg, became less effective in raising blood pressure in the same animal. Thus, there is an apparent inhibition of epinephrine response by gramine. In no instance, however, did a reversal of blood pressure occur in contrast with epinephrine-ergotoxine antagonism. The same phenomenon was previously observed by Raymond-Hamet.[7]

Eight tests on the blood vessels of 3 frogs were made by Trendelenburg's method of perfusion. There was a slight decrease of perfusing rate with a concentration of 1:20,000, an evidence of vasoconstriction. The effect became less noticeable with a solution of 1:40,000, but disappeared with a concentration of 1:80,000.

Eleven observations were made on 5 frogs' hearts perfused by the method of Howell and Cooke.[8] A solution of 1:40,000 produced a slight decrease in amplitude and rate of ventricular contractions, but one of 1:5,000 resulted in a very marked decrease in amplitude which was easily reversed by returning to Ringer's solution. A typical record is shown in Fig. 2. Dilute solutions such as 1:100,000

[8] Howell, W. H., and Cooke, E., *J. Physiol.*, 1893, **14**, 198.

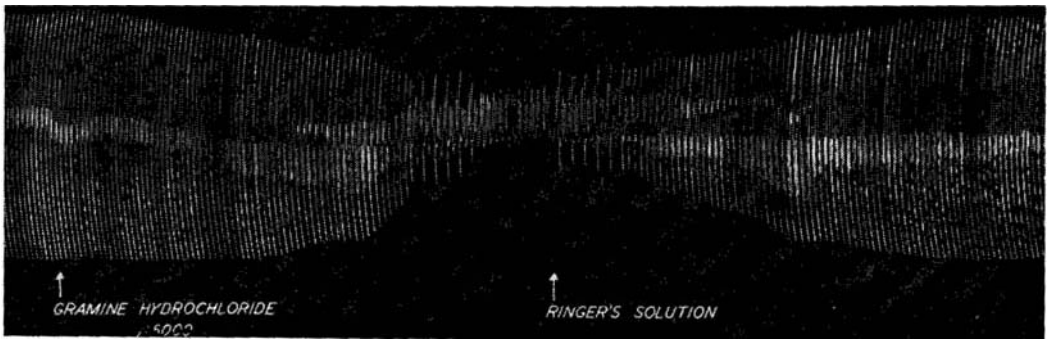


FIG. 2.
Action of Gramine on Frog's Heart.
Leopard frog, female, weighing 84 g, was perfused through inferior vena cava. The depressing action of gramine HCl is obvious, which is reversible by substitution with Ringer's solution.

were ineffective. No distinct stimulation of cardiac contractions was noted with any of the concentrations.

2. *Action on Smooth Muscle Organs.* When solutions of gramine HCl, as high as 4%, were instilled in the rabbit's eye, no change in the diameter of the pupil took place.

Ten tests were made with gramine HCl on strips of isolated rabbits' small intestines immersed in Tyrode's solution warmed to 38°C. Inhibition of peristaltic movements promptly occurred. The effect was definite with concentrations varying from 1:20,000-1:50,000. In most instances, a brief, slight stimulation appeared before inhibition took place. Recovery from inhibition was rapid and complete. Although epinephrine also inhibited peristaltic movements of the same intestines, its response was definitely reduced by a previous application of gramine HCl. The 2 substances are again antagonistic to each other, and certainly not synergistic.

The isolated uteri of 3 species of animals were studied with gramine HCl—rabbits, guinea pigs, and Syrian hamsters (*Cricetus auratus*). Thirty-five assays were made with 12 strips of rabbits' uteri immersed in Tyrode's solution. A dilution of 1:100,000 produced stimulation of contractions as shown in Fig. 3, A. The guinea pig's uterus immersed in Locke-Ringer's solution required larger doses, such as 1:20,000, as shown in Fig. 3, B. Seventeen tests on 5 strips were carried out in that species. The hamster's uterus immersed in Locke-Ringer's solution, like that of the rat, has rhythmic contractions normally. Upon application of gramine HCl, spastic contractions resulted as indicated in Fig. 3, C. Concentrations of 1:20,000-1:10,000 were most suitable.

As is well known, epinephrine also contracts the isolated rabbit's uterus. Our results indicate that when the latter was first treated with gramine HCl, its response to epinephrine was diminished. Again, there is evidence of antagonism between epinephrine and gramine.

3. *Effect on Blood Sugar.* Majority of pressor substances have a hyperglycemic action when administered intravenously. It was thus interesting to ascertain whether or not gramine might have a similar action. Six rabbits were injected intravenously, 2 each, with doses of 5, 10, and 20 mg of gramine HCl per kg of body weight. Their blood sugar was determined at 30-minute intervals according to the procedure of Hagedorn and Jensen.[9] No significant changes in blood sugar were found.

4. *Toxicity.* The median lethal doses (LD_{50}) of gramine HCl were determined in albino mice and rats by intravenous injection. The results are summarized in Table I. Mice, having an $LD_{50} \pm$ S.E. of 44.6 $\pm$ 1.4 mg per kg, are more susceptible than rats, which have an $LD_{50} \pm$ S.E. of 62.9 $\pm$ 2.7 mg per kg. A series of tonic convulsions with an apparent increase of respiratory rate, protrusion of eye-

[9] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46.

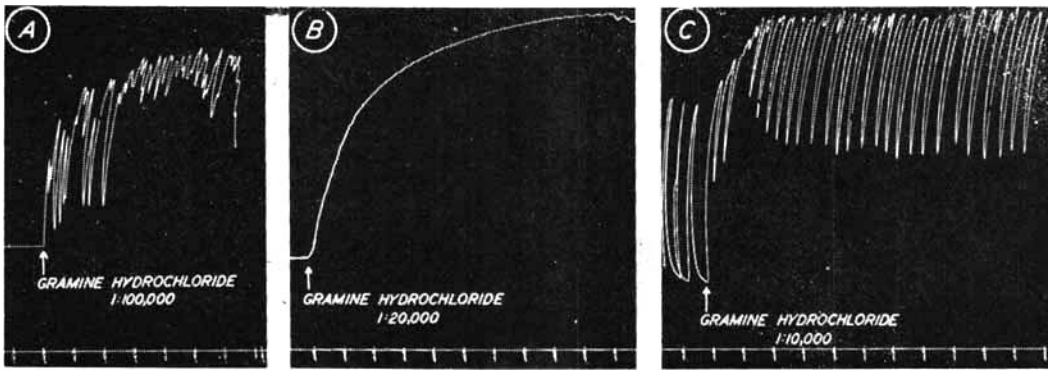


FIG. 3.
Action of Gramine on Isolated Uteri.
A. Rabbit's uterus immersed in Tyrode's solution at 38°C.
B. Guinea pig's uterus immersed in Locke-Ringer's solution at 38°C.
C. Hamster's uterus immersed in Locke-Ringer's solution at 38°C.

balls, and suggestive cyanosis occurred following the administration of lethal and near-lethal doses. With mice, there was a persistent erection of the tail—a phenomenon very similar to that following the administration of morphine.

Six frogs were injected in the lymph sac with gramine HCl in doses ranging from 0.2-1.0 mg per g. These are relatively large doses if expressed on a kilogram basis. All the animals showed depression and prostration, with stretching of hind legs and sagging of front legs. When placed on their backs, they made no attempt to right themselves, even upon the application of mechanical stimulation. This is in contrast with warm-blooded animals, as represented by mice and rats which exhibited evidence of central stimulation following the intravenous injection of gramine HCl.

TABLE I.
Acute Toxicity of Gramine HCl.

Animal	Dose, mg/kg	No. died / No. used	$LD_{50} \pm$ S.E. mg/kg
Mice	36.5	1/10	44.6 ± 1.4
	40.0	4/10	
	45.0	3/10	
	50.0	8/10	
	60.0	10/10	
Rats	50.0	0/5	62.9 ± 2.7
	56.0	4/10	
	62.0	5/10	
	70.0	6/10	
	80.0	9/10	

Summary. 1. Gramine hydrochloride raises blood pressure in anesthetized cats in small doses but lowers it, with a secondary rise, in doses of 30-40 mg per kg. 2. In appropriate concentrations, gramine HCl by perfusion in frogs causes slight constriction of peripheral blood vessels. 3. Gramine HCl when perfused into the inferior vena cava of frogs decreases the amplitude and rate of heart contractions. 4. Gramine HCl inhibits, frequently preceded by a brief stimulation, peristaltic movements of isolated rabbits' small intestines. 5. Gramine HCl contracts the isolated uteri of rabbits, guinea pigs, and Syrian hamsters. 6. Gramine HCl reduces the effect of epinephrine on blood pressure, intestinal movements, and uterine (isolated) contractions, but causes no reversal. 7. Gramine HCl in doses of 5-20 mg per kg does not alter the concentrations of blood sugar in rabbits. 8. The toxicity of gramine HCl has been determined by intravenous injection in both mice and rats, the latter being less susceptible.

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