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THE MODE OF ACTION OF GELSEMICINE*

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The roots and rhizomes of the Yellow Jessamine, *Gelsemium sempervirens,* have been repeatedly subjected to chemical investigations, but the results have varied from laboratory to laboratory. A glance at table 1 will reveal the state of discordance. For more than sixty years gelsemine remained the only crystalline alkaloid that was ever isolated, although other amorphous bases were known to exist and be more toxic. The "gelseminine" of Wormley [1870], Spiegel [1893], and Goeldner [1895] undoubtedly referred to what is now called gelsemine. There is a considerable discrepancy regarding the empirical formula of gelsemine as shown in table 1, so that further analytical work on this compound will not be out of place.

In 1931 one of us (T.Q.C.) working at the Pasteur Institute, Paris, succeeded in isolating three crystalline and one amorphous alkaloids from a specimen of *Gelsemium sempervirens* secured in the United States. Of the three crystalline bodies, one was gelsemine which conformed to the empirical formula of Moore [1910], and the other two were named gelsemicine and sempervine. All of them appeared to have a similar pharmacological action, but gelsemicine in contrast with gelsemine and sempervine proved to be extremely potent and toxic An extensive investigation of the latter was undertaken by Hou [1931, 1932], and the unusually high susceptibility of gelsemicine in mice and rats, as compared with guinea pigs and rabbits, was reported by Chen, Anderson, and

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Robbins [1938] The present communication deals with a few features of this new alkaloid which have not been heretofore emphasized The soluble salt, gelsemicine hydrochloride, m.p. 139.5-140°C (corrected), was used exclusively

TABLE 1.

Active principles of gelsemium sempervirens

Authors	Alkaloid	State of purity	Proposed formula
Wormley [1870]	"Gelseminine"	Crystalline	none
Sonnenschein [1876]	Gelsemine	Crystalline	$C_{11}H_{19}O_2N$
Gerrard [1882-1883]	Gelsemine	Crystalline	$C_{12}H_{14}O_2N$
Thompson [1886-1887]	Gelsemine	Crystalline	$C_{54}H_{69}O_{12}N_4$
	Gelseminine	Amorphous	none
Cushny [1892-1893]	Gelsemine	Crystalline	$C_{49}H_{63}O_{14}N_5$
	Gelseminine	Amorphous	$C_{42}H_{47}O_{14}N_3$
Spiegel [1893]	"Gelseminine"	Crystalline	$C_{22}H_{26}O_3N_2$
Goeldner [1895]	"Gelseminine"	Crystalline	$C_{22}H_{26}O_3N_2$
Moore [1910]	Gelsemine	Crystalline	$C_{20}H_{22}O_2N_2$
	Gelseminine	Amorphous	none
	unnamed	Amorphous	none
Sayre et al [1911, 1919]	Gelsemine	Crystalline	$C_{14}H_{15}ON$
	Gelsemidine	Amorphous	none
	Gelsemoidine	Amorphous	none
	Sempervirine	Reddish brown needles	none
Chou [1931]	Gelsemine	Crystalline	$C_{20}H_{22}O_2N_2$
	Gelsemicine	Crystalline	$C_{20}H_{25}O_4N_2$
	Sempervine	Crystalline	none
	unnamed	Amorphous	none
Hasenfratz [1933]	Sempervirine	Crystalline	$C_{19}H_{16}N_2H_2.O$

Latent period. Upon intravenous injection of toxic or lethal doses, gelsemicine, unlike many other active substances, causes no immediate response until some time has elapsed. At the beginning, unanesthetized animals appeared perfectly normal, but gradually they developed signs of intoxication. For example, in mice and rats there was a latent period of 8-10 minutes before convulsions occurred. Similarly in rabbits, trembling, restlessness, and drooping of the head took place in 15-35 minutes,

on the average 24 minutes, following intravenous injections of lethal or sublethal doses of gelsemicine. The delay in appearance of toxic symptoms was shorter with cats, dogs, and monkeys. Cats showed licking of lips and wabbly gait within 3-9 minutes (average 5 minutes), dogs displayed incoördination in 4-10 minutes, and monkeys exhibited drooping of eye-lids in 1.5-10 minutes (average 4 minutes).

In etherized cats with respiratory excursions as the criteria, the postponement of poisoning from intravenous injection of gelsemicine (0.05-0.1 mg per kg) was very evident. The augmentation of respiratory movements, the decrease in rate, and later irregularity occurred approximately 10 minutes after the administration. The kymographic picture was therefore different from those of morphine, barbiturates, and cyanides, which cause immediate changes of respiration in the same animals.

TABLE 2.

Emetic action of gelsemicine hydrochloride in pigeons by intravenous injection (solution 1:10000)

Dose	Number vomited/ number used	EmD50 ± standard error
mg/kg		*mg/kg*
0.03	0/1	
0.06	1/5	
0.09	2/5	
0.12	1/5	0.108 ± 0.0154
0.15	4/5	
0.18	4/4	

It is difficult to account for the latency of the action of gelsemicine. The simple molecule of the alkaloid as compared with the complicated structures of proteins, and the ease of absorption upon local application to the conjunctival sac make it improbable that gelsemicine migrates with hindrance to the site of action. Nor is there any reason to assume that the base must undergo a chemical change before it reaches the reacting cell. Recent investigations in this laboratory (as yet unpub-

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TABLE 3.

Emetic action and toxicity of gelsemicine hydrochloride in cats by intravenous injection (solution 1:1000)

Cat number	Sex	Body weight	Dose	Nausea	Vomiting	Outcome	LD50 ± standard error
		kg	*mg/kg*				*mg/kg*
1579	F	2.884	0.09	0	0	Survived	
1580	F	2.242	0 09	0	0	Survived	
1559	F	1.800	0. 10	+	0	Survived	
1577	M	2.028	0 10	0	0	Survived	
1578	F	2.368	0. 10	+	0	Survived	
1556	F	1.663	0. 20	0	0	Died	
1573	F	1.811	0. 20	0	0	Died	
1574	M	1.907	0.20	0	0	Died	
1575	M	2.108	0. 20	+	0	Survived	
1576	F	2.136	0. 20	+	+	Died	0.176±0.027
1558	M	2.151	0. 30	+	+	Died	
1563	M	2.181	0. 30	0	0	Died	
1564	F	2.006	0. 30	0	0	Died	
1565	M	2.818	0. 30	+	0	Died	
1572	F	2.194	0. 30	0	0	Died	
1560	F	2.156	0. 40	0	0	Died	
1561	M	2.390	0. 40	0	0	Died	
1562	F	2.400	0.40	0	0	Died	
1557	M	1.822	0. 50	+	+	Died	
1556	F	2.508	0. 80	+	0	Died	

lished) indicate that tutin also has a latent period before the onset of convulsions in mice and guinea pigs, respectively, after intravenous injection.

Emesis. One of the common signs which occur in certain animals by vein is nausea and vomiting. This is relatively constant in pigeons

with appropriate doses, but much less certain in other animals. As shown in table 2, the median emetic dose in pigeons ($EmD_{50} \pm$ standard error) was determined and computed to be 0.108 ± 0.0154 mg per kg according to the method of Bliss [1938]. This is probably less than one-fifth of the lethal dose, for the animals died following injections of 0.6-0.7 mg per kg.

Cats which are prone to nausea and vomiting appeared to be less susceptible than pigeons as indicated in table 3. Doses approaching the LD_{50} did not uniformly cause emesis. Similar irregularity in responses was observed in dogs. In monkeys toxic but non-fatal doses did not produce any evidence of retching or vomiting as seen in table 4.

TABLE 4.

Action of gelsemicine hydrochloride in monkeys by intravenous injection (solution 1:1000)

Monkey number	Sex	Body weight	Dose	Ptosis	Head droop	Tremor and ataxia	Convulsions	Time required for recovery
		kg	*mg/kg*					*hr*
1	F	6.840	0.01	+	+	0	0	0.50
2	F	6.615	0.02	+	+	0	0	0.75
3	M	6.137	0.03	+	+	+	0	1.08
4	M	5.115	0.04	+	+	+	0	2.83
5	M	6.107	0.05	+	+	+	+	2.22
6	M	7.120	0.07	+	+	+	+	>1.88

No clean-cut evidence has been obtained as to the etiology of emesis. It is unlikely that gelsemicine directly stimulates the vomiting center, since the alkaloid in general depresses the central nervous system. Nor is it due to local irritation of the gastric mucosa, for intravenous injections almost uniformly induce vomiting. Perhaps cyanosis due to respiratory depression plays an important role in the act.

Muscular weakness. Upon subcutaneous and intravenous injections of appropriate doses of gelsemicine hydrochloride, muscular weakness

was always observed. This was true in both amphibians and mammals. The action is undoubtedly central since the peripheral motor nerves are not effected as proved by Hou [1931]. Both the brain and spinal cord are depressed as judged by the symptomatology. In monkeys *(Rhesus macaque)* and pigeons, ptosis was one of the most constant signs of intoxication as exemplified in figs. 1 and 2. This was followed by drooping of the head, which was well illustrated in the same animals.

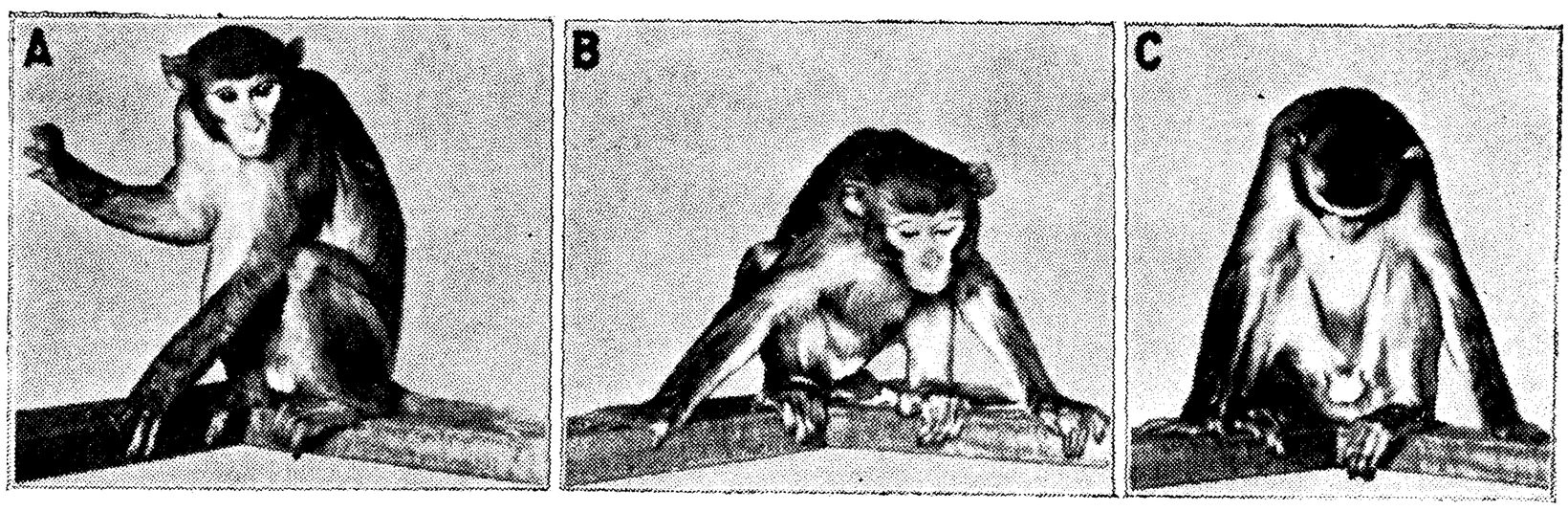

Fig. 1. Action of gelsemicine in monkeys. Monkey numbered 4, male, weighing 5.115 kg, was injected intravenously gelsemicine hydrochloride in the dose of 0.04 mg per kg. Pictures were taken at various intervals: A, before injection; B, 6 minutes after injection; and C, 28 minutes after injection. Note ptosis and head droop.

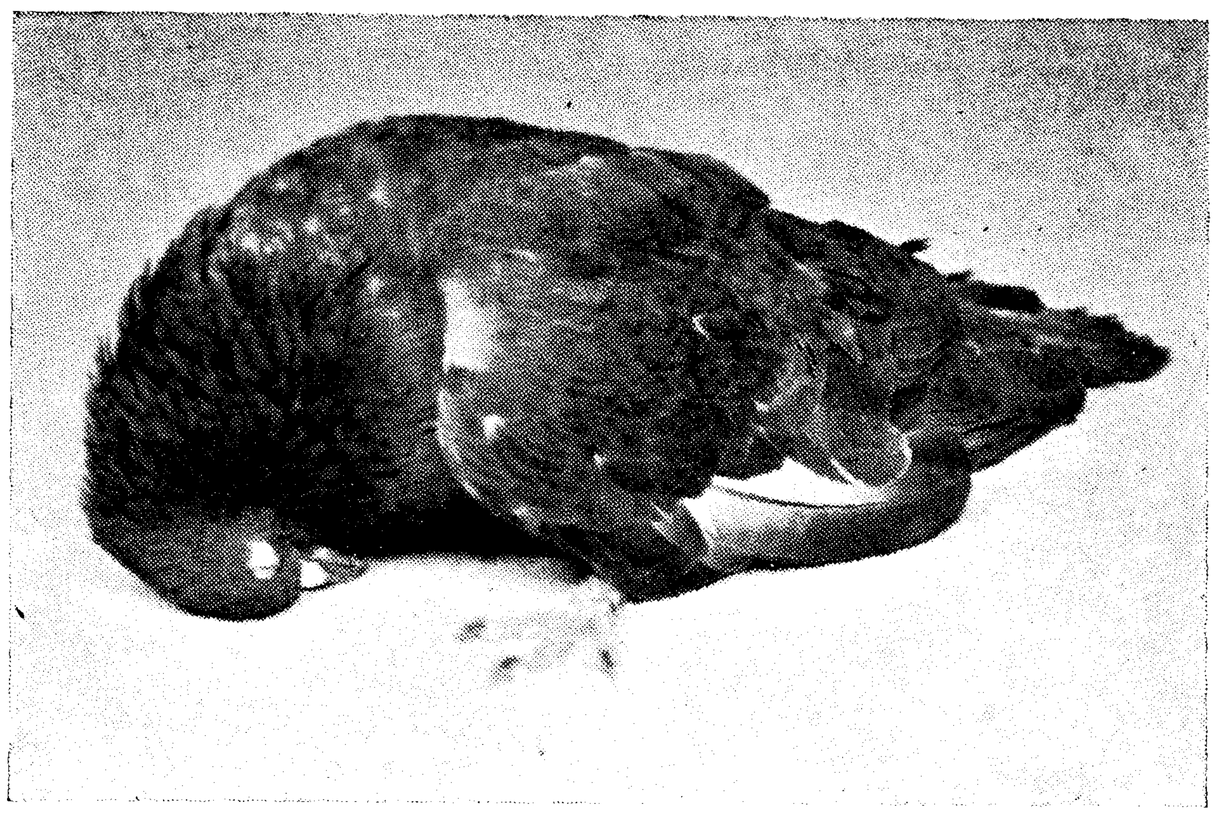

Fig. 2. Action of gelsemicine in pigeons. Pigeon numbered 1884, weighing 395 g, was administered by the wing vein gelsemicine hydrochloride in the dose of 0.15 mg per kg. The picture was made 5 minutes after the injection. The eye-lids were closed and the head rested on the floor.

Weakness of legs, hanging down of the caput, and trembling of the body were the most frequent signs in mice, rats, guinea pigs, rabbits, cats, and dogs, after intravenous injection of near lethal doses of gelsemicine hydrochloride. Frogs lay flat on their abdomen and were motionless following administration of the alkaloid to the ventral sac.

Depression of respiration. The fatal termination of all mammals poisoned with gelsemicine was due to respiratory failure. Cardiac arrest always arrived after the respiration had stopped. Artificial respiration, however, enabled pithed, decerebrated, or anesthetized cats to tolerate as much as 20 average lethal doses of the alkaloid without the slightest evidence of poisoning. In contrast with morphine or barbiturates, ephedrine, picrotoxin, or coriamyrtin is entirely ineffective in stimulating the respiration or reviving the animal. Artificial respiration was the only measure that abolished the poisonous effects. The action of gelsemicine, therefore, does not appear to be exerted upon the respiratory

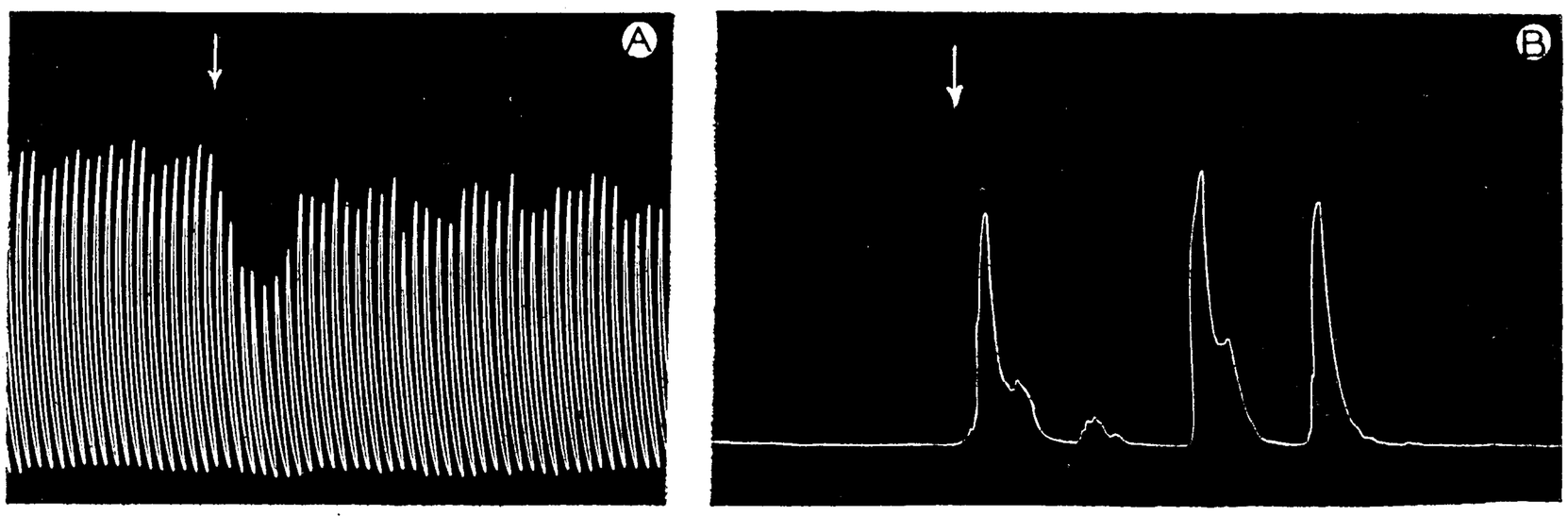

Fig. 3. Action of gelsemicine upon smooth muscle organs.

A. A strip of isolated rabbit's small intestine was immersed in Tyrode's solution maintained at 38°C. At arrow, gelsemicine hydrochloride was added to the bath.

B. A strip of guinea pig's uterus was immersed in Locke's solution kept at 38°C. The arrow indicates the application of gelsemicine hydrochloride.

center, but most likely upon the spinal motor neurons which control the efferent impulses to the diaphragm and intercostal muscles by way of the phrenic and intercostal nerves. The ultimate paralysis of the respiratory muscles is thus brought about by exactly the same mechanism as the weakness and paralysis of the voluntary muscles in other parts of the body.

Vagus. In view of the fact that gelsemine is believed to inhibit the vagus as reported by Ikeda [1916], experiments were designed to test the action of gelsemicine upon the vagus. Mydriasis appeared when a 0.1, 0.2, or 1.0 per cent solution of gelsemicine hydrochloride was applied locally to the rabbit's eye. Light reflex was present during dilatation of the pupil, so that the action is not comparable to that of atropine. Incidentally, it may be mentioned that a 1.0 per cent solution was so promptly and efficiently absorbed from the conjunctival sac that several rabbits in our experiments died with typical symptoms. In anesthetized cats and dogs, gelsemicine injected intravenously did not decrease the vagal irritability as shown by the response of blood pressure upon electrical stimulation of the vagus. Nor did gelsemicine abolish the bradycardia caused by a previous injection of physostigmine. Nine observations were made each with 9 strips of isolated rabbits' small intestines and 4 strips of isolated guinea pigs' uteri. Concentrations of 1:125000 to 1:100000 caused slight but definite relaxation of intestinal movements. The same concentrations, however, excited contractions of the uterus. Examples are shown in fig. 3. No antagonism could be demonstrated on the intestine between gelsemicine and physostigmine. Barium chloride was effective in stimulating peristalsis after gelsemicine. The evidence on hand is thus against any action of gelsemicine upon the vagus. The mydriasis, intestinal relaxation, and uterine contraction speak more for an "adrenergic" action. The enhancement of the pressor effect of adrenaline as observed by Raymond-Hamet [1937] supports our contention.

SUMMARY

1. When gelsemicine in the form of the hydrochloride is injected intravenously in mammals, there is a latent period before toxic symptoms appear. This is particularly pronounced in rabbits.

2. Vomiting occurs uniformly in pigeons but occasionally in cats following the intravenous injection of gelsemicine. The median emetic dose in pigeons is 0.108 ± 0.0154 mg per kg. In cats doses as big as the LD_{50}, which is 0.176 ± 0.027 mg per kg, only rarely induce emesis.

3. Gelsemicine apparently depresses the motor neurons of the brain and spinal cord resulting in generalized muscular weakness.

4. The respiratory failure after the administration of fatal doses of gelsemicine is not due to the paralysis of the center, but is attributable to that of the spinal motor neurons innervating the respiratory muscles.

5. Gelsemicine has no action upon the vagus. The mydriasis, intestinal relaxation, and uterine contraction following gelsemicine rather suggest an action upon the sympathetic nervous system.

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鈎吻素乙之作用

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（一）由靜脉注射鈎吻素乙的氫氯化物於哺乳動物體中，在毒性病症未顯以前，先有一潛伏期，此種現象，在家兔上特著

（二）在注射鈎吻素乙於鴿子體中後，一致發現嘔吐症狀，惟於貓上則間或見之。其在鴿子上之中量吐劑爲每公斤體重 0.108±0.0154 公絲，在貓上之劑量，雖大如每公斤 0.176±0.027 公絲，亦僅於少數中發現嘔吐。

（三）鈎吻素乙能抑制腦及脊髓之運動神經單位，甚爲明顯，結果可發生全部肌肉虛弱。

（四）注射致命劑量之鈎吻素乙所產生之呼吸衰竭，並非由於呼吸中樞之麻痺，乃於抑制脊髓運動神經單位後，而影響於其神經支配之呼吸肌肉所致。

（五）鈎吻素乙對於迷走神經，並無作用。由注射鈎吻素乙後所產生之瞳孔放大，小腸鬆弛及子宮收縮觀之，殊可暗示其作用於交感神經系統。