A Pharmacological Study of the Active Principle of Passiflora Incarnata*

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In a previous paper, the authors have reported a chemical study of a physiologically active principle in *Passiflora incarnata.* No definite conclusions were drawn as to the chemical nature of this substance but it was isolated in relatively pure form as a mercury derivative. In this paper a pharmacological study of the active principle will be reported.

Other investigators (1, 2, 3) have reported certain activities of the crude Passiflora extracts, but the results are inconclusive. Therefore, because of the long use of this drug in medicine it was decided to make a rather extensive pharmacological study, using both the crude fluidextract and the active principle which has been isolated as a mercury derivative.

For this purpose an aqueous solution of the mercury derivative was prepared and the mercury was removed by saturating the solution with hydrogen sulfide. After filtering off the mercuric sulfide, the excess hydrogen sulfide was removed from the filtrate by aerating and heating below 60° C. The final volume was adjusted so that the concentration of active principle was that represented by 50 mg. of the mercury derivative per cc. This solution was used in the pharmacological experiments to be reported.

EXPERIMENTAL

A. GENERAL SYSTEMIC ACTION

In order to check the observations of other workers, a fluidextract of the crude drug was employed in addition to the solution of the active substance described above.

Alcohol was removed from the fluidextract by vacuum distillation and the aqueous residue was made up to volume with water. Doses of the alcohol-free extract equivalent to 2.5 Gm. to 60 Gm. of the crude drug were administered intravenously, intraperitoneally and orally to frogs, guinea pigs, rabbits, cats and dogs. A series of three to six intact animals was used in each case. Observations were made immediately and at the end of 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours and 24 hours. No diminution of either voluntary or reflex activity could be demonstrated in any of the animals tested. A similar series of experiments was carried out using the solution of the active principle prepared as described. A fleeting increase in respiration was observed in those animals who received intravenous injections. Aside from this minor action no other effects were observed. These findings are of particular interest in view of the fact that the use of Passiflora in modern therapeutics is based solely on its supposed sedative action.

B. ACTION ON THE CARDIOVASCULAR SYSTEM

The most intensive study was that made on the cardiovascular system. The carotid blood pressure was recorded to observe the effect of the active substance alone and in conjunction with other drugs having known effects.

1. Active Substance Alone.—Doses of 1–3 mg. per Kg. body weight were injected into the femoral vein of dogs anesthetized with ether. The carotid blood pressure was recorded in the usual manner. Immediately following the injection the blood pressure fell 40–60 mm. and rose slowly to normal. A typical reaction is shown in Fig. 1.

2. Active Substance in Conjunction with Other Drugs.—In an attempt to locate the site of action of the active principle its effects were studied following the administration of nicotine, atropine, adrenaline and pituitrin.

(*a*) *Nicotine:* A 0.4 per cent solution of nicotine was administered in 0.25-cc. doses until the heart no longer responded to vagal stimulation and the pupil no longer reacted to stimulation of the preganglionic fibers of the superior cervical ganglion. If the active substance was then injected, the usual fall in blood pressure occurred. This result indicates that if the action of the drug is through autonomic nerves its site of action is postganglionic.

(*b*) *Atropine:* A 1:1000 solution of atropine sulfate was administered until vagal stimulation no longer produced any effect on the blood pressure. Both vagi were then sectioned. The active principle was administered and a fall in the blood pressure similar to that observed without atropine was recorded (Fig. 2). This result indicates that if the substance acts on the parasympathetic division, the action is beyond the myoneural junction.

(*c*) *Adrenalin:* It was shown that adrenalin and the active substance were mutually antagonistic when administered consecutively. When administered together both a rise and fall in blood pressure occurred, one after the other. The rise occurred, first, if the adrenalin were present in the larger amount and, second, if the active substance were present in the larger amount. If the two were administered in amounts judged to be pharmacologically equivalent both a rise and fall occurred but it was not possible to predict which would occur first. No graphs showing these phenomena are included.

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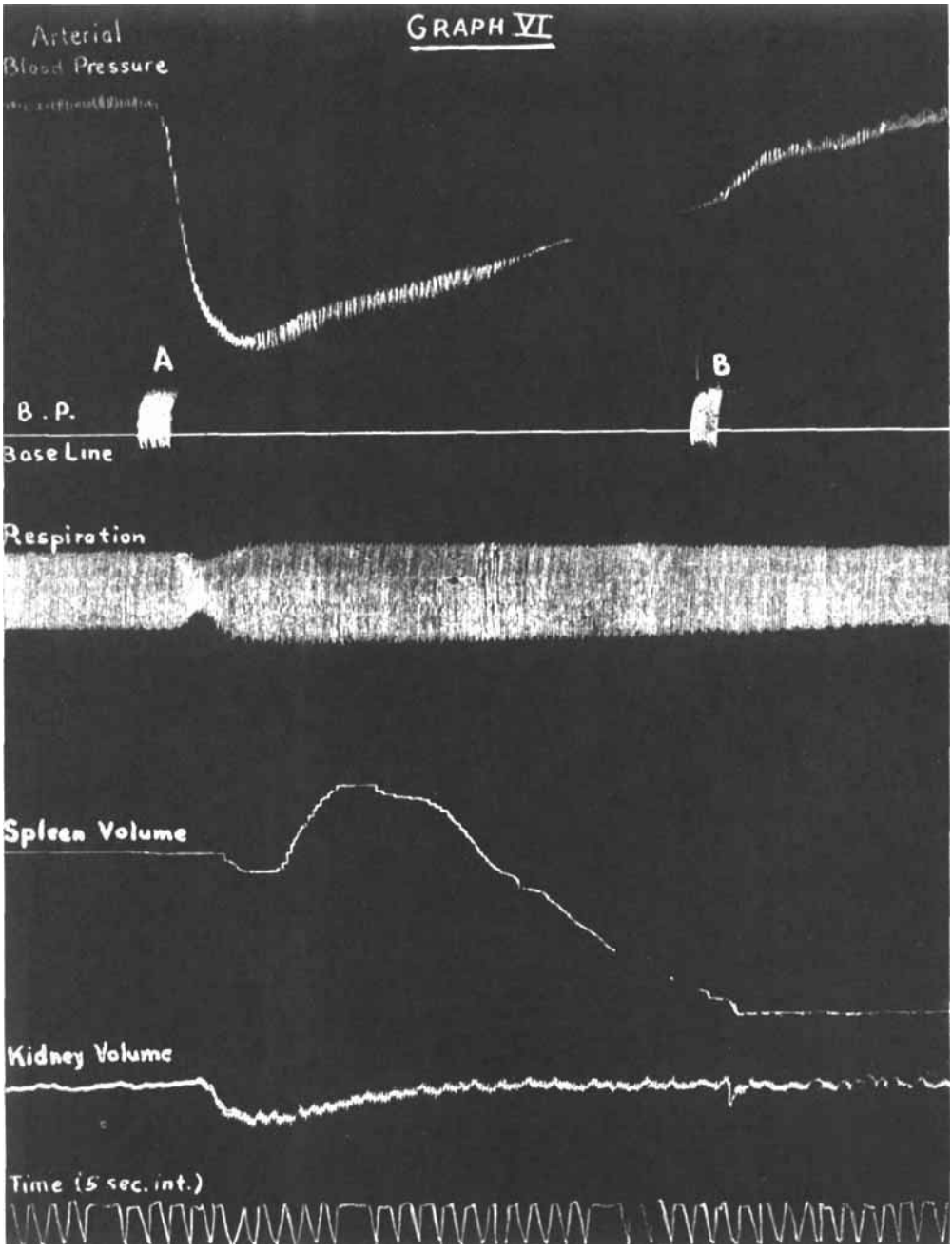

Fig. 1.—Changes in the Blood Pressure, Respiration, Spleen Volume and Kidney Volume Following the Injection of the Active Principle.

A. Injection of the active principle.
B. Stimulation of the peripheral end of the vagus nerve.

(*d*) *Pituitrin:* One cc. of solution of posterior pituitary (U. S. P.) was administered intravenously and at the peak of the rise in blood pressure the active substance was administered. A fall in blood pressure occurred at once. No graphs are included.

3. Action on the Heart.—The effect of the active principle on the heart was studied by means of myocardiograms and electrocardiograms. The myocardiogram showed no change in heart rate but did show that there was dilation of the heart. This was interpreted as being due to more complete filling compensating for the lowered blood pressure. The dilatation followed the fall in blood pressure and disappeared upon the return of the blood pressure to normal. The electrocardiogram revealed no changes in conduction but the conditions of the experiment were such that its interpretation was difficult.

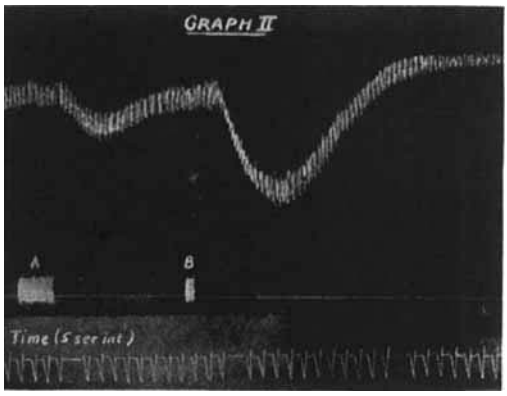

Fig. 2.—Arterial Blood Pressure. Action of the Active Principle in Conjunction with Atropine.
A. Stimulation of Peripheral Vagus.
B. Injection of 1 cc. Active Principle.

4. Portal Injection.—The substance is inactive by mouth, and when injected intravenously its action is fleeting. It was assumed for these reasons that the active principle was rapidly destroyed, possibly in the liver.

In order to test this assumption, injections of the active substance were made into the portal vein of dogs. The minimum dose necessary to produce a 30–40 mm. fall of the blood pressure when injected into the femoral vein was first determined for each animal tested. This dose was then injected into the portal vein, but no change in blood pressure resulted. The dose was increased gradually without effect. If 10 times the minimum dose was injected rapidly into the portal vein, a fall in the blood pressure of 10 ± 5 mm. occurred. From these experiments it was concluded that the active substance exerts only a fleeting action because it is destroyed in the liver.

One of two conclusions with regard to the site of action of the drug may be drawn from the experiments on the cardiovascular system: first, that it is a parasympathomimetic drug acting beyond the myoneural junction, and, second, that it acts directly upon the smooth muscle of the vessel walls to produce vasodilation.

C. ORGAN VOLUME CHANGES

The spleen and kidney were placed in oncometers and were arranged to record changes in volume simultaneously with the recording of the blood pressure. Spleen volume increased greatly and kidney volume decreased (Fig. 1). This result indicated visceral vasodilation. There is some evidence obtained by a leg plethysmograph that peripheral vasodilation also occurred.

D. ACTION ON THE RESPIRATORY SYSTEM (DOG)

Respiration was usually recorded as well as blood pressure. As the blood pressure fell rapidly (Fig. 1), the depth and rate of respiration increased. As the blood pressure arose the respiration returned to normal. DeNito's (2) conclusion that this respiratory effect was due to reflex stimulation of the respiratory center through the carotid sinus seems reasonable since a diminution of the blood pressure does set up respiratory changes probably as a result of concomitant anoxemia of the brain and stimulation of the carotid sinus. Since these respiratory changes were so closely allied to the blood pressure changes and since well-tried theories could explain the respiratory effects on the basis of a decrease in blood pressure, it was thought unnecessary to investigate these changes further.

E. ACTION ON ISOLATED ORGANS

1. Intestinal Strip (Rabbit).—A short piece of the jejunum was placed in a bath of oxygenated Locke's solution at 37–38° C. A modification of the apparatus described by Sollman (4) was used for recording smooth muscle contraction. A normal record of the longitudinal contractions was made and then 1 cc. of unknown solution per 100 cc.

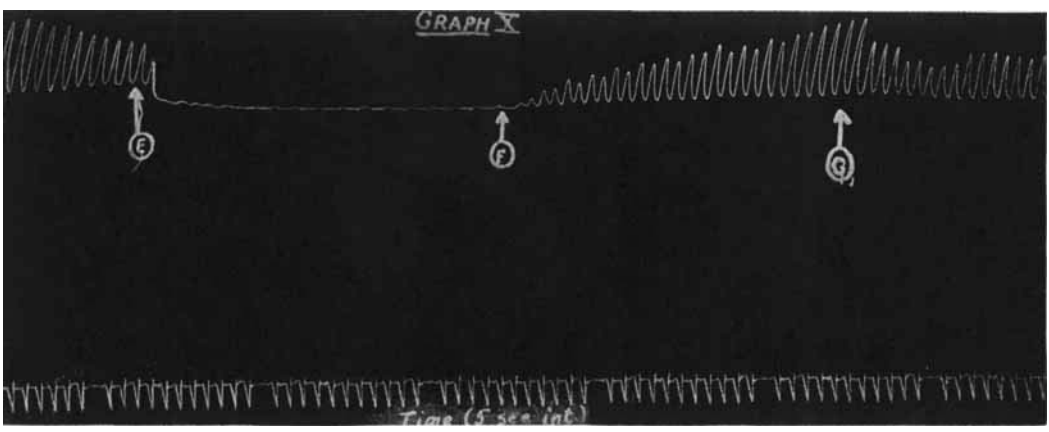

Fig. 3.—Isolated Intestinal Strip. Action of the Active Principle in Conjunction with Adrenalin.
E. 0.25 cc. 1:1000 adrenalin.
F. 5.0 cc. active principle.
G. 0.25 cc. 1:1000 adrenalin.

of Locke's solution was added. A very slight increase in tonus, recorded as a rise in the base line, occurred. The bath was changed and 3 cc. of unknown per 100-cc. Locke's solution were added. A slightly greater increase in tonus occurred. Neither the amplitude of the contractions nor the rhythmicity of contraction changed. Apparently the whole effect was one of increased tonus. Recovery was complete in about five minutes.

In an effort to check the site of action of the active substance on the intestinal strip a series of experiments was carried out using the unknown in conjunction with adrenalin. Using the set-up described above and fresh strips of jejunum, a normal record was secured and a check made to be sure that the unknown was exerting the effect previously observed. The Locke solution was then changed and 0.25 cc. of a 1:1000 adrenalin solution was added. Contractions stopped immediately. After about thirty seconds had elapsed, 5 cc. of the solution of active substance were added and within 20 seconds contractions were resumed. The reaction produced was typical in that the tonus increased above normal and then returned to normal. In order to be sure that normal recovery from the effects of adrenalin would not have begun just at the time of exposure to the active substance, another series of tests was run in which the muscle was permitted to remain under the influence of the adrenalin for varying periods of time before the addition of the active substance. The results of a typical test which was allowed to run for about three minutes are shown in Fig. 3. Similar experiments were performed using atropine and the active substance with almost identical results as those obtained with adrenalin. No graphs are included of the action with atropine.

2. Uterus.—(*a*) *Guinea pig:* Isolated uterine horns of virgin animals were studied using the same set-up as was employed for the intestinal strips. A normal record was obtained and then 3 cc. of the solution of active substance per 100 cc. of Locke's solution were added. An immediate and sharp contraction occurred (Fig. 4) raising the base line enormously. Rhythmicity of contraction remained practically unchanged but the amplitude was markedly diminished. The solution was now changed and adrenalin added. A markedly diminished activity followed but when the active substance was added a sharp contraction followed at once. The results obtained with the guinea-pig uterus were almost identical with those obtained with the intestinal strip.

(*b*) *Rabbit:* Similar experiments were made using the uterine horns of virgin rabbits. The uteri of three rabbits were used. All of these uteri re-

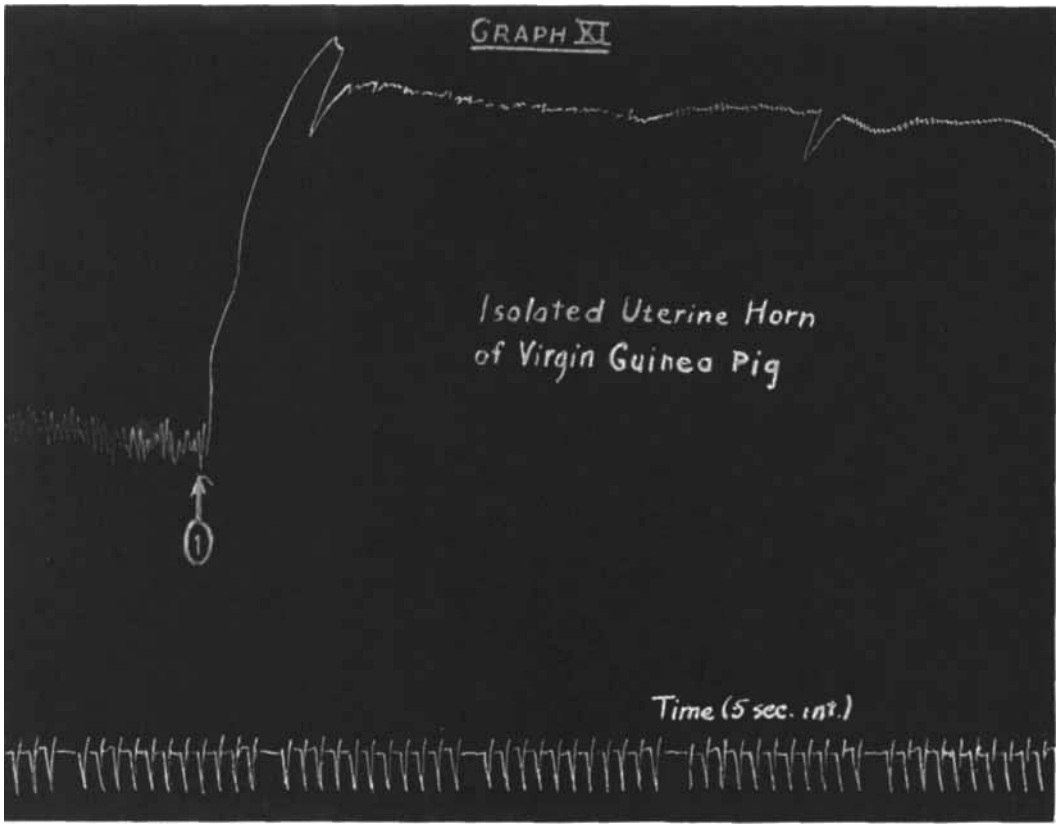

Fig. 4.—Response of Guinea-Pig Uterus to the Active Principle.
1. Addition of 3 cc. of solution to 100 cc. Locke Solution.

acted to adrenalin with a strong contraction. On the rabbit uterus the active substance acts in the same way as adrenalin. No graphs are included.

3. Eye (Cat, Rabbit, Dog).—The solution of active substance was instilled into the conjunctival sac of the cat, rabbit and dog. Time of exposure to the solution was varied as well as the lighting conditions. Absolutely no effects on the pupil were noted and no impairment of accommodation resulted as far as could be determined.

DISCUSSION

The effect of the active principle in producing a fall in blood pressure, dilatation of the heart and constriction of the smooth muscle of the intestine would seem to indicate that the active principle was parasympathotropic. However, this is not universally true. For example, there was no action on the pupil and at no time was any increased salivation observed although no special experiments were done to check this point. Furthermore, the undiminished activity after vagotomy, nicotine and atropine argue against a parasympathetic mediation. The action of the drug in causing contractions of virgin uteri regardless of the previous action of adrenalin on those uteri suggested a musculotropic action.

The assumption of musculotropic action means that the substance must act on smooth muscle in different locations in opposite fashion, that is, to relax the muscle of the vessel walls and constrict that of the uterus and intestine. This seems unusual but it can be pointed out that synephrine and one or two other closely related sympathomimetic compounds have similar paradoxical actions and these drugs have been shown almost surely to be musculotropic (5).

SUMMARY

1. A pharmacological study of a physiologically active compound from *Passiflora incarnata* has been made.

2. Neither the active substance itself nor the fluidextract of the crude drug had any action which could be construed as sedative.

3. The active substance caused lowering of the blood pressure and contraction of smooth muscle of the gut and uterus.

4. The activity of the substance has been shown to be unaffected by vagotomy, atropine, nicotine or pituitrin.

5. The drug probably exerts its characteristic activity by direct action on smooth muscle.

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Toxicity of Selenium-Cystine and Some Other Organic Selenium Compounds*

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Earlier studies on the selenium problem (1, 2, 3) have demonstrated that the toxicity as well as the selenium is carried in the protein fraction of seleniferous grains. The selenium is thought to occur in organic combination (4, 5) and it has been postulated that selenium could be replacing sulfur in the amino acids: cystine and methionine (6). Jones, Horn and Gersdorf (5), by a special enzymatic hydrolysis of gluten from seleniferous wheat, have isolated two fractions which contained practically all of the selenium and most of the cystine. The remaining fractions were practically free from selenium and contained only traces of cystine.

The toxicity of a number of organic selenium compounds has been investigated (7) and it was found that the toxicity of the selenium in the particular compounds studied did not approach the toxicity of sele-

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