The action of an active principle from apocynum / by H.H. Dale and P.P. Laidlaw.

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By H. H. DALE and P. P. LAIDLAW.

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I. Historical and Introductory.

Apocynum has for many years enjoyed a somewhat varying reputation in therapeutics, chiefly as an alterative to digitalis. The earlier clinical observers (Knapp, Griscom[12]) who appear to have tried the drug on the strength of its reputation among the American Indians, describe it as emetic, cathartic, diaphoretic, and diuretic. Later clinical observers, such as Dabney[5], laid special emphasis on its diuretic action. Dabney's paper contains a number of references to similar clinical observations. Husemann[13] appears to have first pointed out that this drug, like other members of the Apocynacæ (Oleander, Strophanthus), contained a heart-poison belonging to the Digitalis group. Bradford is reported by Murray[19] to have found that it resembled Strophanthus in its action on the heart, but that its powerful effect on the vagus mechanism, and the absence of a vaso-constrictor effect, prevented it from producing any rise of blood pressure. In 1883 Schmiedeberg[22] separated from it two indefinite products which he termed Apocynin and Apocynëin. Apocynin was an amorphous non-glucosidal body, almost insoluble in water, readily soluble in alcohol and ether, which caused systolic arrest of the frog's heart in very small doses. Apocynëin was an amorphous glucoside resembling digitalein.

In 1888 Sokoloff[23] examined the action of Apocynum on mammals. He found that an extract (he used an 8 per cent. watery infusion of the rhizome), when injected intravenously in doses of from 3 to 10 cc., caused, in the first place, a pronounced retardation of the heart's action, the pulse wave being enlarged, and the blood pressure raised; later, the initial retardation gave way to a secondary acceleration of the heart, the arterial pressure rising still further. He stated that the initial slowing of the heart was caused by a stimulation of the cardio-inhibitory centre, and of the peripheral inhibitory mechanism. The subsequent acceleration was not due to actual paralysis of the inhibitory apparatus, since injection of another dose of the drug could again produce retardation. Large doses produced a third stage of arrhythmia

* The experiments at Guy's Hospital were performed by P. P. Laidlaw only.

of the heart, with undulating blood pressure, which ultimately fell to zero with failure of the heart. Sokoloff attributed the rise of blood pressure, during the first and second stages of the action, to stimulation of the vaso-motor centre in the medulla, of the spinal vaso-motor centres, and also, to some extent, of the heart and blood-vessels themselves.

In 1904 H. C. Wood, Jr.[26], reinvestigated the drug physiologically and chemically. His physiological results were in the main similar to those of Sokoloff. Wood, however, concluded that the constriction of the arterioles was mainly, if not entirely, due to direct stimulation of their muscular walls. He also stated that the vagus became refractory to stimulation during the second stage of cardiac acceleration, and pointed out the characteristic death by sudden heart-failure caused by large doses of the drug. Chemically he advanced but little beyond Schmiedeberg's results; but he made the significant observation, that a crystalline substance, obtained commercially as Apocynin, was practically inert, and that the corresponding crystalline substance which he prepared himself, though very active when first isolated, gradually lost activity with successive purifications. He conjectured, rightly as now appears, that the activity was due to some very highly active impurity adhering to the crystalline Apocynin. Because the activity of the mother liquors and extracts was destroyed by boiling with dilute acids he supposed that the active substance was glucosidal in nature, but he did not succeed in separating it. In 1908 H. Finnemore[9] described his identification and synthesis of the crystalline Apocynin, which proved to be Acetovanillone. One of us (P.P.L.) found that this synthetic Apocynin had only a very slight activity, thus confirming Wood's suggestion with regard to the pure Apocynin.

Early in the present year Finnemore[10] published a preliminary note on the isolation, from *Apocynum cannabinum*, of a crystalline bitter principle, which he called "Cynotoxin," and which one of us (P.P.L.) had found to possess, in a very intense degree, the characteristic action of Apocynum. Finnemore believes that it is "a dilactone, either of Kiliani's digitic acid or of a closely related isomeride." C. W. Moore[18] published almost simultaneously the results of an independent and very exhaustive investigation of the constituents of *Apocynum androsæmifolium*, from which he had also isolated the bitter principle, to which the name "Apocynamarin" was assigned. This substance had also been ascertained by one of us (H.H.D.) to be the essential active principle. Moore considers it possible that Apocynamarin is the "dilactone of Kiliani's oxy-digitogenic acid, or of an isomeride."

A comparison of our results, when we discovered the duplication, showed us that the principles thus separately isolated were identical in physiological action, and we have thenceforth conducted the investigation together. Unfortunately, it is impossible, at present, to assume the chemical identity of the bitter principles from the two species. Though they agree in physical properties, the formulæ assigned to them by their respective discoverers are not the same. Cynotoxin, according to Finnemore's preliminary

note, has the formula $C_{29}H_{28}O_6$. Apocynamarin, according to Moore, has the formula $C_{28}H_{36}O_6 \cdot 2H_2O$.* In our further physiological investigation of the two preparations we were unable to detect any qualitative or quantitative difference between them, and ultimately used one or the other indifferently. While the question of chemical identity remains open, we have no choice but to use, in describing any experiment, the name indicating which preparation was actually employed. But it must be understood that whenever we attribute an action to Cynotoxin an identical effect, in sign and degree, is obtainable with Apocynamarin, and *vice versâ*. It may be noted incidentally that the identity of action disposes of the statement, which has obtained currency in the literature of the subject, to the effect that *Apocynum androsæmifolium*, which is often used instead of *Apocynum cannabinum*, is devoid of the specific activity of the latter†.

II. The action of apocynin (acetovanillone).

The experiments have been made with synthetic and natural preparations made by Finnemore, and a specimen of the natural substance obtained by Moore from *Apocynum androsæmifolium*. These were not perceptibly different in action.

The only definite effects which we have been able to detect as a result of the administration of Apocynin were upon the vascular system. When 10 mg. of Apocynin are injected into an intact frog the only effect to be noticed is that the frog remains quiet and motionless for a time and then recovers. Reflexes persist throughout. If the dose is injected into the dorsal lymph sac of a pithed frog, with the heart exposed, there is observable a gradual slowing of the heart-beat without much increase in strength ; both systole and diastole are prolonged, but the latter more conspicuously. Larger doses (up to 25 mg.) cause a still further slowing of the heart by prolongation of the diastole, and the beat becomes weaker. In no case does Apocynin cause systolic arrest of the heart. The effects produced by direct application of the drug, in 1 per cent. solution, to the exposed heart are similar to those resulting from injection. Fig. 1 shows the effect on the frog's heart.

The isolated mammalian heart, perfused with oxygenated Ringer's solution by Locke's method, shows similar results, viz., a slight increase in amplitude of beat with very slight slowing. Large doses produce a more marked slowing with considerable diminution in force (see Fig. 2).

Injected intravenously into anæsthetised animals (cats, dogs, and rabbits were used), Apocynin was found to cause a slight, transient rise of

* It appears that the difference between the formulæ really depends for the most part on the water of crystallisation (cp. Moore, loc. cit.).

† The United States Pharmacopœia, under the title "Apocynum," recognises "The dried rhizome of *Apocynum cannabinum*, or of closely allied species of Apocynum."

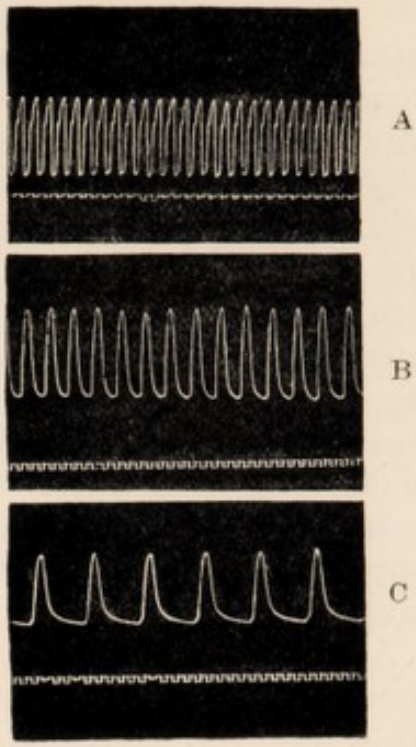

Fig. 1. × $\frac{2}{3}$ linear. Frog's heart, suspension method. Upstroke = systole. A—Normal. B—30 sec. after intravenous injection of min. viii of 0·5 per cent. Apocynin in Ringer's solution. C—After further injection of min. v of the same solution.

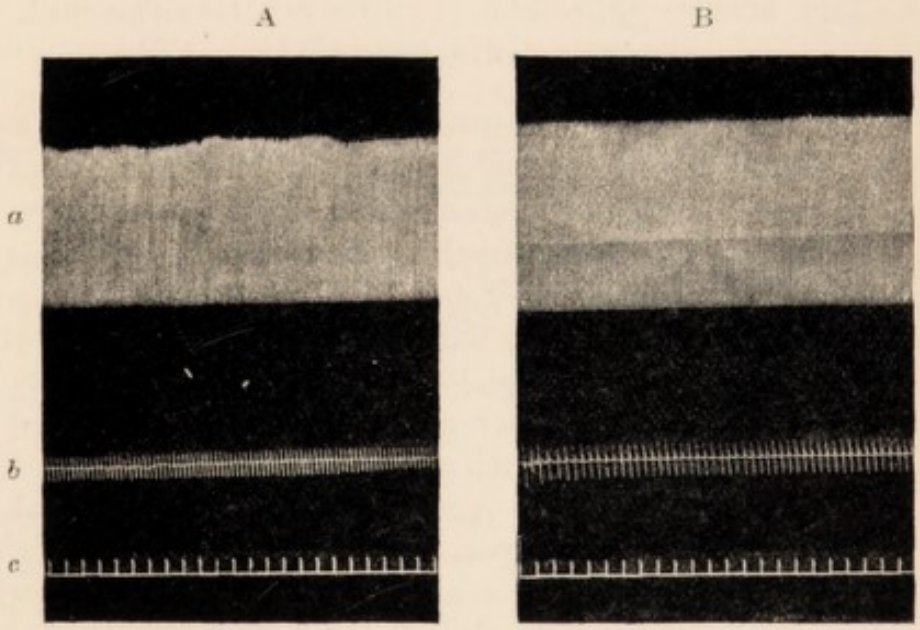

Fig. 2. × $\frac{2}{3}$ linear. Isolated rabbit's heart, perfused with Locke-Ringer solution. A—Perfusion with plain Locke-Ringer. B—One minute after substitution of 1 : 15,000 Apocynin. *a*—Ventricular beat; *b*—Drop-record of coronary outflow; *c*—Time in seconds.

blood pressure. A small acceleration of urinary flow was noticed in one experiment.

It is somewhat unfortunate that the name Apocynin has been applied to several different substances. It is clear that the crystalline substance, known commercially as Apocynin, and identified by Finnemore as Acetovanillone, plays practically no part in the specific action of the drug. Schmiedeberg's "Apocynin," on the other hand, appears to have been physiologically potent, but was evidently a mixture of substances, among which were probably both acetovanillone and the true active principle described in the next section. The resinous products called "Apocynin" by early observers, such as Griscom, were chemically even more indefinite. The fact, first demonstrated by Wood, that the natural crystalline Apocynin is purified only with some difficulty from traces of the true active principle, might be regarded as accounting even for the small degree of activity which we detected. Such a supposition, however, is excluded by the identical activity of synthetic acetovanillone.

In addition to free Apocynin (Acetovanillone) Moore isolated from *Apocynum androsæmifolium* a glucoside of that substance to which he gave the name "Androsin." This, however, we found to be even less active than the Apocynin itself. The observation has, therefore, no connection with Wood's suggestion that the active principle is a glucoside which loses its activity on hydrolysis. The true explanation of Wood's experience is given in the next section.

III. The bitter principle [cynotoxin (finnemore), apocynamarin (moore)].

It will be clear from the descriptions, reproduced above, by various observers of the action of extracts of Apocynum, that the essential active principle must possess in a high degree an action of the general type known as a "digitalis" action. It will be shown that this is true of the bitter principle isolated independently by Finnemore[10] and by Moore[18] from the two species. It is a neutral crystalline principle, with an intensely bitter taste, and is only slightly soluble in water or physiological saline solution at the ordinary temperatures, though its solubility increases rapidly with rise of temperature. It is not a glucoside, though boiling with acids or alkalies destroys its activity; this fact forms the basis of Wood's suggestion as to the glucosidal nature of the active principle. Solutions for experiment were made by dissolving 20 mg. of the substance in 0·5 cc. of absolute alochol; this was mixed with 40 cc. of hot physiological saline. Such a solution (1 : 2,000) was stable on cooling to room temperature. With higher concentrations crystallisation always took place after a time.

As stated above, the action belongs to the digitalis type. We have experimented on the frog and on mammals.

A. Experiments on the frog.

An intact frog when a lethal dose of the principle (about 0·2 mg. per 100 grm.) is injected into the dorsal lymph sac, shows no symptoms for some time after the injection. At from 1 to 1½ hours after the injection it becomes less active, squats on the table with its head touching either the fore-limbs or the table itself. When disturbed it moves slightly and assumes a normal attitude only to sink back in the course of a minute or so to its former position. A little later it will no longer recover when placed upon its back, and respiration fails. Within three hours reflexes disappear. Post mortem the ventricle is found to be in systole, the auricles engorged with blood. The minimal lethal dose per 100 grm. of frog is about 0·2 mg.

Determination of lethal dose of apocynamarin for the frog. (R. temporaria).

Weight.	Dose.	Dose per 100 grm.	Result.	
26 grm.	·066 mg.	·25 mg.	†1st hour	
26 ,,	·066 ,,	·25 ,,	†1st hour & half	Strophanthin (from Strophanthus gratus) has an M.L.D. of between ·07-·08 mg. per 100 grm. frog.
24 ,,	·05 ,,	·21 ,,	†1st hour	
24 ,,	·05 ,,	·21 ,,	†2nd hour	
27 ,,	·06 ,,	·22 ,,	†2nd hour	
30 ,,	·06 ,,	·2 ,,	†2nd hour	
30 ,,	·06 ,,	·2 ,,	†2nd hour	
30 ,,	·06 ,,	·2 ,,	*lived	
30 ,,	·057 ,,	·19 ,,	lived	Apocynamarin or Cynotoxin has, therefore, about ½-⅓ of the activity of Strophanthin on the frog's heart.
30 ,,	·055 ,,	·18 ,,	†2nd hour	
30 ,,	·055 ,,	·18 ,,	lived	
30 ,,	·055 ,,	·18 ,,	lived	
26 ,,	·047 ,,	·17 ,,	lived	
26 ,,	·047 ,,	·17 ,,	lived	
25 ,,	·047 ,,	·17 ,,	lived	

* died next morning.

Its action on the frog's heart is readily demonstrated by pithing a frog, injecting a few drops of 1 in 2,000 solution in physiological saline into the dorsal lymph sac, and exposing the heart. The suspension method will give a graphic record of the results. The first effect is to increase the contraction of the ventricle, systole being more complete. As the substance continues to be absorbed the ventricle is gradually thrown into complete systole. There are noticeable areas of systole, usually more marked at the apex, which persist during diastole of the remainder. These systolic areas increase in size until they involve the whole ventricle. Congestion of the auricle and great veins becomes very marked towards the end, as the output of the ventricle diminishes. Just before the final stage of complete systole one receives the impression that the auricle by its systole is distending a tonically contracted ventricle, and the ventricular contraction, when it develops, is like a slow peristaltic wave. The auricle continues to beat for a

short time after the ventricle remains firmly contracted. Fig. 3 shows the result of recording by the suspension method at various stages.

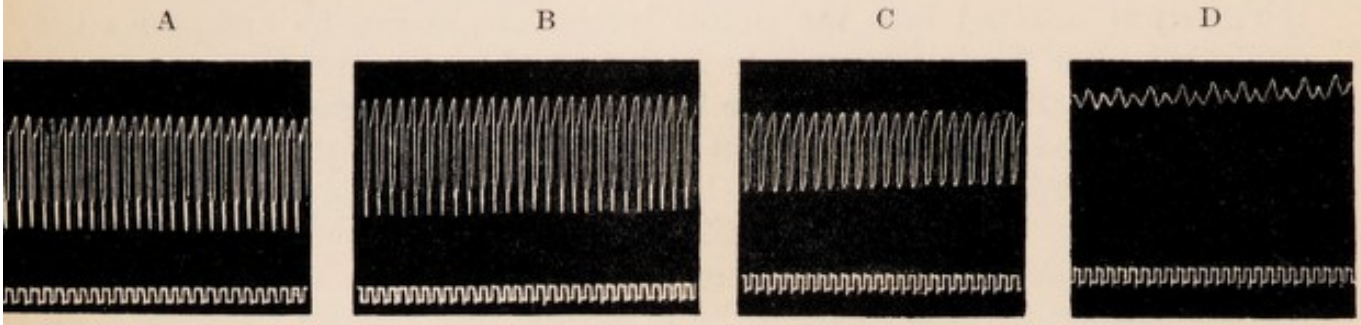

Fig. 3. Heart of decerebrate frog. Suspension method. Upstroke = systole. Min. vii 1 : 5,000 Cynotoxin injected into dorsal lymph sac. A—Normal. B—3 minutes after injection. C—6 minutes after injection. D—10 minutes after injection. Time in seconds.

Tracings of isolated frogs' hearts, perfused with Ringer's solution (through the sinus venosus), were also taken; addition of Cynotoxin caused the development of a precisely similar series of phenomena, to those observed in the pithed amphibian. To obtain a measure of the ventricular volume under the influence of the drug, we used Locke's modification of Williams' apparatus*. This gives an accurate record of the output of the ventricle while the heart perfuses itself. We found Dixon's[6] frog-intestine volume-recorder very useful with this apparatus. Cynotoxin or Apocynamarin in quite weak concentration (1 in 500,000 to 1 in 200,000) produces an increased output, and a very similar sequence of effects to those observed in pithed animals, concluding with systolic arrest (Fig. 4). One variation should be mentioned; the initial decrease of volume frequently gives way to a secondary dilatation, the tonus subsequently recovering, and then increasing till systolic arrest becomes complete. This difference is doubtless due to the fact that, in the perfusion apparatus, the pressure on the interior of the ventricle remains constant, and may secondarily distend the ventricle in spite of its systolic effort. Indeed, with a sufficiently high inflow pressure, a final quiescence in a condition of distension, simulating true diastole, may be obtained. In the pithed frog, on the other hand, the pressure falls with the failing output, and nothing but the efforts of the auricle hinders the passage of the ventricle into complete contraction.

In addition to this characteristic digitalis-like action on the frog's heart Cynotoxin or Apocynamarin has a pronounced effect on the plain muscular tissues of that animal. The action was examined on :—

1. The plain muscle of the arteries;
2. The plain muscle of the stomach.

* This apparatus was shown at a meeting of the physiological society, but a full description has not yet been published. Through the kindness of Dr. Locke we have been able to use it in the incomplete form.

For recording the former a fine cannula was inserted into the aorta, and the vessels washed out with Ringer's solution. The sinus was laid open freely. The frog was placed in a funnel and its vessels perfused with Ringer's

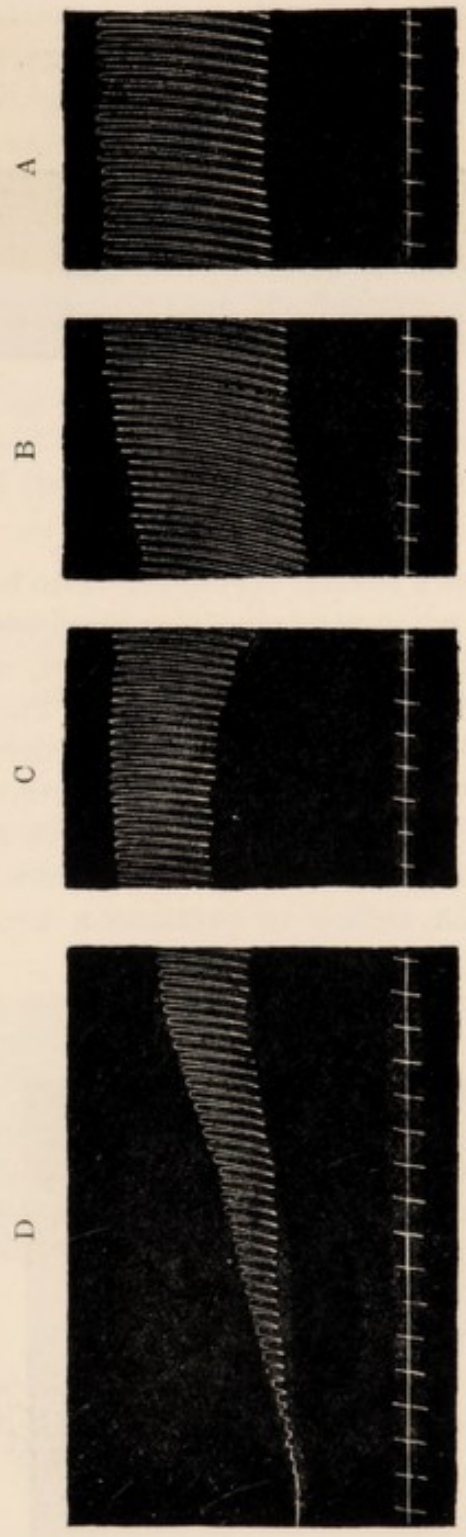

Fig. 4. $\times \frac{6}{11}$ linear. Frog's heart (Locke-Williams apparatus, Dixon volume-recorder). Perfusion with Ringer's solution, changing to 1 : 100,000 Apocynamarin in Ringer. Downstroke = systole. A—Normal. B—Just after Apocynamarin reaches the heart. C—Later (secondary dilatation). D—Passing into permanent systole.

solution under constant pressure. The rate of outflow from the funnel was recorded with a drop-counter or by collecting in a graduated cylinder. The injection of Apocynamarin or Cynotoxin into the perfusion cannula caused a

pronounced diminution of venous outflow. Fig. 5 shows the result of a comparison between 1 mg. of Strophanthin (known to be a very active specimen) and $\frac{1}{3}$ mg. Cynotoxin. It will be seen that the Strophanthin caused a very slight slowing, while Cynotoxin diminished the venous outflow by about one-half. It is, therefore, a much more powerful vaso-constrictor than Strophanthin. The dose given was, of course, large.

Fig. 5. × $\frac{4}{7}$ linear. Perfusion of frog's blood-vessels at pressure of 30 cm. Ringer's solution. Drop record of outflow, drops averaging 6 to 1 cc. At A—1 mg. Strophanthin injected into perfusion cannula. B—$\frac{1}{3}$ mg. Cynotoxin.

The frog's stomach showed a similar response. The technique employed was that described by Dixon[7] when studying the innervation of this organ. A frog's stomach, carefully filled with Ringer's solution, was connected with a U tube and a slight positive pressure maintained. The other limb of the U tube was connected to a Dixon volume-recorder. When 1 : 5,000 Apocynamarin was applied to the outside of the stomach the rhythmic movements were replaced by a prolonged intense contraction (Fig. 6). It should be noted that larger doses are required to induce good responses in plain muscle than those which suffice to produce a pronounced effect on cardiac muscle.

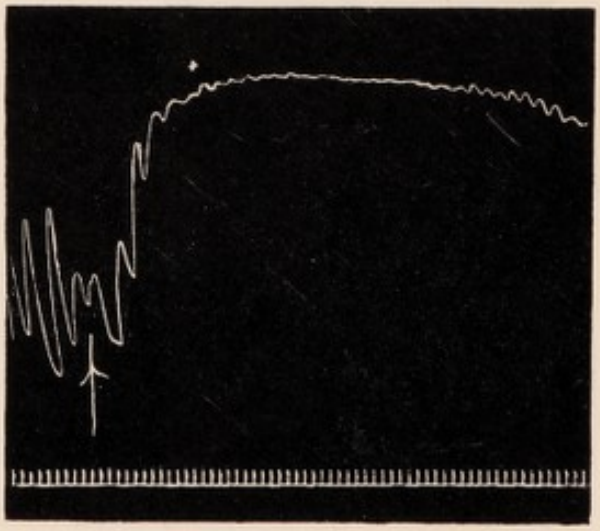

Fig. 6. × $\frac{3}{4}$ linear. Volume-record of frog's stomach. Distension-pressure = 18 cm. of water. Upstroke = contraction. At ↓ 1 : 5,000 Apocynamarin in saline solution applied to the exterior.

On skeletal muscle Apocynamarin or Cynotoxin has no effect in small doses. A muscle-nerve preparation from a frog killed with Apocynamarin will respond normally when stimulated through the nerve or directly. Reflexes in the spinal frog are still obtainable for some time after the heart is systolised. But, like other members of the digitalis series, Apocynamarin presents a curare-like action if a muscle-nerve preparation is immersed in a solution of the substance of sufficient concentration. A nerve-muscle preparation was soaked for half an hour in physiological saline solution, 0·6 per cent., containing a little alcohol (½ cc. in 20 cc.), and sufficient Apocynamarin to give a concentration of 1 in 2,000. At the end of this time the muscle would no longer respond to any stimulus applied to the nerve, but it was almost normally responsive to direct stimuli. Control muscles, soaked in physiological saline with the same proportion of alcohol only, responded normally to both direct and indirect stimulation. The curve obtained by direct stimulation of the poisoned muscle was slightly flatter than that given by the control, and showed a trivial prolongation of the relaxation period.

Note on the action on the toad's heart.

It has been known for many years that the heart of the toad is relatively very resistent to the poison which the animal's skin secretes (Vulpian[25]), and which is present in a recognisable amount in its blood (Phisalix and Bertrand[20]). In its action on other animals this toad poison, "Bufotalin" (Faust[8]), resembles the members of the digitalis series, and it was observed

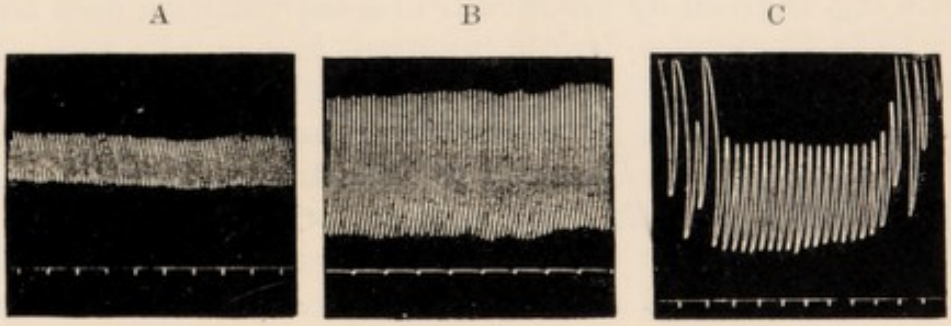

Fig. 7. × ½ linear. Toad's heart (Locke-Williams apparatus; Dixon recorder). Perfusion with a mixture of blood and Ringer's solution. The heart had already been perfused with 1 : 22,000 Apocynamarin, and in 1 : 5,000 Strophanthin. A—After return to plain blood—Ringer. B—After 2 hours perfusion with 1 : 50,000 Apocynamarin in blood—Ringer. C—After 16 hours continuous perfusion with 1 : 50,000 Apocynamarin.

that the toad's heart is similarly resistant to other members of that series (Vulpian[25]). It was not surprising, therefore, to find that the toad's heart is far less sensitive to Apocynamarin than is the frog's heart. In one case a toad's ventricle continued beating for 18 hours in the Locke-Williams apparatus, being perfused for the whole time with 1 : 50,000 Apocynamarin,

the only effect of which was to increase the size of the output with some irregularity towards the end of the prolonged perfusion (Fig. 7); whereas a solution of one-tenth this concentration (1 : 500,000) rapidly produces systolic arrest of the frog's ventricle. Other toads' hearts also possessed a similar partial immunity to Apocynamarin.

B. Experiments on mammals.

The mammals used were rabbits, cats, dogs, and a monkey.

The animals were fully anæsthetised, except for simple observation of the effects of hypodermic injection or administration by the mouth. The anæsthetics used were usually chloroform followed by ether. In a few experiments in dogs the combination of morphia (0·01 grm. per kilo) hypodermically and paraldehyde (1·5 cc. per kilo) by the stomach tube was employed, a little chloroform being subsequently given by inhalation during the preliminary dissection. The general results have been very similar in the different types.

I. *Observations on the intact animal.*

If a large dose be given by the mouth, e.g., 0·1 grm. to a dog, the chief result is to cause vomiting in a few minutes, which is repeated at intervals for an hour or two, and removes all but traces of the quantity administered from the possibility of absorption. The rapid onset of the vomiting under these conditions suggests that local action on the mucous membrane of the stomach is chiefly responsible. It will be shown later, however, that this is not the only mechanism by which vomiting is induced by the drug. If a dose of 1 or 2 mg. is given by the mouth to a cat vomiting does not usually result. For 15 to 20 minutes, indeed, no result is seen, as a rule. It is then noticed that the pulse is somewhat diminished, and this retardation is gradually accentuated. The heart-beat becomes perceptibly more forcible as the rate decreases. If vomiting occurs with this smaller dosage it usually happens about 30 minutes after the administration.

If the Apocynamarin (or Cynotoxin) is given hypodermically the symptoms appear more rapidly. 1 or 2 mg. injected into a cat caused vomiting in about 10 minutes.

In a large dog injections, amounting to 4 mg. in all, caused pronounced slowing of the pulse with a feeling of increased tension, and a similar result was obtained with a monkey.

The following protocols illustrate these effects :—

(1) Effect of administration by the mouth, continued for 8 days. Cat, male, weighing 2,300 grm. :—

May 6th.

Before any Apocynamarin was given the rate of the heart-beat, when the cat was kept quiet, varied from 200-190 per minute.

3.4 p.m. 2 mg. Apocynamarin in capsule given by mouth.

4.5 p.m. Heart-beat 160 per minute. Perceptibly more forcible.

4.30 p.m. Heart-beat 172 per minute, becoming quicker if the cat is excited.

May 7th.

9.51 a.m. Cat quite normal. Heart-beat 170 per minute when quiet. 2 mg. Apocynamarin in capsule given by mouth.
10.20 a.m. Heart-beat 120 per minute. Regular and forcible. No signs of nausea.
10.22 a.m. Cat suddenly vomited without any premonitory symptoms.
10.55 a.m. Heart-beat 140 per minute. No further sickness.
5.10 p.m. Heart-beat 148-152 per minute. Cat appears to be quite well. 2 mg. Apocynamarin in capsule given by mouth.
5.55 p.m. Heart-beat 100-112 per minute. The animal is quiet.

May 8th.

10.20 a.m. Cat appears to be quite well. Heart-beat 140 when the animal is quiet; it becomes a little more rapid after walking about the laboratory.
10.23 a.m. 2 mg. Apocynamarin in capsule given by mouth.
10.58 a.m. Heart-beat 128 per minute. No signs of discomfort or uneasiness.
11.30 a.m. Defæcation and micturition. Heart-beat much the same. No second dose was given on this day.

May 9th.

11.45 a.m. Cat seems well. Heart-beat 176-184 per minute.
11.50 a.m. 2 mg. Apocynamarin in capsule given by mouth.
12.24 p.m. Heart-beat 144 per minute. No symptoms of discomfort or nausea.
1.10 p.m. Heart-beat 156 per minute. Micturition.
3.40 p.m. Heart-beat 148 per minute. Cat seems quite well. 2 mg. Apocynamarin in capsule given by mouth.
3.45 p.m. Heart-beat 140 per minute.
4.25-4.35 p.m. Four readings of heart-beat during this period were, 128, 124, 136, 140.

May 10th.

9.50 a.m. Heart-beat 148 per minute.
10.0 a.m. 2 mg. Apocynamarin in capsule given by mouth.
10.30 a.m. Heart-beat 108 per minute.
5.0 p.m. Heart-beat 136 per minute. 2 mg. Apocynamarin in capsule given by mouth.
5.35 p.m. Heart-beat 108-112 per minute.

May 11th.

9.55 a.m. Heart-beat 148 per minute. 2 mg. Apocynamarin in capsule given by mouth.
10.45 a.m. The cat basking in sunlight. Heart-beat 128.
4.44 p.m. Heart-beat 140 per minute. 2 mg. Apocynamarin in capsule given by mouth.
6.0 p.m. Heart-beat 108 per minute.

May 12th.

9.45 a.m. Cat appears to be quite well. Heart-beat 148 per minute. 2 mg. Apocynamarin in capsule given by mouth.
5.15 p.m. Heart-beat 148 per minute. 2 mg. Apocynamarin in capsule given by mouth.
5.58 p.m. Heart-beat 140 per minute.

May 13th.

10.0 a.m. Heart-beat 128 per minute when asleep. 148 when disturbed.
10.26 a.m. 2 mg. Apocynamarin in capsule given by mouth.
11.20 a.m. Heart-beat 120 per minute.
2.5 p.m. Heart-beat 144 per minute. 2 mg. Apocynamarin in capsule given by mouth.
2.25 p.m. Heart-beat 120 per minute.

May 14th.

Heart-beat 148. Cat appears to be quite well. Experiment discontinued.

May 18th.

Heart-beat 180 per minute. Cat appears to be quite normal.

It is clear, then, that, when the drug is repeatedly administered by the mouth in small doses, the pulse rate of the cat, which was normally 180-200 per minute (the normal average of a number of cats was found to be about 180), could be kept down to an average rate of about 140. When the animal was fully under the influence of the drug, from 30 minutes to 3 hours after the administration, the rate fell on several occasions to about 110. This small dose (2 mg.) by the mouth produced vomiting only on one occasion. There was no evidence of any cumulative effect. In fact, on the morning after the day (8th May) on which only one dose was given, the pulse had regained almost its normal rate (176). It soon became again permanently normal when the administration was discontinued. During the period of administration the animal took its food well and rapidly gained weight. When it was killed, 19 days after the commencement of the experiment, no signs of gastric or intestinal irritation were found post mortem.

(2) Cat—Weight 2,810 grm.

The animal was very excitable and the pulse rate continuously above 200.

10.5 a.m. Pulse rate 212; counting difficult.
10.25 a.m. 1 mg. of Apocynamarin in 2 cc. saline injected hypodermically. Pulse rate practically unaltered, and cat quite normal till
10.37 a.m. when vomiting occurred.
10.51 a.m. Cat vomited again; much salivation. Pulse 188.
11.0 a.m. Cat quiet. Salivation continues. Pulse 148.
11.15 a.m. Much collapsed. Respiration very rapid; cat pants with its mouth open.
11.23 a.m. Condition the same. Defæcation. Pulse 104 (irregular).
11.37 a.m. Condition the same. Pulse 92 (very irregular; intervals of 4 sec. occurred without any beat). Another count of the pulse was 108.
11.45 a.m. Condition improving. Pulse more regular but still very slow.
12.0 noon Great improvement. Cat walks about. Pulse 160.
12.10 p.m. Cat almost normal again. Pulse 186.

From this time no further symptoms occurred, and after effects were absent.

In this experiment the rapid onset of the symptoms and their comparatively short duration indicate a very rapid absorption and excretion or destruction of the drug. The slowing of the pulse was probably delayed by the vomiting. The pronounced retardation first appeared after the vomiting ceased. The panting respiration with the mouth open was probably due to high blood pressure.

(3) Monkey, male, April 27th, 1909—A full grown Macacus Rhesus.

12.55 p.m. Pulse in femoral artery 188 per minute. 2 mg. Apocynamarin hypodermically.
1.2 p.m. Pulse in femoral artery, 196 per minute.
1.10 p.m. Pulse in femoral artery, 184 per minute. Micturition during slight struggle.
1.15 p.m. Pulse in femoral artery, 184 per minute. Micturition during slight struggle.

1.25 p.m. A further 2 mg. Apocynamarin hypodermically.
1.29 p.m. Pulse in femoral artery, 168 per minute; less compressible.
1.37 p.m. Pulse in femoral artery, 152 per minute.
1.41 p.m. Pulse in femoral artery, 112 per minute; irregular; drops beats occasionally.
1.44 p.m. Pulse in femoral artery, 96 per minute some counts—others as high as 120.
1.46 p.m. Pulse in femoral artery 107 per minute.
1.47 p.m. Micturition.
1.52 p.m. Pulse in femoral artery, 108 per minute. Put back in cage.
2.30 p.m. Vomited.
3.24 p.m. Pulse rapid and weak, but regular—210 per minute.
3.26 p.m. Breathing slightly wheezy. Râles in chest. Pulse 200 per minute.

Next day it had recovered completely, and is still living and well.

A study of these protocols in detail shows three stages of action on the heart. In the case of the cat to which repeated doses of 2 mg. were given by the mouth, the therapeutic stage of the action is illustrated by the minor degrees of slowing, accompanied by strengthening of the heart-beat. The doses were, however, in most cases absorbed with sufficient rapidity to produce the further stage of excessive retardation, the beat falling in several instances to about 110 per minute and becoming somewhat irregular. This stage of excessive inhibition is illustrated more strikingly in the next protocol, where it is seen that, after 1 mg. hypodermically, the pulse rate sank from over 200 to as low as 92, being at that time markedly irregular. In the case of the monkey, which received 4 mg. hypodermically altogether, this stage of excessive inhibition gave way to the third stage of excessively rapid pulse, from which, however, the animal recovered. These are, of course, the well-known stages of digitalis action, and they have been so completely analysed by Cushny[3] and others that we have contented ourselves with showing that the action of the Apocynum principle follows the same general lines. Before passing, however, to the experiments under anæsthetics, certain other features of the protocols deserve mention. Vomiting and purging have been described as effects of administering Apocynum; on the one hand, they have been regarded as drawbacks to its use as a heart tonic, and, on the other hand, have even led some to advocate the use of the drug as an emetic and cathartic. Using the pure principle we have seen no reason to conclude that Apocynamarin in therapeutic doses is a gastric or intestinal irritant. Vomiting, following an heroic dose, such as 100 mg. of the intensely bitter substance, cannot be regarded as significant. Smaller doses by the mouth produce vomiting, if at all, only after the appearance of action on the heart indicates that absorption has taken place; such vomiting occurs earlier, and far more constantly, if the dose is injected hypodermically. It is clearly, then, an effect produced after absorption, and must be attributed either to an action on the centre, or, possibly, on the musculature of the stomach. Such an action, in producing increased tonus of plain muscle, probably accounts for the defæcation and micturition which, in some cases, followed and appeared to be causally related to the administration. There

was no evidence of irritation of the subcutaneous tissues any more than of the alimentary mucous membrane. It should further be pointed out that we were, in all cases, using hypertherapeutic doses, and it is unlikely that vomiting or purging would result from the use of the pure principle in practical medicine. It seems probable that the irritant effects attributed to extracts of Apocynum are due to the presence of some other constituents than the active principle. Such experiments as we have made with various crude extracts lend support to this conclusion.

Such small variations of respiratory movements as we observed may probably be regarded as secondary to changes in the blood pressure. In a few cases, especially in those in which nausea and vomiting were prominent features, moist sounds were audible on auscultation of the chest. These are probably attributable to a reflex hypersecretion, secondary to the nausea, and comparable to that produced by Squill.

II. *Experiments under anæsthetics.*

The three stages described above, with a further stage leading to death, can be well seen in records of the arterial blood pressure from an anæsthetised animal which has received a hypodermic injection of 5 to 6 mg. of the active principle. In the first stage it can be seen that moderate slowing of the heart is accompanied by rise of blood pressure. In the second stage, which cannot always be clearly differentiated, the heart-beat becomes often so slow that the blood pressure falls below the initial level, in spite of the peripheral arterial constriction to which we shall refer later. Sooner or later the retardation disappears and the heart-beat becomes extremely rapid, the blood pressure rising continuously. The change may take place abruptly, as in Fig. 8. Later the blood pressure shows marked fluctuations corresponding to irregular output of the ventricle. A fall of the blood pressure to zero, which is frequently quite sudden, indicates failure of the heart-beat, and post mortem the left ventricle is found in systolic contraction, the systole of the right ventricle being masked by engorgement. The degree of systole of the left ventricle varies, but is usually complete after such doses as that indicated. The different stages are shown in Fig. 8. Fig. 9 illustrates the second stage of excessive vagus inhibition.

If the drug is injected intravenously the same sequence of stages can be seen, but they follow one another more rapidly, and are less easily differentiated, so that, as described by Cushny[3], with other members of the digitalis series injected intravenously, the main distinction which can be drawn is between a first stage in which the beat is slowed by inhibition, and a second in which the heart escapes from inhibition, becomes very rapid and then irregular, and stops in systole.

The interpretation of the different stages is easily made on familiar lines. The retardation of the heart-beat in the earlier stages is eliminated by

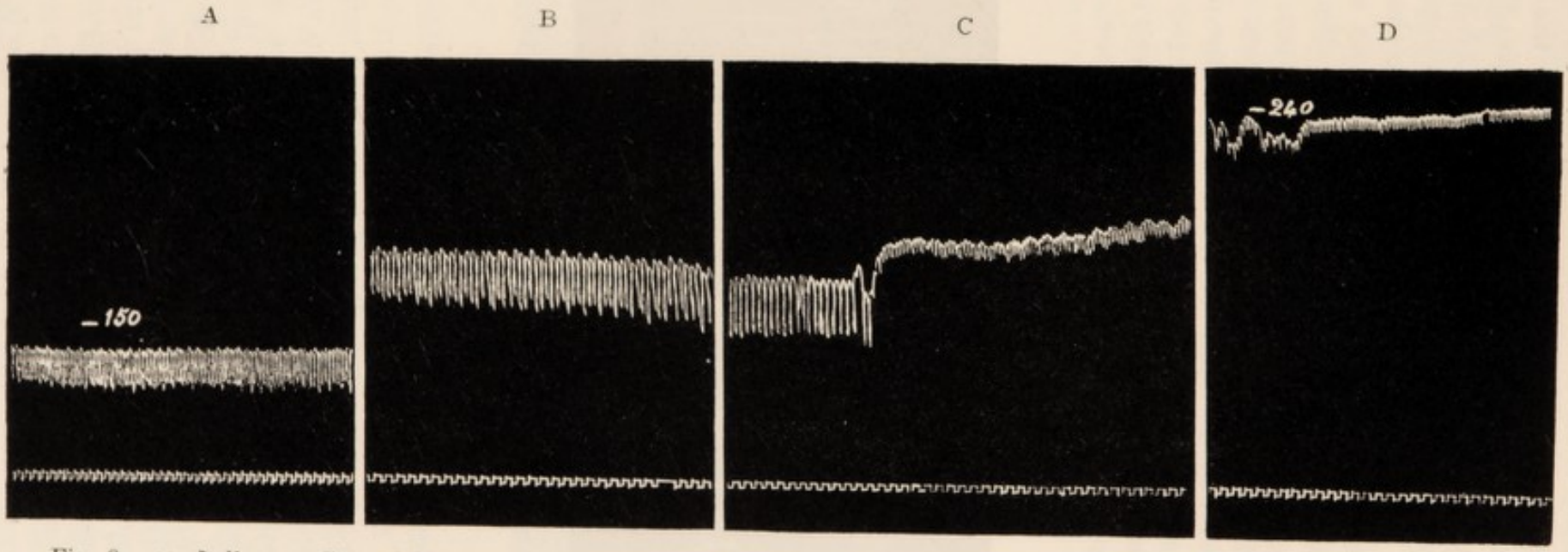

Fig. 8. × $\frac{7}{12}$ linear. Cat. 1,800 grm. ether. Carotid blood pressure. Four stages of the action of 4·5 mg. Cynotoxin, injected hypodermically. A—Normal. B—12 minutes after the injection. C—6 minutes later. D—3 minutes later.

destruction of the medullary centres, by section of the vagi, or by administration of atropine. The fact that, in the early stages of the action of the drug on the isolated heart, a very slight slowing accompanies the increase in amplitude* suggests that there may be a slight action on the peripheral inhibitory mechanism, as has been stated in the case of other members of the digitalis series. This action, however, is of altogether subordinate importance to the effect through the centre, and the effect of cutting the vagi is almost equivalent to giving atropine as regards the elimination of the inhibitory effect. The quickening of the rhythm, accompanied by rise of blood pressure, in the third stage is clearly due to escape from the inhibition. We have found no evidence to support Wood's statement that actual paralysis of the vagus mechanism occurs. Sokoloff[23] pointed out that with Apocynum, as with Digitalis, a further injection in the stage of acceleration causes a temporary renewal of the inhibition. We have ourselves observed

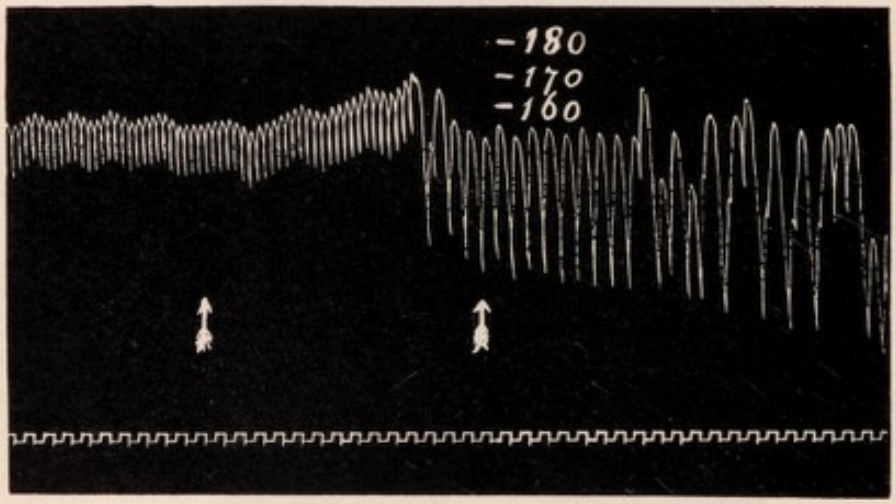

Fig. 9. × ⅔ linear. Cat. 3,592 grm., ether. 1 mg. Cynotoxin injected intravenously between ↑—↑.

that electrical stimulation of one vagus quite late in this third stage is still capable of producing a distinct slowing of the beat accompanied by fall of blood pressure. The explanation originally advanced by Cushny[3], and more recently adopted by Lhotak von Lhota[15], for the escape from vagus inhibition of hearts poisoned by other members of the digitalis series, may be applied to this case also. The heart muscle becomes so excitable that the impulses which reach it through the vagus mechanism are no longer adequate to produce an obvious retardation. They are not, however, without effect, for we have convinced ourselves that the heart passes more rapidly into fatal delirium when the vagi are cut, or when atropine has been injected, than when the inhibitory mechanism is left intact.

* An actual count made on the record reproduced in Fig. 17, shows 53 beats before, 51 shortly after the beginning of the drug's action in equal time intervals.

Nature of the effects on the circulatory system.

In analysing the rise of blood pressure during the later stages of the action, two factors have to be taken into account—increased peripheral resistance due to vaso-constriction, and increased output of the ventricles.

Action on plain muscle.

The occurrence of very pronounced vaso-constriction can be detected without graphic appliances ; mere inspection of the exposed intestines, which become extremely pale during the rise of pressure following injection, is sufficient to show that vaso-constriction not only occurs, but is so intense that it must play a large part in the production of the rise of pressure. Plethysmographic records indicate the same. Fig 10 shows such a record from

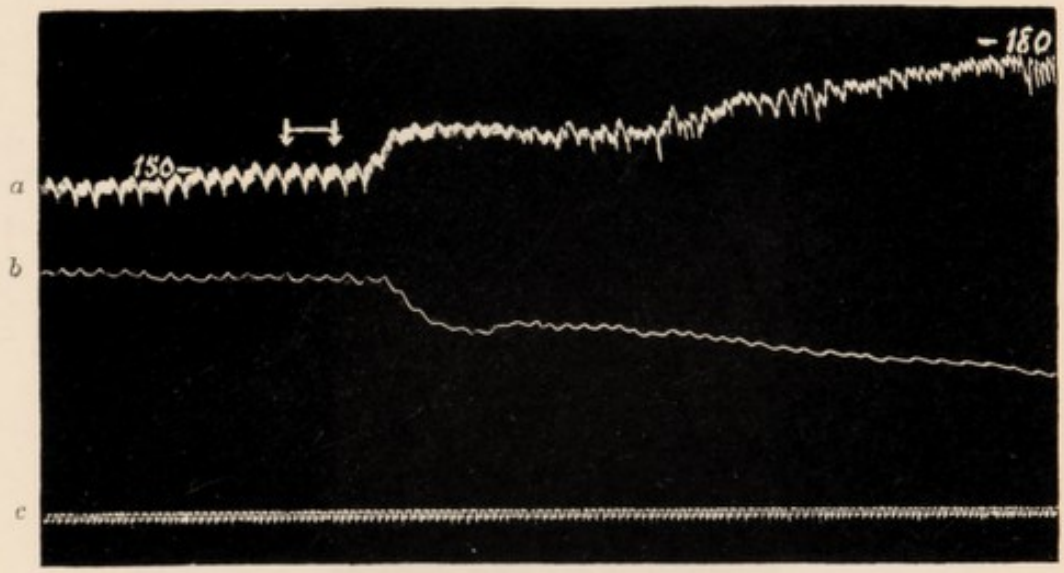

Fig. 10. × $\frac{3}{5}$ linear. Cat. Ether. (*a*) Carotid blood pressure. (*b*) Intestinal plethysmograph. (*c*) Time in seconds. Between ↕—↕ 0·5 mg. Cynotoxin injected intravenously.

a loop of intestine. We have found nothing to support Sokoloff's statement that the action is produced by stimulation of the medullary vaso-motor centre, or of the spinal cord. Destruction of the whole brain and spinal cord did not, in our experiments, diminish the vaso-constrictor action. On the contrary, by reducing the arterial tone and blood pressure to a low initial level, this procedure made the effect of Apocynamarin or Cynotoxin more striking, the pronounced vaso-constriction which it produced, together with the alteration in the heart-beat, causing a sudden rush of the blood pressure from its low level to the maximum, as soon as the drug was injected (Fig. 11). Nor is the action on any nervous structure between the spinal cord and the arterial musculature, since, after doses of ergotoxine sufficient to abolish the

vaso-constrictor effect of adrenaline, which now produced only a fall of blood pressure, the vaso-constrictor effect of Apocynamarin was unimpaired, as could be seen from the rise of blood pressure and the pallor of the intestines which it still produced. Measurement of the rate of outflow from the coronary vessels of hearts perfused by Langendorff's method show that marked constriction of these vessels also is produced by Apocynamarin and Cynotoxin. Since there is no clear evidence of the possession of a vaso-constrictor nerve supply by these vessels this fact points again to the action of the principle being on the actual plain muscle of the arteries. In this respect our conclusions are in agreement with those of Wood[26], and differ

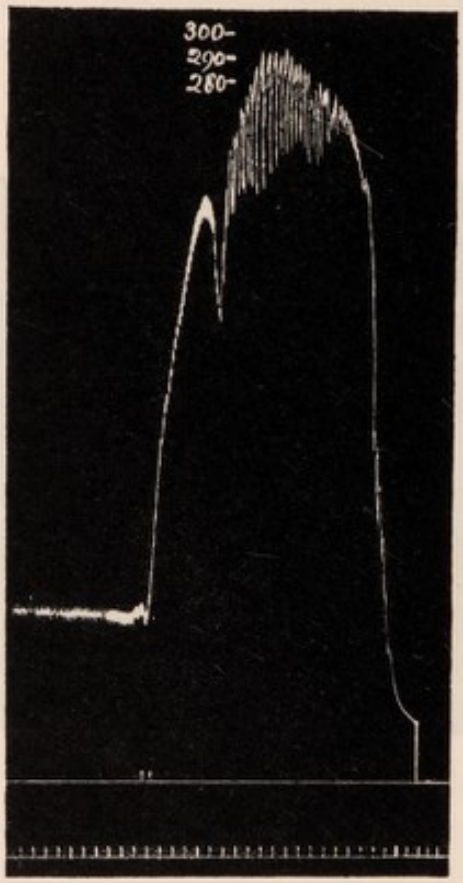

Fig. 11. × ½ linear. Cat. Cervical cord cut and brain destroyed. Artificial respiration. Carotid blood pressure. Time in 10 sec.. 2·5 mg. Apocynamarin injected intravenously.

from these of Sokoloff[23]. Other organs containing plain muscle are similarly affected, contraction of the muscular walls of the stomach intestine, urinary bladder, spleen, and uterus being produced. Fig. 12 shows the effect of adding 1 mg. of Apocynamarin to 250 cc. of warm oxygenated Ringer's solution in which a short length of rabbit's jejunum was suspended so as to pull on a recording lever. Fig. 13 shows the effect of the same dose on the isolated horn of a cat's uterus under identical conditions. In both cases it is seen that the tonus is increased to such an extent as practically to obliterate

the spontaneous rhythm. Contraction of the spleen was shown in plethysmographic records, and in one case wide, rhythmic variations of volume appeared, quite independent of the blood pressure. Intense tonic contraction of the urinary bladder is caused by intravenous injections, and, after

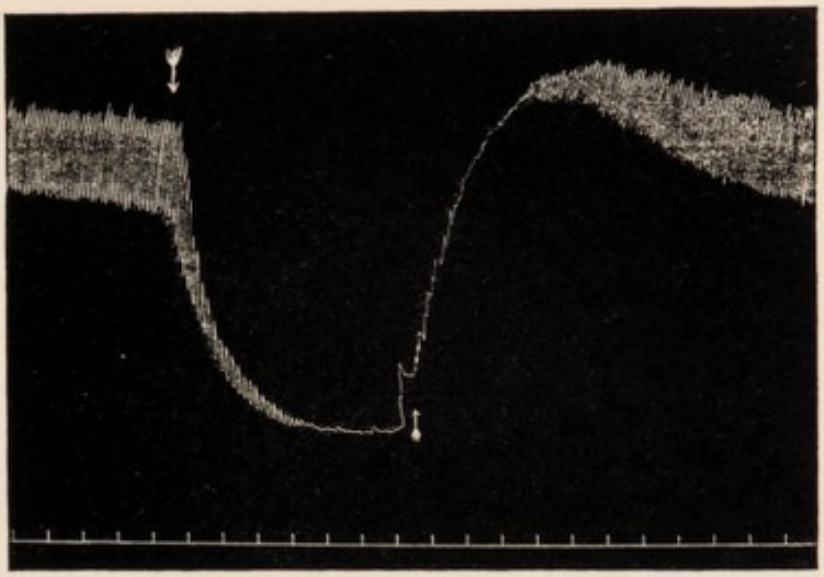

Fig. 12. × ½ linear. Isolated loop of rabbit's jejunum. Downstroke of lever = contraction. At ↓ 1 mg. Apocynamarin added to the 250 cc. of warm oxygenated Ringer's solution in which the loop was suspended. At ↓ change to pure Ringer.

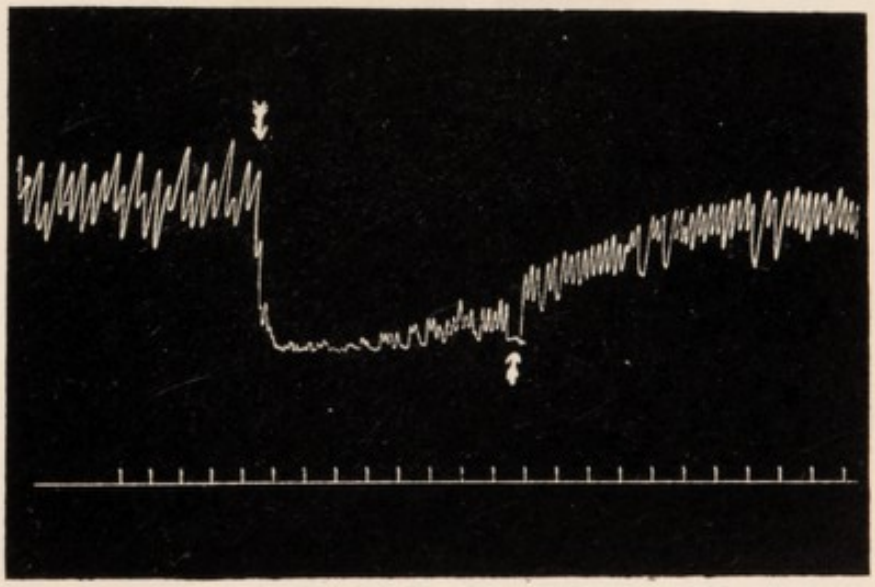

Fig. 13. × ½ linear. Isolated horn of cat's uterus (non-pregnant). Conditions as in Fig. 12. At ↓ added 1 mg. Apocynamarin ; at ↓ change to pure Ringer.

a large intravenous injection, the lumen was practically obliterated. The effect on the peripheral arteries is, therefore, merely one instance of the general stimulation of plain muscle fibres.

Action on the heart.

For studying changes in the output of the heart some form of cardiometer would appear to be the ideal instrument. Some of the defects of Roy and Adami's instrument have been indicated by Cushny[3], who pointed out that a decrease in ventricular volume may be masked by simultaneous engorgement of the auricle. This objection would be overcome by using an instrument which enclosed the ventricle only. Such can be made, and has been used by several observers, by covering the end of a thistle-funnel with thin rubber, burning a central hole, and pushing the annular rubber diaphragm thus formed over the ventricles, so that its edge grips the auriculo-ventricular ring. We failed to adjust this instrument so as to exclude, at the same time, leakage and hampering of the heart's action. In several cases, when the instrument seemed successfully adjusted, injection of Apocynamarin produced a surprisingly small effect both on the cardiometer record and the blood pressure. In such cases withdrawal of the instrument was succeeded immediately by the rise of blood pressure characteristic of the action of the drug. We were more successful with a simple thistle-funnel slipped inside the opened pericardium, which was tied securely round the rim. The record shown in Fig. 14 was thus obtained, a small Brodie's bellows being the

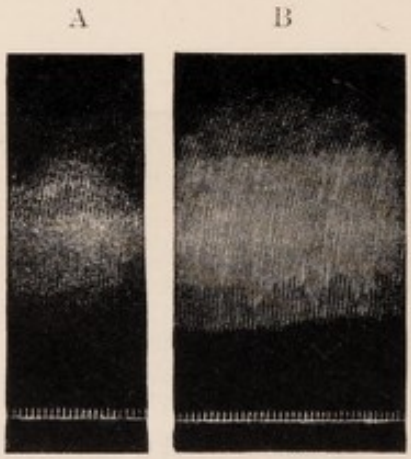

Fig. 14. × ½ linear. Cat. Cardiometer-record of ventricular volume. A—Normal. B—After injection of 0·5 mg. Apocynamarin.

recording instrument. Though we were able thus to demonstrate directly the increase of output, the cardiometer, in our hands, proved less suitable for following the effect of the drug on the heart through its various stages, for a very small difference in the adjustment is sufficient to vitiate the record completely. For this purpose we found methods which recorded the muscular contraction of the heart wall much more suitable.

The course of events is so clearly similar to that described for other members of the digitalis series that we contented ourselves with recording

contractions of the ventricles, since there is little room for doubt that Cushny's[3] analysis of the effect of various members of the series on different chambers of the heart will apply to this case also. We recorded the contractions of the dog's left ventricle, in situ by means of Cushny's modification of the Roy-Adami myocardiograph, and of the left ventricle in isolated perfused hearts of cats and rabbits by the ordinary method of suspending from the aorta, and connecting the lever to a hook in the tip of the left ventricle. In the experiments on dogs, which were anæsthetised with morphia and paraldehyde, a little chloroform being added during the operative preparation, the blood pressure was also recorded from the carotid artery. Fig. 15 shows samples of a continuous myocardiographic record, the

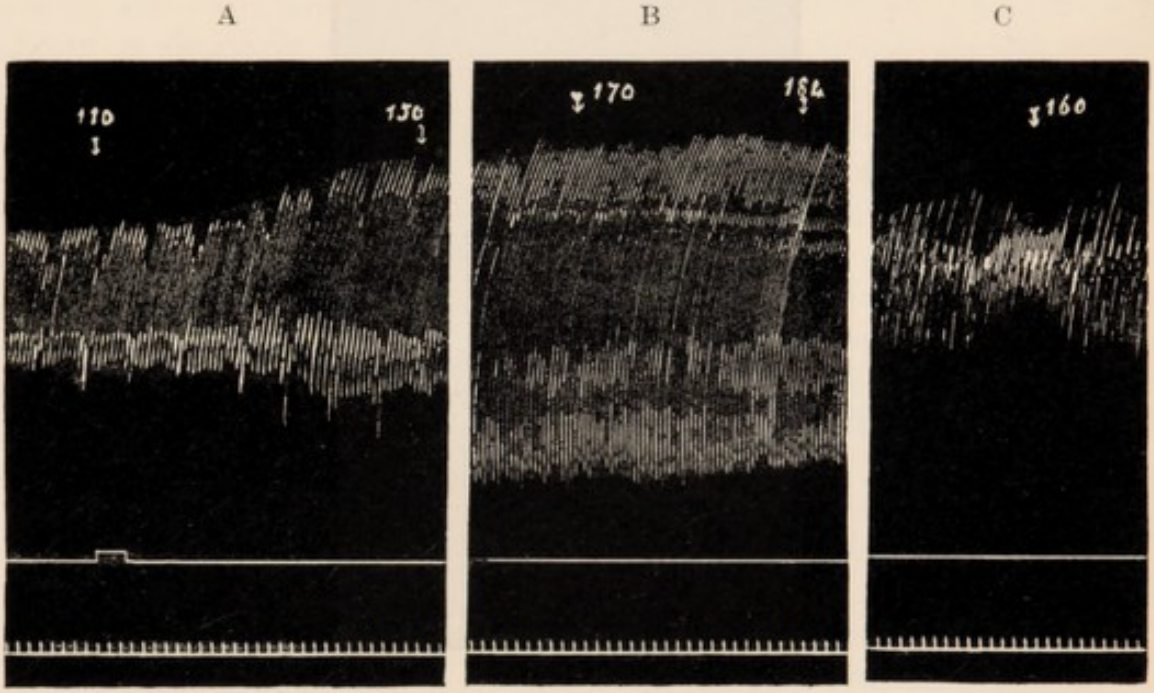

Fig. 15. × ½ linear. Dog (5½ kilos.). Morphia and paraldehyde. Chloroform. Myocardiographic record. Injection of 2 mg. of Apocynamarin intravenously. Time = 2 sec.. A—Before and just after injection. B—36 sec. later than end of A. C—96 sec. later than end of B.

changes in blood pressure being indicated by the figures placed above the tracing. In this instance the preliminary slowing is almost absent though the vagi were intact. Probably the cardio-inhibitory centre was greatly depressed by the anæsthetic. The apparent periodic irregularities in the ventricular tracing before the injection of Apocynamarin were due to the artificial respiration. It will be seen that the injection of Apocynamarin caused, from the first, increase both of diastolic relaxation and of systolic contraction. As the systole continues to gain in vigour the respiratory undulations become less marked. Section (B) of the tracing shows the period of maximum cardiac efficiency under the action of Apocynamarin, systole and diastole being both increased, and the rhythm accelerated, but as yet

perfectly regular. It will be seen that during this period the blood pressure rose from the original 110 to 184 mm. Period (C) shows a later stage where the contractions have become irregular and weak, diastole and systole being both imperfect. Fig. 16 from a similar experiment shows the stage of un-

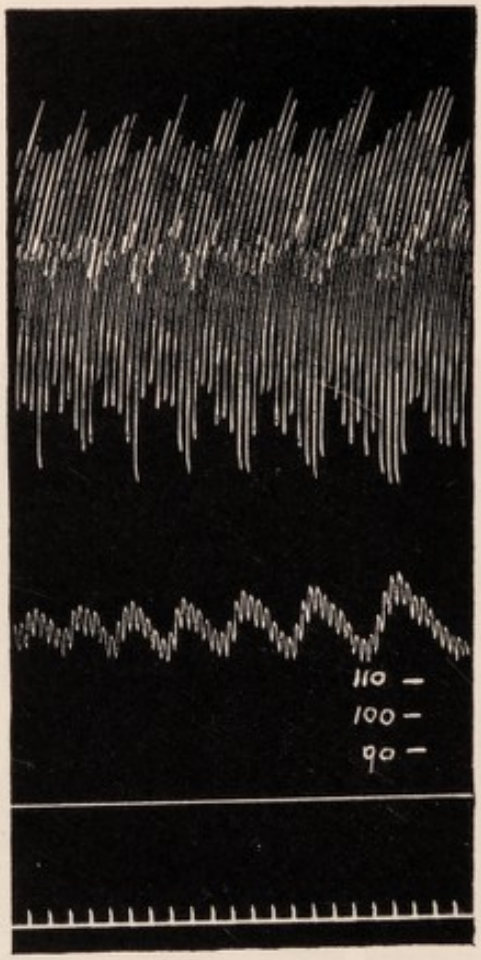

Fig. 16. × ⅔ linear. From an experiment similar to that of Fig. 15, to illustrate the stage of undulatory blood pressure.

dulatory blood pressure, the undulations corresponding to rhythmic variations in the amplitude of the ventricular contractions. The condition, which has been the subject of elaborate analysis by Cushny[3, 4], and has been produced by him by other means of excitation as well as by digitalis poisoning, is preliminary to the stage of delirious irregularity. The undulations of blood pressure, which are usually very regular when they first appear, may disappear for a time, the heart-beat becoming temporarily uniform, and may then reappear with a different rhythm. They are well seen in cardiometric as well as in myocardiographic records. These rhythmic variations have been shown by Cushny to be due to the acquisition by the excessively excited ventricle of a rhythm of its own, independent of that of the auricle. In his earlier paper he regarded the variations as being due to the interference in each chamber of the two rhythms, that initiated in the chamber itself and

that transmitted from the other. A maximum ventricular beat would occur when a transmitted impulse from the auricle coincided with an impulse originating in the ventricle itself; a minimum beat, when the transmitted impulse fell wholly within a refractory period of the idioventricular rhythm. A similar explanation applied to the rhythmic variation of auricular contractions. In a later paper[4], dealing wholly with this phenomenon, Cushny states that he has been able to induce it after destroying conductivity between auricle and ventricle, and attributes it to a periodicity in the filling of the ventricle by the auricular systole. This would be more complete as the auricular systole approached its normal relation to the ventricular systole, and less complete as it diverged from this. Since the two rhythms, though different, are both regular, there will be a regular recurrence of the optimum relation of the two, giving a maximal ventricular output. The auricular periodicity is similarly explained by a rhythmic variation in the freedom of the flow of blood into the ventricle.*

It cannot be doubted that this later theory offers an adequate explanation of the phenomenon as seen in the heart in its natural relations. On the other hand, it clearly fails to explain the occurrence of the rhythm in the ventricular contractions of the isolated perfused heart. Fig. 17 shows that, in such a heart, perfused with Ringer's solution containing Cynotoxin in high dilution, the ventricular contractions present this periodicity in a very marked form in the stage of action preceding delirium. Under such conditions the left auricle, at least, is quite empty, and the phenomenon can have no connection with the filling of the ventricle. This observation suggests that the interference of excitation waves, in accordance with Cushny's earlier explanation, is a sufficient cause of the periodicity, though the filling of the ventricle may also be concerned when the heart is in its normal relation. Possibly a simultaneous record of auricular and ventricular contractions on the isolated, perfused heart would throw further light on the subject.

The records from isolated hearts, for the perfusion of which Locke's[16] method was used, correspond also in other respects closely with those obtained with the myocardiograph. The final systole, as might be expected from the fact that interior of the ventricle is exposed to no increasing pressure, is more complete than that usually seen in an animal killed by the drug.

Diuretic action.

The clinical accounts of the action of Apocynum emphasises its diuretic action, and its consequent value in dropsy. It has even been called the "vegetable trocar" (cp. Dabney[5]).

Though the diuretic action of the drugs of the digitalis series is a familiar clinical phenomenon, many experimental investigations of the effect have

* The same phenomenon has quite recently been studied by Straschesko[38], whose conclusions as to its cause are practically identical with those of Cushny's later paper.

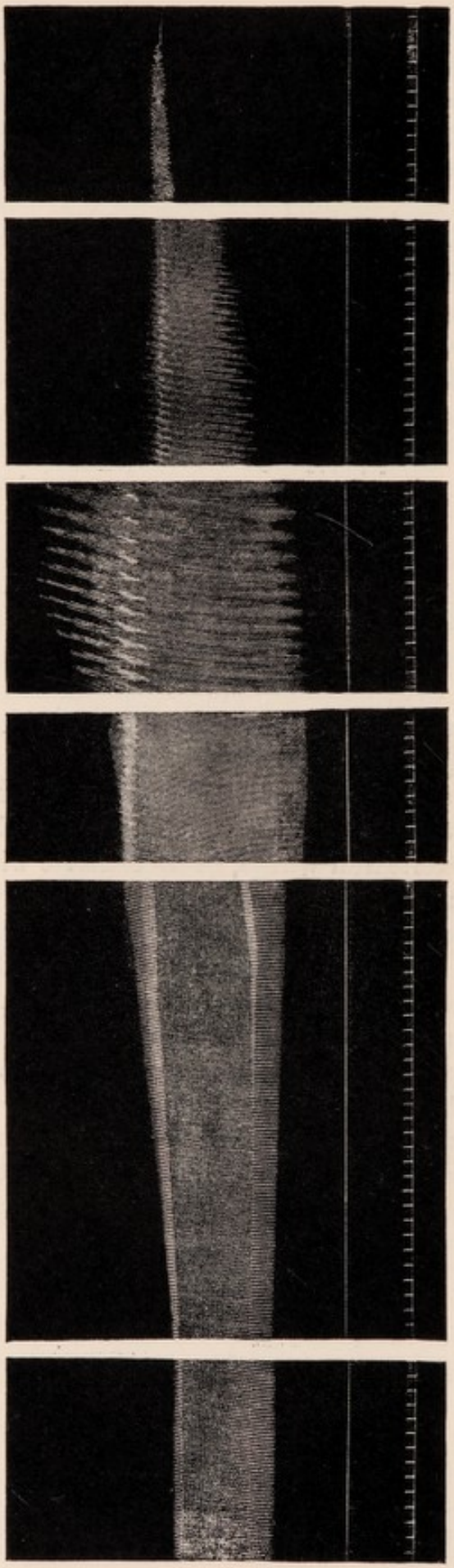

Fig. 17. × ½ linear. Isolated heart of Cat, perfused with oxygenated Locke-Ringer solution. Upstroke = systole. Time = 2 sec.. A—Normal. B—Just after change to 1 : 150,000 Cynotoxin. C—52 sec. after end of B. D—112 sec. after end of C (note ventricular undulations). E—136 sec. after end of D. F—110 sec. after end of E.

yielded results not immediately accordant with the clinical observation. Bradford and Phillips[1], and Gottlieb and Magnus[11] found that the kidney vessels did not escape the general vaso-constrictor effect of this drug, and Brunton and Power[2], Pfaff[21], and Marshall[17], all found that diuresis was decreased by the large doses which they administered. It has recently been pointed out by Jonescu and Loewi[14] that therapeutic doses of these drugs produce but the slightest, if any, rise of the normal blood pressure, but that they, nevertheless, produce, especially in rabbits, a greatly increased diuresis, accompanied by increased kidney volume.

Our experience with Apocynamarin has been, on the whole, of a similar nature. In the cat and dog large doses (1 mg. or more) intravenously cause a primary diminution of the flow of urine, which usually, however, gives way to acceleration. The kidney volume diminishes during the initial retardation of the urine flow, to increase again beyond the original as the secondary acceleration occurs. If the drug is very slowly absorbed after subcutaneous injection it is possible to observe a considerable increase in the flow of urine, even though cardiac inhibition prevents the rise of the blood pressure beyond the initial level. Fig. 18 illustrates such a condition in an experiment on a cat.

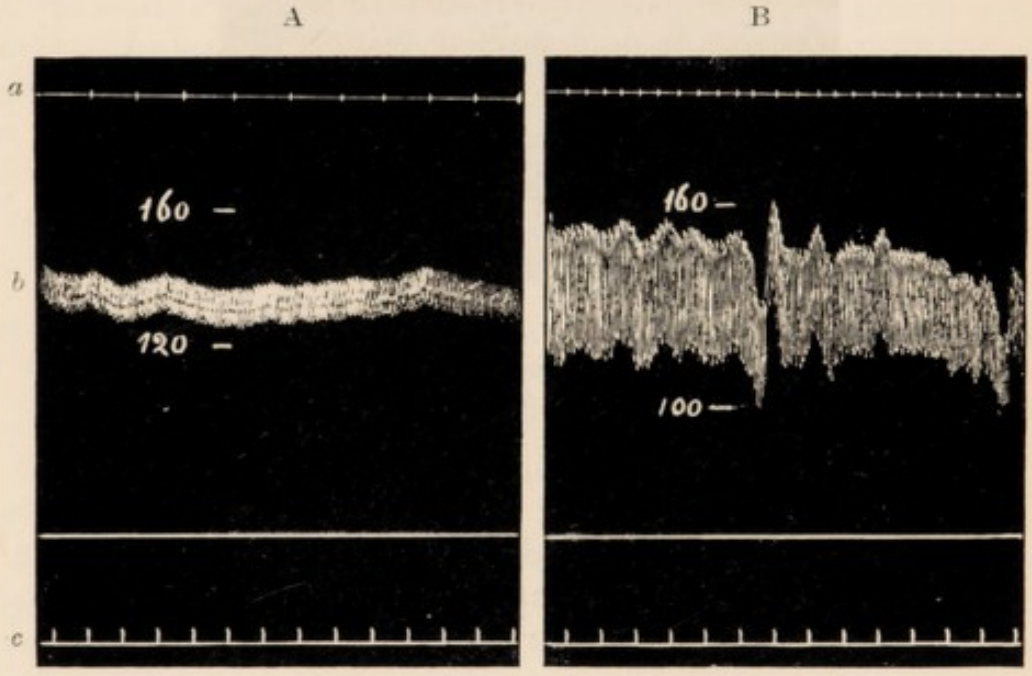

Fig. 18. × $\frac{2}{3}$ linear. Cat. Chloroform and ether. (*a*) Drop record of urinary secretion (bladder cannula); (*b*) Carotid blood pressure; (*c*) Time in 10 sec. A—Normal. B—6 minutes 40 sec. after hypodermic injection of 2 mg. Apocynamarin.

We found, also, in accordance with the statements of Pfaff and of Jonescu and Loewi, concerning the digitalis drugs, that the rabbit is far more sensitive to the diuretic effect of small doses of Apocynamarin than the dog or cat. Fig. 19 shows the effect of intravenous injection of 0·5 mg. of Apocynamarin on the flow of urine and kidney volume in this animal. One other point deserves mention. It has been mentioned by Pfaff, and by Jonescu and

Loewi, that, after an injection of Digitalis, even when this of itself produced no diuresis, as is frequently the case in the dog, a subsequent injection of Caffeine, which of itself has also unusually little effect in the dog, produces a profuse diuresis. In the case of Apocynamarin the converse seems also to be true. In a rabbit, after an injection of Caffeine which was in itself but feebly diuretic, an injection of Apocynamarin produced a diuresis far more

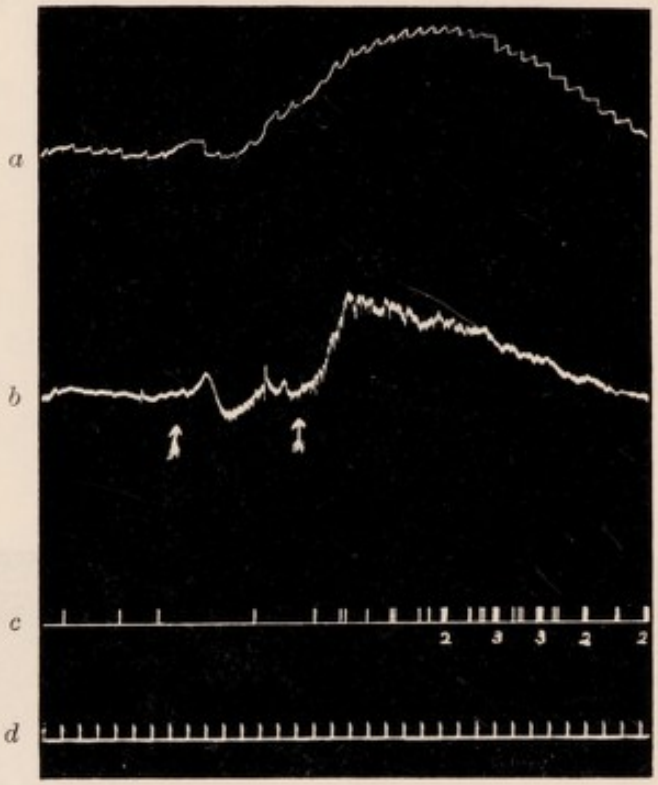

Fig. 19. × ⅔ linear. Rabbit. Urethane. (*a*) Kidney volume; (*b*) Carotid blood pressure; (*c*) Drop record of urine flow, and zero of blood pressure; (*d*) Time in 10 sec.. Between ↑ — ↑ 0·5 mg. Apocynamarin injected intravenously.

profuse than could be accounted for by the addition of the effects which the two drugs would separately produce. This appears to be one of the rare instances where polypharmacy has an experimental, though not, as yet, a completely rational basis, and deserves further investigation.

Excretion.

Attempts were made to detect by physiological tests the presence of Apocynamarin in the urine from the cat which received two doses of 2 mg. *per diem* by the mouth for eight days. Three separate samples of urine were used, but all gave negative results. The question as to whether it is excreted as such, or destroyed in the body, must, therefore, remain open, though the negative evidence is, on the whole, in favour of the latter.

Conclusions.

It is clear that the characteristic effects of the two species of Apocynum under consideration are due to the bitter principles named Cynotoxin and Apocynamarin by their respective discoverers. There is no pharmacological indication of any difference between the two. The action is in all respects a characteristic digitalis effect. Careful quantitative comparison with other pure principles would be needed to establish the position of the drug in the digitalis series. We have only determined that its vaso-constrictor action is considerably more powerful than that of Strophanthin; its action on the heart seems but little weaker than that of the latter. Its diuretic effect seems to be similar to that of other drugs of the series. On the other hand it appears to be excreted or destroyed with comparative rapidity, and there seems to be experimental basis for the statement that Apocynum is not cumulative in its action. This fact should tell in favour of the use of Apocynum in practical therapeutics, and the employment of the pure active principle should eliminate the drawbacks which have hitherto restricted its use, and which seem to be due to the presence of other constituents of an irritant nature in the crude extracts. We have observed no irritant effects with either oral or subcutaneous administration of the pure principles. The rapidity of the action enjoins caution with regard to subcutaneous dosage.

It is a pleasure to acknowledge our indebtedness to Messrs. Finnemore and Moore for the specimens of active principles; also to Dr. F. B. Power for kindly criticising our account of the chemical investigations.

Summary.

1. Crystalline Apocynin (Acetovanillone) has but the feeblest physiological action when pure.

2. The true active principle of Apocynum is a definite crystalline non-glucosidal substance, to which the names "Cynotoxin" and "Apocynamarin" were given by the investigators who independently obtained it from *A. cannabinum* and *A. androsæmifolium* respectively. Its action is in all respects that of the digitalis series, but its effect is apparently not cumulative.

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