THE PHARMACOLOGICAL ACTIONS OF PLANTS OF THE GENERA COTYLEDON AND CRASSULA, N.O. CRASSULACEAE, WITH OTHER PAPERS.

By

Norman Sapeika,
B.A., M.B., Ch.B. (Cape Town).

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(From the Department of Pharmacology, University of Cape Town.

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HISTORICAL.

The disease known as "Krimpsiekte" (Nenta, Kraamsiekte, cerebrospinal meningitis, Cotyledonosis) has been recognised in South Africa for many years. It appears in arid parts of the Cape Province, and affects animals which have ingested certain members of the genus Cotyledon (N.O. Crassulaceae).

Although the disease was officially recorded by Brown (1) in 1864 and erroneously attributed by him to Lessertia spp., it was not until 1891 that a Mr. Weyer of Darlington drew attention to a plant actually responsible for the malady (Soga (2)). This plant was recognised as Cotyledon Ventricosa by Macowan, who was surprised that a member of the Crassulaceae could be toxic. By feeding experiments C.Ventricosa was proved to be a cause of the disease. Other plants e.g. Lessertia annularis Burch, and other legumes had been incriminated by Macowan (3) but feeding experiments carried out with these by Tomlinson (4) and others were all negative.

The fact that members of Crassulaceae have more recently been shown to vary in toxicity according to locality, season, etc. probably accounts for the earlier differences of opinion as to the causative agent of Krimpsiekte.

Other members of this genus were subsequently proved to be toxic. Thus Kehoe (5) demonstrated the toxicity of C.Orbiculata to fowls, goats, while Henning (6) concluded from his experiments that C.Wallichii Harv., (from Matjesfontein) was a cause of this disease, and in fact, the main cause of the disease under natural conditions. Further, Steyn (7) found C.Decussata Sims. toxic to rabbits (by mouth) and to

guineapigs (by hypodermic injection). Cotyledon Eckloniana has also been shown to be toxic (8), (9).

Kamerman (10) has isolated an active principle from several species - C.Orbiculata, C.Wallichii, C.Decussata, C.Paniculata, (but not from C.Reticulata) - which produces Krimpsiekte when injected into goats. He points out that C.Orbiculata from Slangkop near Cape Town was found to be nontoxic, while specimens from Magaliesberg near Pretoria were highly toxic (11). Steyn has also shown the marked variation in toxicity in specimens of C.Orbiculata (12).

No extensive pharmacological investigation of this group of plants has been reported. Several observers have described the toxic effects in intact animals produced experimentally by feeding or by hypodermic injection to determine the minimal lethal dose.

The symptoms in the larger animals are described by Henning (13).

Gunn (14) investigated a sample of C.Ventricosa Burm., and found the chief actions of 70% alcohol extracts of tubers and leaves to be on the nervous and circulatory systems. These extracts were purified by precipitation with lead acetate, filtering, and again filtering after precipitation of the lead from the filtrate by hydrogen (or ammonium) sulphide.

The present work deals with a more extensive pharmacological investigation of several including the more important members of the genera Cotyledon and Crassula, N.O. Crassulaceae.

PRESENT INVESTIGATION.

The experimental work described in the following pages was first undertaken with Cotyledon Wallichii, Cotyledon Reticulata, Cotyledon Paniculata in 1933 and continued on plants of the same and other related species in 1935.

The following specimens were obtained from "Whitehill", Little Karroo, through the courtesy of Prof. R. Compton (Dept. of Botany, University of Cape Town) and were identified by him before they were prepared for pharmacological study.

Cotyledon Wallichii	Crassula Portulacea
" Reticulata	" Lycopodoides
" Orbiculata	" Ericoides
" Paniculata	" Canescens
" Ventricosa	
" Decussata	
" Glutinosa	
" Mammilaris	

The Crassulaceae do not contain many species considered as poisonous; there are 16 genera, with over 400 species, of which 8 genera and more than 300 species occur in South Africa; they are herbs or undershrubs, often fleshy or succulent (Philipps (15)).

Botanical details of the toxic varieties of Cotyledon are given by Philipps (15), Steyn (16), Henning (13).

Various colloquial names have been assigned to these plants and they have been put to use in diseases of the most diverse nature. A full account is given by Watt and Breyer-Brandwijk (17). Thus, amongst other uses preparations of the plants have been taken internally in the treatment of dysenteries and diarrhoea, in epilepsy, in bilharziasis, and have been applied externally as corn cures and poultices, and as drops for ear-ache and toothache. There is no mention of their use by the native tribes as arrow poisons.

The experimental results obtained in this investigation varied with different batches of plant. This is not surprising

since it is known that plants of the same species obtained from different sources or in different stages of development at different periods of the year may vary greatly in toxicity. This has been conclusively demonstrated by Steyn (12) in toxicity experiments on guineapigs with C.Orbiculata; plants growing adjacent to each other may vary in toxicity to a marked degree. Comparison of the pharmacological actions on a quantitative basis has therefore proved difficult since the same species of plant exhibited differences in potency at different times. The potency also varies according to the type of extract prepared.

Thus an extract of one specimen of C.Orbiculata, prepared with 96% alcohol, proved inactive even in big doses, while another specimen previously examined (prepared as a 20% tincture) was found to be potent and to produce all the actions to be described for C.Wallichii.

C.Wallichii showed marked action on the heart resembling the action of digitalis, and must be regarded as the type of poisonous plant to be found in this genus.

Preparations of stems of C.Reticulata generally had pronounced action on the nervous system, while those of the peduncles (reticulum) had marked action on the heart. C.Reticulata is generally regarded as nontoxic though Henning (6) observed incoördination of movements in fowls fed on the plant. Kamerman failed to isolate the toxic principle from it.

One sample of C.Paniculata was found to be practically inactive as was the case with a specimen of C.Ventricosa, though these plants may at times be pharmacologically active.

PREPARATION OF MATERIAL.

(a) Drying of plant.

A characteristic feature of these plants, especially of the fully developed leaves, is their high water content ("fleshiness") a fact borne out by the small amount of dried residue obtained after incubation of the minced fresh plant, e.g. in a particular sample,

	Wt. of fresh plant.	Wt. after drying.
C. Wallichii	leaves 35 G.	4 G.
	stems 100 G.	22 G.
C.Reticulata	leaves 140 G.	7 G.
	stems 120 G.	18 G.
C.Paniculata	leaves 17 G.	2 G.
	stems 130 G.	25 G.

On account of the different methods adopted for preparing extracts, doses have been expressed as the number of grammes of the original fresh leaves or stems of the plant.

(b) Preparation of extracts.

Several extracts of the fresh and dried leaves or stems were prepared, especially in the case of the commonly described toxic varieties of Cotyledon. Alcohol was the solvent generally used. A few aqueous preparations of dried plant were also prepared but were found to be practically inactive.

The alcoholic extracts were made using 96% alcohol as the solvent in the majority of cases, the general procedure being as follows:

A weighed quantity of minced leaves or stems was allowed to dry in a drying oven the temperature of which never exceeded 60°C; the dried residue was finely pulverised in a grinding machine and then allowed to stand in 96% alcohol for 5-6 days with occasional shaking of the mixture. The extract

was then filtered, the residue being squeezed through muslin and the expressed fluid added to the filtrate. The volume was then adjusted with 96% alcohol so that 1 c.c. of final tincture represented a definite number of grammes of the original fresh leaves or stems.

In some cases the residue was further extracted with 50% alcohol; in others the dried plant was extracted with 50% alcohol only, giving very potent preparations as a rule.

A similar procedure was adopted for extracting fresh leaves or stems which had not been allowed to dry previously.

The tinctures were stored in the refrigerator. Before use in any experiment the alcohol was driven off by gentle heat on a waterbath and the residue made up to required volume with frog's Ringer solution or Locke's solution according to the type of animal tissue used in the particular experiment.

Most of these aqueous solutions showed persistent froth on shaking and were acid in reaction; they were always neutralised with decinormal NaOH using litmus as indicator, and filtered prior to intravenous injection or perfusion of an isolated organ.

A uniform procedure was adopted throughout all the experiments so that comparable results should be obtained; also for the reason that Steyn (12) has shown that differences in extraction of the active principle depend on such factors as degree of agitation of the alcoholic mixture, degree of division of leaves, etc.

EXPERIMENTAL DATA.

Local Actions.

The aqueous acid preparations made neutral to litmus have very slight irritant properties as shown by mild inflammatory redness occasionally following injections into the ventral lymph sacs of frogs, or into the subcutaneous tissues or peritoneal cavities of guineapigs. Intraperitoneal injection caused a certain amount of free fluid, and in some cases a little fibrinous exudate especially noticeable on the diaphragmatic surface of the liver in animals autopsied some 48 hours after injection.

No conjunctival redness and no loss of the corneal reflex appeared in rabbits after the instillation into the conjunctival sac of high concentrations of neutralised aqueous preparations. Application to the skin and tongue also revealed no irritant action or paralysis of sensory nerve endings. The members of the digitalis series which the plants investigated closely resemble are known to vary in their local actions.

General Actions.

A. Action on intact animals.

On account of limited quantities of material for investigation at any one time, toxicity experiments by feeding or injection could only be carried out on small laboratory animals of which the guineapig is especially suitable. In actual veterinary practice the goat is most often the victim of Krimpsiekte.

Poisoning is more readily produced by feeding on the dried plant in which the toxic principle is more concentrated. Similarly, extracts of dried plant are more potent than those prepared from fresh specimens whose water content is so high.

(a) FROGS.

The relative toxicity of crassulaceous plants for frogs may be gathered from the following table indicating the "hour dose" for Xenopus Laevis (the South African clawed toad or frog) and also from the results of perfusion of the isolated frog's heart (v. Circulatory system). 96% alcohol extracts of the dried plant were used in these "hour dose" estimations; the doses express the number of grammes of original plant used per gramme body-weight of frog.

C. Wallichii -	leaves stems	0.5 0.04
C.Reticulata -	peduncles	0.012
C.Paniculata -	leaves stems	0.4 0.8
C.Ventricosa -	stems	0.08
C.Decussata -	leaves	0.2

(b) MAMMALS.

1. Cotyledon Wallichii.

A large number of experiments were carried out on different animals, examples of which are detailed below.

The most prominent symptoms following the administration of extracts of leaves and stems are on the circulatory system. Weakness, tremor, dyspnoea are notable features.

Guineapigs.

Feeding experiments.

Guineapig 318 G. fed 3 G. dried stems after 24 hours starvation. No convulsions were observed. Marked trembling appeared when it was disturbed. Dead in 2 days. Similarly, guineapig 320 G. fed 3 G. C.W. stems, dead in 2 days.

Parenteral Administration.

Dosage is expressed as G. of original plant per G. body-weight of animal.

i. Intraperitoneal injection.

Within a few minutes the animal became weak, lay on its side, dyspnoeic, unable to rise, hindlimbs extended backward, generalised tremor. No convulsions were observed. Death rapid, depending on preparation and dose.

Guineapig 220 G. 0.04 G/G (stems) intraperit.

½ hr. limbs suddenly gave way, the animal struggled to move, fell on its side and became motionless; there were no clonic jerkings of the limbs; corneal reflexes present. Occasional fruitless attempts to rise. Dyspnoea. Just before death, corneal reflex gone, few feeble gasps.

1 hr. Dead. P.M. heart in systole.

Similar results with preparations of leaves and stems (1933).

ii. Subcutaneous injection.

The minimal lethal doses of extracts of leaves and stems were estimated with 96% alc. extracts of the dried plant as shown in the following tables. Symptoms appeared after a greater interval and were not so marked as with intra-peritoneal injections.

Dosage is expressed in G. of original fresh plant per G. weight of guineapig.

C.Wallichii (leaves). M.L.D. 0.04 G/G.

No.	Wt. of Guinea-pig.	Dose G/G.	Remarks.
1	260 G.	0.04	1 hr. Tachypnoea, tremor, restless, head swaying as if dizzy. 1½ hrs. Dyspnoea, tremors. 5 hrs. same. 11 hrs. On side, flaccid; occasional body movements with inspiratory gasps. Running movements of limbs ? attempt to rise. No convulsions. 11½ hrs. dead. P.M. nil.
2	317 G.	0.03	
3	305 G.	0.02	All alive after 48 hours.
4	265 G.	0.01	

C.Wallichii (stems). M.L.D. 0.03 G/G.

1	310 G.	0.04	1 hr. Weakness. Trembling on movement. Dyspnoea. Intermittent movements. Very excitable to stimuli. 1½ hrs. On belly, restless. 2 hrs. Intermittent tremor. Micturition. Defaecation. Head on the ground, animal restless and using extra-ordinary muscles of respiration. No convulsions. 2½ hrs. Dead. P.M. nil.
2	348 G.	0.03	10 hrs. Obviously ill. Disinclined to move. 18 hrs. Dead.
3	330 G.	0.03	4 hrs. Sluggish, less responsive. 9 hrs. limbs weak, difficulty in walking. 20 hrs. Trembling on movement. Unable to walk, forelimbs paresed, guinea-pig pushing itself forward with its hindlimbs with head on the ground. Husky voice. 70 hrs. Dead.

C. Wallichii (stems) M.L.D. 0.03 G/G. (Contd.)

4	230 G.	0.02	No effects observed.
5	330 G.	0.01	
6	350 G.	0.005	

Rat.

Feeding.

Rat 123 G. died in 30 hours after eating about 4 G. dried stems.

Injection experiments.

Rat 222 G. injected intraperiteneally with 0.1 G/G C.Wallichii stems (1935).

In 10 minutes lay on its belly with no effort to move, nocuous stimuli failing to elicit any motor response. Corneal reflex present.

2 hours. Few slight muscular contractions were observed but these were irregular and intermittent.

3½ hrs. dead. P.M. periteneum congested, otherwise nil.

Similarly, 0.05 G/G C.Wallichii leaves (1933) produced death in a rat in 36 heurs.

Rabbit.

The limited amount of material prevented extensive investigations on this animal.

2.5 G/Kgm. dried stems (50% ext.) intraperit. inj. produced no effects apart from temporary increase in respirations, and disinclination to move.

2.36 G/Kgm. intraveneous injection.

Within 5 mins. animal was sitting erect on hindlimbs with forelimbs extended and supporting it; there were nodding movements of the head, head being thrown backwards in jerky fashion. Dyspnoea.

10 min. Suddenly leaped forward into the air, with convulsions, defaecation. Death.

P.M. heart in diastole; lungs collapsed.

Intravenous inj. of 0.25 G/Kgm. dried leaves (50% ext.) (= 5 G/Kgm. fresh leaves) produced irregularities in heart-action with cessation in 65 minutes; this occurred in the anaesthetised animal during the course of another experiment.

Fowl.

Cotyledon Wallichii. 96% ext. fresh stems (1933), ("hour-dose" for the frog 0.03 G/G).

(1) Intraperitoneal injection.

Fowl 1.25 Kgm. 10 G/Kgm.

During the first half hour following the injection the fowl sat still with head erect. After 30 mins. it stood up and walked about, eating food and showing no abnormal activity.

70 mins. Suddenly started to shake and roll its head, fell over as if struck by a blow, made a few feeble movements with its wings and limbs. A few inspiratory gasps with a guttural noise preceded death which came on in a few seconds.

P.M. heartmuscle - fibrillary tremors on the surface; lungs collapsed; peritoneum nil.

Fowl 1.7 Kgm. 8.25 G/Kgm.

features similar. Death in 75 minutes.

(2) Subcutaneous injection.

Fowl 1.4 Kgm. 10G/Kgm.

For the first 30 hours the bird seemed unaffected but gradually became less active and died during the night (about 36 hours) -

P.M. serous, nonpurulent fluid at site of injection; heart in systole.

The following data derived from various experiments indicate the relative toxicity to various animals of a preparation of C.Wallichii stems (1935 specimen dried, extracted with 96% alc. q.v.). The time indicates the moment of cessation of the heartbeat.

Dosage is expressed as G original stems per G animal.

Animal.	Route of Administration.	Dose.	Cardiac arrest.
Frog	Lymphsac.	0.04	60 mins.
Guineapig	intraperit. inj.	0.04	60 "
	subcut. inj.	0.04	210 "
Cat	intraven. inj.	0.0024	15 "
Rat	intraperit. inj.	0.1	210 "

The relatively large dose required to produce toxic effects in the rat shows the tolerance of its heart to the active principle in C.Wallichii; this principle has an action on the heart resembling digitalis.

The relative toxicity of 50% alcohol ext. of dried C.Wallichii leaves and stems (1933) is shown in the following table compiled from various experiments.

Dosage - G original plant per G of animal.

		Leaves.	Stems.
Frog	Lymphsac.	0.047 (60')	0.005 (60')
Guineapig	intraperit.	0.015 (40')	0.0021 (45')
Cat	intravenous.	0.0035 (8')	0.00234 (8')
Rabbit	intraperit.	0.005 (65')	0.00236 (10')

C.Wallichii stem preparations are thus seen to be more potent than preparations of the leaves. This fact was also observed in the results obtained with 96% alc. ext. of the 1935 specimens.

2. Cotyledon Reticulata.

Preparations of the stems, and also of the leaves, produced characteristic persistent clonic spasms especially in guineapigs, as a result of stimulation of the central nervous system.

Preparations of the peduncles were however found to have an action on the circulatory system.

Guineapigs.

Feeding experiments.

Guineapig 240 G starved 24 hours fed on 1 G dried stems made up with a little bran and moistened with water.
3 hrs. By this time there were twitchings, generalised tremor, diarrhoea, twisting of the head to one side then to the other. The animal was unable to walk.
Later, there appeared clonic movements of the hindlimbs, which all the time remained extended backwards.
7 hours. Dead.

This same preparation of C.Reticulata was found to have retained its toxicity after 2 years, e.g.

Guineapig 270 G fed on 1½ G was unable to move within a period of 12 hours, and was dead in 18 hours.
P.M. nothing unusual was noted.

Injection experiments.

(1) Intraperitoneal injection.

Guineapig 290 G 0.04 G/G.

10 mins. Defaecation, then gradually onset of trembling, limbs weak.

1 hour. There gradually developed a marked rhythmical nodding of the head, intermittent and aggravated by attempts at movement.

1 hr. 20'. Spasmodic jerkings of the trunk also appeared, the head bobbing intermittently. The hindlimbs were paresed, and the animal unable to go forward.

1½ hrs. Generalised body-spasms still continuing; corneal reflex present.

2 hrs. Animal lying on its side with hindlimbs extended backwards, teeth grinding, all limbs jerking irregularly as well as contractions of the trunkmuscles. Left forelimb showing more clonic movement than the other limbs.

2½ hrs. Still showing generalised twitchings. Feeble grunts and gnashing of the teeth. Corneal reflex present. Lying on its side, unable to rise.

If the proximal part of a limb was held firmly, the distal parts still showed rhythmical movement. The animal also showed occasional "kicking", apparently voluntary, movements while trying to rise presumably, showing that it was not unconscious.

3 hours. Frothy mucus at mouth and nostrils - a few gasps - death.

P.M. peritoneal cavity contained excessive free fluid, serosa congested; heart in diastole; lungs collapsed.

Similarly guineapig 240 G 0.08 G/G intraperit. showed the same sequence of events with death in 1½ hours.

These convulsive movements were observed with preparations of dried C.Reticulata stems in the case of all guineapigs injected, both in 1933 and 1935.

50% alc. ext. of leaves (1933) produced the same features, e.g. 0.05 G/G - death in 8 hours.

Symptoms following subcutaneous injection were similar though the effects came on more slowly owing to the slower absorption (v.i.).

(2) Subcutaneous injection.

Guineapig 200 G 0.04 G/G (stems).

1 hour. At this time there were observed - tremors, marked trembling on attempting to move. Defaecation.

2 hrs. Lying on belly, hindlimbs extended, but not paralysed; unable to move forwards. Placed on its back, it was unable to rise. General tremor.

3 hrs. On its side. Now showing intermittent rhythmical movements of the limbs, left hindlimb occasionally moving more than the others. Teeth grinding. Respiratory noises, jaw movements. Generalised tremor of body and limbs lasting about 2 secs. and occurring every 2 secs., were observed when the clonic movements were less marked.

3½ hrs. Defaecation. Generalised tremor with continued limb movements and occasional "running" movements when the animal appeared to be making efforts to rise.

4 hrs. Same. 4½ hrs. Dead.

Rat.

Feeding.

White Rat 166 G weight - fed 1½ G dried stems, after previous starvation for 24 hours.

24 hours. Animal remained motionless unless disturbed,

when it rolled over and over and twisted itself to get into the erect position. In between these efforts it lay on its belly. There were occasional attacks of head retraction, or the hindlimbs were extended forward, while the animal tried to push itself backward in this position. Altogether there was a certain stiffness about its movements. Heart rapid and regular (stethoscope).

30 hours. Writhing, "stiff" movements were made by the rat when stimulated.

48 hours. Dead.

Rat 190 G fed after 24 hours starvation on 2G dried C.Reticulata stems.

9 hours. No inclination to move even with nocuous stimuli; back hunched; when it tried to go forward it fell over on its side and moved around in circles; placed on its side it had the greatest difficulty in turning on to its belly.

12 hours. Dead. P.M. nothing abnormal.

Rat 144 G fed 2 G; similar; dead 36 hours.

Injection experiments.

0.05 G/G (1933) intraperitoneal inj.

24 hrs. Rat lying on its side; showed occasional muscular spasms keeping its tail rigid and erect, rolling over and over, with head retracted, until death - 60 hours.

(0.04 G/G C.Retic. stems (1935) intraperit. - nil 24 hrs.)

In the case of C.Reticulata the stems were found to be very potent (neurotoxic), e.g. C.Reticulata (1933) 50% ext.-

Guineapig intraperit. inj. 0.05 leaves (8 hrs.);
0.016 stems (3 hrs.).

A 20% tincture of C.Retic. dried stems did produce systolic arrest of the frog's heart (0.07 fresh stems per G frog) but with the other extracts only the peduncles showed marked action on the heart.

The experiments with Cotyledon Reticulata show this specimen to be toxic, though this variety is generally regarded as having no toxicity, e.g. Henning (6) was unable to produce poisoning by feeding sheep and goats with the plant though fowls showed incoördination of movement, and Kamerman (10) (11) failed to isolate 'cotyledontoxin'.

A decerebrate guineapig showed no convulsions with a characteristic preparation of C.Reticulata. These convulsive movements would therefore appear to arise from excitation of the motor cerebral cortex. During the whole period of poisoning the animal appears to be conscious, as shown by its response to a noise, and by its occasional fruitless efforts to regain the erect position.

The symptoms produced by C.Reticulata were compared with those following the intraperitoneal injection in the guineapig of strophanthin and picrotoxin.

Strophanthin produced restlessness, weakness, twisting and rolling of the whole body, inspiratory gasps, and terminal convulsions quite different from the long continued rhythmical limb movements produced by C.Reticulata; these movements produced by Strophanthin are probably efforts to 'get more air' and are more closely resembled by those following the administration of C.Wallichii, C.Paniculata.

The "running" movements produced by an injection of Picrotoxin were more related to those produced by C.Reticulata, though the forelimbs were more rapid and running in their movement and there was much bending of the head backwards.

3. Cotyledon Paniculata.

Active preparations of this plant produce symptoms similar to those following the administration of C.Wallichii.

Rat.

(1933) 20% Tinct. dried stems produced death in 12 hours (0.05 G/G).

Guineapigs.

Feeding.

1933 specimen produced same effects as C.Wallichii.

Injection experiments (intraperitoneal).

1933 sample (leaves and stems) gave similar results - weakness, jerkings especially of the hindlimbs, occasionally torticollis.

e.g. 0.05 G/G (leaves) death in 50' } 50% extract of
0.02 G/G (stems) " " 2½ hrs. } the dried plant.

These results could not be repeated with the 1935 preparations (96% alc. ext.), nor with any of the following similarly prepared extracts in a dose of 0.05 G/G.

C.Orbiculata — Crassula Portulacea (leaves, stems)
C.Decussata — " Canescens.

Cotyledon Decussata has toxic properties however as shown by the hour-dose for the frog (0.2 G/G) and also from the work of Steyn (7).

Absorption and elimination.

The results of the feeding experiments above described show that the active principles are slowly absorbed from the alimentary canal. The persistence of symptoms over a period of many hours after a single dose points to slow destruction or elimination of the drug.

In nonfatal poisoning in animals the disinclination to move about is probably due to the fact that any effort produces increased circulatory embarrassment and increases the animal's uneasiness.

Some animals may recover after manifesting typical symptoms, provided they are left undisturbed and judiciously fed; these measures may save the life of the animal unless it has had an overdose of cotyledon (13).

Tolerance.

In the feeding and injection experiments described above it was noted that greater amounts of C.Wallichii were required to produce toxic effects in the rat than in the other animals.

The action of C.Wallichii is chiefly on the heart resembling digitalis (see circulatory system). It is well known that the rat's heart is tolerant to digitalis (18) (19).

It will also be shown later that the toad Bufo regularis is unaffected by doses at least ten times as great as that required to produce systolic arrest in 1 hour in the frog Xenopus laevis.

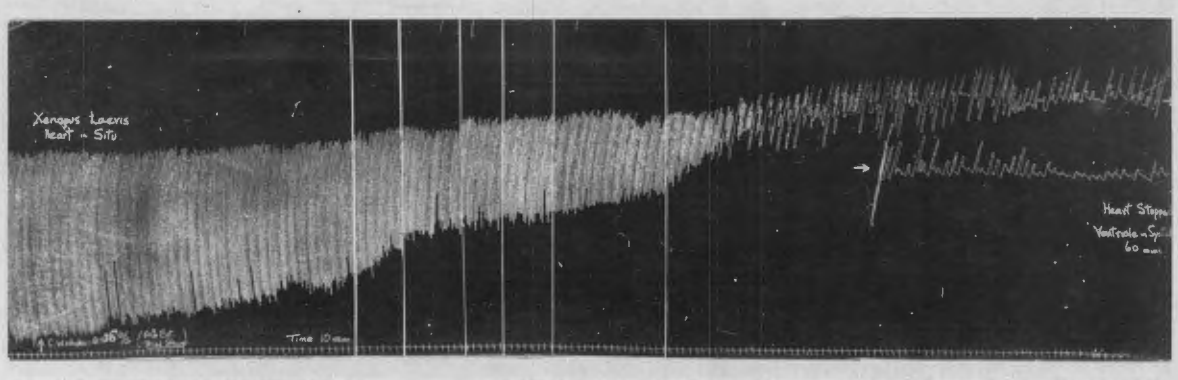


Fig.1. Frog's heart in situ. Effect of C.Wallichii. Gradual increase in systolic tonus, heartblock.

B. Circulatory System.

Action on the HEART.

(a) Frog's heart (Xenopus laevis).

i. Effect of C.Wallichii on the frog's heart in situ.

50% alcohol extract 0.05 G/G.

A pithed frog was pinned to a board; the pericardium was exposed through a small window in the chestwall and the sac incised. The ventricular apex was then connected to a delicately balanced writing lever by means of a clip and thread, and the cardiac contractions recorded on a revolving smoked drum.

The drug was injected into the thigh lymph sac. Details are given in the following table, measurements being taken from the tracing shown opposite. (Fig.1).

Time. (mins.)	Heartrate beats per min.	Ventricular amplitude. (mm.)	Remarks.
0	21	75	Constant.
5	25	65	Rate increasing.
10	23	52	Beats diminishing in size; gradual increase of systolic tonus.
15	23	41	
25	21	39	
30	20	37	
35	20	19	Onset of A-V dissociation, at first 4:1 block; heartblock increasing. Less and less ventricular relaxation Ventr. systole 60'.
40	7	19	
50	4	Irregular 6-16 diminishing	
55	2-3		

Thus slight quickening at first is followed by slowing as the ventricle goes gradually into complete and permanent systole, the auricles being unable to empty into the contracted ventricle.

ii. Effect of other cotyledons on the frog's heart in situ.

Some specimens of C.Paniculata and C.Orbiculata (1933) and C.Reticulata peduncles, produced similar changes - slowing of the

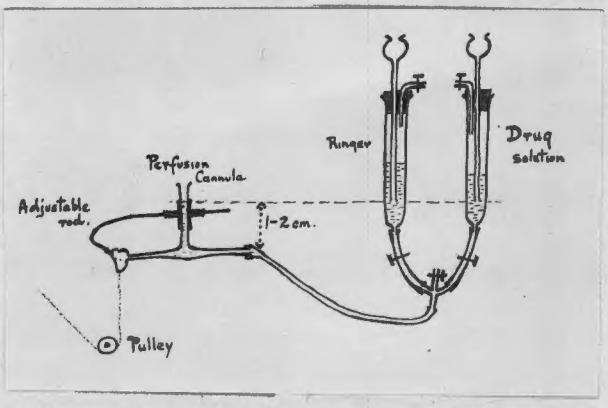


Fig.2. Perfusion apparatus for isolated frog's heart.

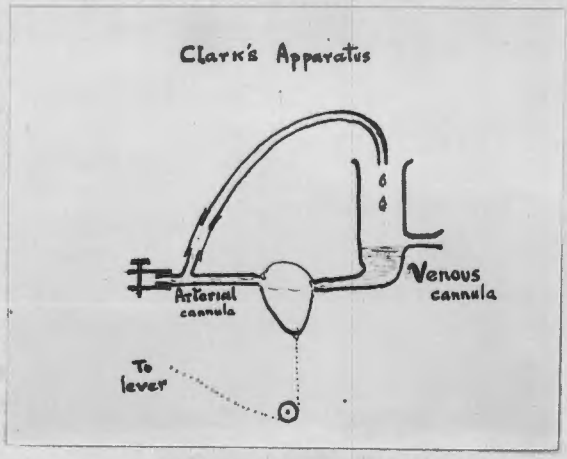


Fig.3. Clark's apparatus for perfusing isolated frog's heart.

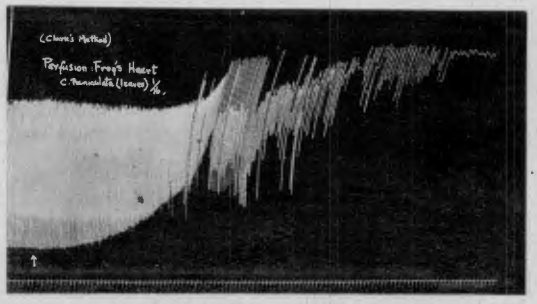


Fig.4. Tracing showing rise in systolic tonus and auriculoventricular block, produced in isolated frog's heart by C.Paniculata leaves.

heartbeat with less and less relaxation, until the ventricle finally ceased in systole. Various irregularities were observed before terminal standstill, e.g. auriculo-ventricular block, periodic grouped beats.

iii. Isolated frog heart.

The excised heart of Xenopus laevis was perfused through the inferior vena cava with neutral filtered solutions made up in different concentrations in frog's Ringer solution flowing from a Mariotte bottle at room temperature and at a pressure of 1-2 cms., fluid escaping from a cut branch of the aorta, the latter being tied to an adjustable rod (Fig.2). The drug solution was allowed to perfuse the heart after normal Ringer's solution had been perfusing for some time, with identical conditions of temperature, rate of flow, pressure. Movements of the ventricle were recorded by connecting its apex with a writing lever.

In a lesser number of experiments, especially where the extract was limited in amount but potent, the heart (auricle and ventricle) was perfused by Clark's modification (20) of Hartung's method (21), as illustrated opposite (Fig.3).

Gradual diminution of ventricular amplitude was observed with several extracts provided a sufficiently strong concentration was used. The beat gradually became smaller and smaller with a rise in systolic tonus. This alone in many instances might be attributed to the presence of saponins (Gunn and Epstein (22)), but the occurrence of various degrees of sino-auricular or auriculo-ventricular block and characteristic terminal standstill of the heart demonstrate the presence of digitaloid drugs, especially in the case of the following. (Fig.4).

Fig.5. Perfusion of isolated frog's heart with C.Paniculata stems showing initial increase in size of the beats, gradual tendency to systole, periodic grouped beats.

	Concentration.	Time of stoppage of heart.
C.Wallichii (leaves)	1 in 30	30 minutes
" stems	1 " 300	20 "
C.Reticulata peduncles	1 " 500	42 "
C.Paniculata (leaves)	1 " 16	15 "
" stems	1 " 50	40 "

In these cases immediately following the entry of drug into the heart there is generally an increase in amplitude and rate of the beat, followed by gradual slowing and incomplete relaxation; finally the ventricle ceases in systole, the auricles remaining fully dilated with solution which they are unable to empty into the ventricle.

A typical sequence is as follows (C.Paniculata stems 1 in 50 (1933)), measurements obtain from the tracing. (Fig.5).

Time. (mins.)	Amplitude. (mm.)	Rate/min.	Remarks.
-	55	34	Normal contractions, uniform.
3	60	42	Increasing rate and amplitude.
8	65	36	Maximum rate and amplitude
12	65	35	lasting 12 minutes.
20	55	33	Rapid decrease in rate and height of the beat.
23	40	28	Increase in systolic tonus.
26	25	25	Beats diminishing in size,
30	13	21	sinus block; groups of beats diminishing in number - 16, 13, 10, 8, 6, 6, 4, 4, 2,
40	0		0, separated by an interval of 25 seconds.

In the perfusion experiments the concentrations (expressed as dilution of G of original plant) appear rather high. This is due to the fact that the original leaves or stems have a high watercontent and the amount of active drug present in them is small.

iv. Estimation of the "Hour-dose".

To compare the activity of the different Cotyledon preparations the "hour-dose", the amount of drug required to stop the heart in one hour, was determined.

In these experiments frogs which had been kept for a few weeks in the laboratory were used. They were weighed after being dried with a towel, and with stomach and bladders emptied, and those of weights from 15-30 G were selected.

The aqueous extracts of the plants were prepared by driving off the alcohol and making up volume with frog's Ringer solution and neutralising with NaOH if acid. Injections were made into the ventral lymphsac through the floor of the buccal cavity, and observations made on the exposed heart in pithed animals at the end of 1 hour. The amount of fluid injected never exceeded 1 c.c.

Using 96% alc. extracts typical effects of digitalis action were observed with C.Wallichii, but also with C.Reticulata peduncles, C.Decussata, C.Paniculata, C.Ventricosa.

In these animals movements became less frequent with later little or no response to stimuli. The selective cardiac action is shown by "systolic arrest" of the heart even when the frog appeared unaffected as shown by its spontaneous active movements. The ventricle is colourless and in a condition of systole; the auricles, less sensitive to the action of the drug, remain purple and distended since the circulation ceases before the concentration is high enough for the development of auricular contracture (23).

A white slimy skin secretion often appeared after injection, chiefly on the back and extensor aspects of the limbs.

In the following table doses represent number of G of the original leaves or stems per G of frog weight. Doses in

brackets are the maximum doses injected which failed to produce any effect.

96% alc. extracts were used in these "hour-dose" estimation so that the relative potency of the same preparations of the various plants might be compared.

Table. "Hour-dose" for Xenopus laevis (96% alc. ext.).

		1935 Sample.
C.Wallichii	leaves	0.5
	stems	0.04
C.Reticulata	peduncles	0.012
	leaves	(0.8)
	stems	(0.4)
C.Paniculata	leaves	0.4
	stems	0.8
C.Ventricosa	leaves	(0.015)
	stems	0.08
C.Orbiculata	leaves	(0.2)
C.Mammilaris	"	(0.8)
C.Decussata	"	0.2
C.Glutinosa	"	(0.5)
Crass.Lycopodoides	"	(0.8)
" Ericoides	"	(0.5)
" Canescens	"	(0.6)
" Portulacea	"	(0.5)
	stems	(0.5)

Cf. Strophanthin 0.00075 mg/G Gunn (24)
0.0008 mg/G Epstein (25)

The toad shows a remarkable tolerance to the action of digitalis (c.f. the rat). This was also observed in this investigation. Bufo regularis showed no response to large doses of the potent preparations, exhibiting no effect after the injection of C.Wallichii, C.Paniculata, C.Reticulata in quantities 10 times the equivalent (hour) dose for the frog.

The minimal lethal dose of digitalis preparations for the toad is given as from 30-100 times as large as that for the frog of the same weight (23). Epstein (25) found Bufo 1000 times more tolerant to strophanthin than the frog Xenopus laevis.

As has already been mentioned, different preparations of the same plant vary in potency according to the method of extraction with different concentrations of alcohol. This is well demonstrated in the following table.

C.Wallichii (1933).	Preparation.	Hour-dose (G original plant per G frog).
Leaves	96% alc.ext. fresh leaves	0.16
	50% " " " "	0.01
	96% " " dried "	(0.14)
	50% " " " "	0.047
Stems	96% " " fresh stems	0.03
	96% " " dried "	0.04
	50% " " " "	0.005

From these figures and from experiments to be described later it would appear that 96% alcohol extracts are not the most potent and that extraction with lower concentrations of alcohol gives more active preparations.

Several of the more active preparations when retested were found to have retained their potency after 2 years.

(b) Mammalian heart.

i. In situ.

Cats were used in these experiments. They were anaesthetised with paraldehyde administered by mouth through a stomach tube (dose 1.5-2.5 mil/kgm. body weight), anaesthesia being occasionally maintained with small amounts of ether.

The movements of auricle and ventricle were recorded by means of Cushny's myocardiograph (26). The heart was exposed by cutting through the thoracic wall to the left of the sternum and by incising the pericardial sac, the cut margins being drawn laterally with hooks. Respiration was artificially maintained by means of a pump connected with the tracheal cannula. The 2 levers of Cushny's myocardiograph were sewn to the wall of the right ventricle so as to include about half an inch of muscle, the movements being recorded by a thread passing to the writing lever. The movements of right atrium were recorded by attaching the auricle to the auricular recording lever.

Bloodpressure was recorded from a carotid artery. Solutions were injected intravenously through the external jugular vein.

The effects set in almost immediately and tend to be cumulative, outlasting the administration for a considerable time.

C. Wallichii.

With adequate doses there was first produced increase in ventricular amplitude and rise in bloodpressure, then decrease of auricular and ventricular contractions, with slowing of the rate to a variable degree and subsequently the onset of irregularity, auriculoventricular block, ectopic rhythms, fibrillation, the bloodpressure falling to zero with cessation of the beat.

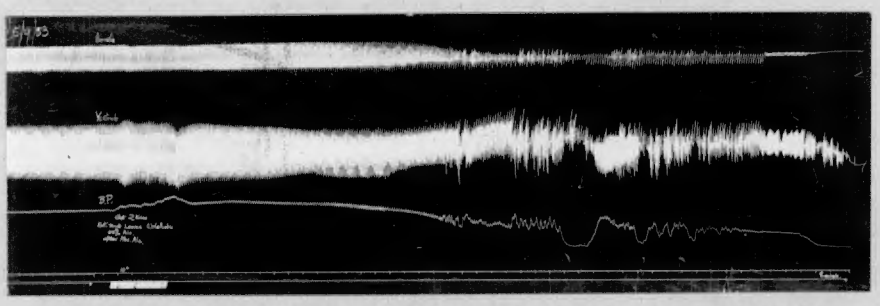


Fig.6. Mammalian heart (auricle and ventricle) and bloodpressure. Effect of C.Wallichii leaves. Marked irregularity in auricle and ventricle. Bloodpressure - slight rise, then marked irregularity.

The following are the details of a typical experiment using C.Wallichii (1933) 50% ext. 3.5 G leaves/kgm. (fig.6).

Time (min.)	B.P. (mm. Hg.)	Rate per min.	Ventric. amplitude (mm.).	Remarks.
-	64	234	43	Normal beats.
1	79	228	44	Increased amplitude.
2	74	234	38	
3½	56	240	26	Auricular and Ventric. beats diminishing in size and becoming irregular.
5	50	c.222	irreg.	
7	28	c.198	"	B.P. fluctuating.
7½	22	216	0-22	Auricular contractions very small. Ventric. rhythm regular but beats rapid and irregular in size. A-V. dissociation. B.P. gradually fell to zero.
8	-	-	-	

The measurements (after injection) could only be approximate owing to the irregularity of the beats.

The changes produced by a typical preparation of C.Wallichii may be described in three stages cf. Cushny's "three stages" for the action of digitalis on the heart (27).

1st stage - Following the entry of the drug into the heart there may be an increase in amplitude of the ventricular beat with slight rise in bloodpressure. The ventricular rate is often slowed, due to vagal action. This stimulation of the vagus does not occur in all the cats used.

2nd stage - The heart becomes more markedly slowed and irregular to a variable degree in different animals. It is due to excessive stimulation of the vagus centre in the medulla oblongata since an injection of atropine or cutting of the vagi

(and in one experiment a big dose of nicotine) results in less slowing of the heart or it may even be absent. The stimulation of the inhibitory centre is direct and not due to carotid sinus reflexes produced by the rise in bloodpressure; the bloodpressure may be below normal during the period of marked vagus action.

The auricles being more subject to vagus control show relatively greater inhibitory effects. The irregularities are often exaggerated variations of the respiration.

The auricles and ventricles may contract independently the ventricle beating with faster or slower irregular rhythm than the auricles.

3rd stage - The excitability of the heartmuscle increases and the ventricle beats rapidly, the inhibitory nerves being unable to hold it in check. Conduction from the auricles diminishes so that they beat independently of the ventricles. The A-V dissociation results in a difference in rhythm leading to periodic grouped beats with periodic variations in the strength of both auricle and ventricle. Extra-systoles occur and many of these premature contractions may expel no blood into the aorta. The contractions become irregular and ineffective.

This irregularity of the ventricle occurs even after the vagi are divided or paralysed with atropine. The bloodpressure shows marked fluctuations at this stage with a rise and fall, sometimes of 20-30 mm.

With terminal fibrillation the bloodpressure falls to zero level.

The cat was used in these experiments on the heart and the changes produced were not always so typical as to be described in three stages.

C. Reticulata.

Preparations especially of the stems produced a diminution of auricular and ventricular amplitude with a fall of blood-pressure - these changes being of short duration with recovery after a short time. Occasional cardiac irregularity occurred which could be abolished by atropine, indicating excessive vagal action arising from central i.e. medullary stimulation (v. other actions of C.Reticulata).

However preparations of the peduncles (reticulum) of C.Reticulata produced actions as described for C.Wallichii; the potent digitalis action is also shown by its effects on the frog's heart.

C. Paniculata.

Similar effects as described for C.Wallichii were obtained with preparations of stems and leaves (1933).

The following plants (1935) failed to produce any changes in cardiac action with the (intravenous) doses mentioned (G/kgm. cat) using similarly prepared extracts (96% alcohol).

C.Paniculata 5.4.

C.Ventricosa leaves 7.3.
slight diminution in auricular and ventricular beat; slight fall in B.P. cf.: Gunn (14).

C.Orbiculata 6. C.Mammilaris 8.

C.Glutinosa 6. C.Decussata 5.4.

Crassula Canenscens 7.3

" Portulacea 5 (stems, leaves).

" Lycopodoides 5 (slight diminition A. and V.; slight fall in B.P.)

" Ericoides 5.

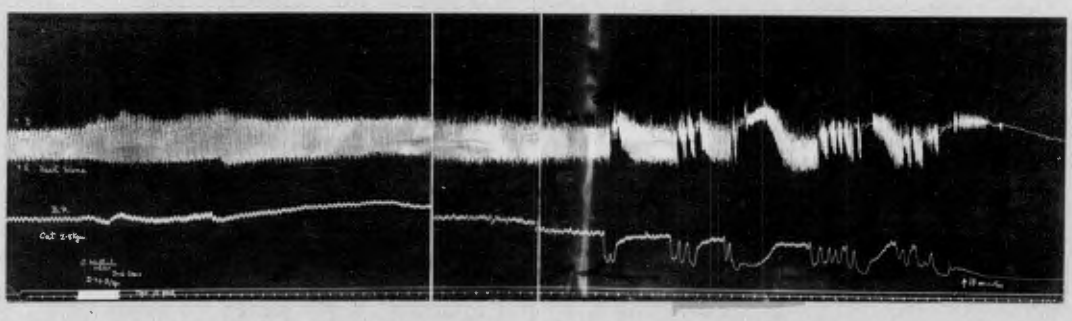


Fig.7. Record of changes in heartvolume and bloodpressure produced by C.Wallichii stems.
In order to avoid too great a reduction in the size of the photograph, two portions of the tracing (2' each) were removed.

Heart volume.

Investigated in the cat by means of the cardiometer (cardioplethysmograph).

The pericardium was exposed after cutting through the anterior chestwall of the anaesthetised animal, respiration being maintained artificially by means of the respiratory pump. The pericardial sac was incised and a thistle funnel of suitable dimensions slipped into it and over the heart; the funnel was then connected with a tambour recording by a writing lever.

The drug solution was injected intravenously while blood-pressure was recorded from the carotid artery.

The volumetric changes in the heart are thus recorded, cardiac dilatation causing the rubber membrane of the tambour to rise, the lever then writing at a higher level; diminished heart volume causes the lever to record at a lower level.

The detailed results measured from an experiment with an extract of C.Wallichii stems are given below. (Fig.7).

C.Wallichii dried stems (50% ext.) 2.34 G/Kgm.

Time (mins.)	B.P. (mm.Hg.)	Ventricular rate/min.	Amplitude mm.	Remarks.
0	80	198	21	Normal.
3	96	204	27	
4	100	216	27	Bloodpress. maximum.
6	88	210	27	B.P. beginning to show fluctuations,
9	62	174	24	increasing in extent
12	56	192	21	Gradually tending to fall to zero level.
13-18	B.P.falling; sometimes a sudden drop to zero with recovery in a few secs. Fall to zero 18'.	192		Heartvolume was so reduced at times that no record was made, returning to normal. Rapid alterations in volume, heart ceasing in diastole — 18 min.

Whereas the frog's ventricle passes gradually into complete systolic standstill, the mammalian heart was in most cases in a condition of diastole both in toxicity experiments and experiments on the heart.

This has been attributed in the intact animal to the accumulation of metabolic poisons during cardiac activity, but the ventricles were often dilated even when the isolated heart was perfused through the coronary arteries.

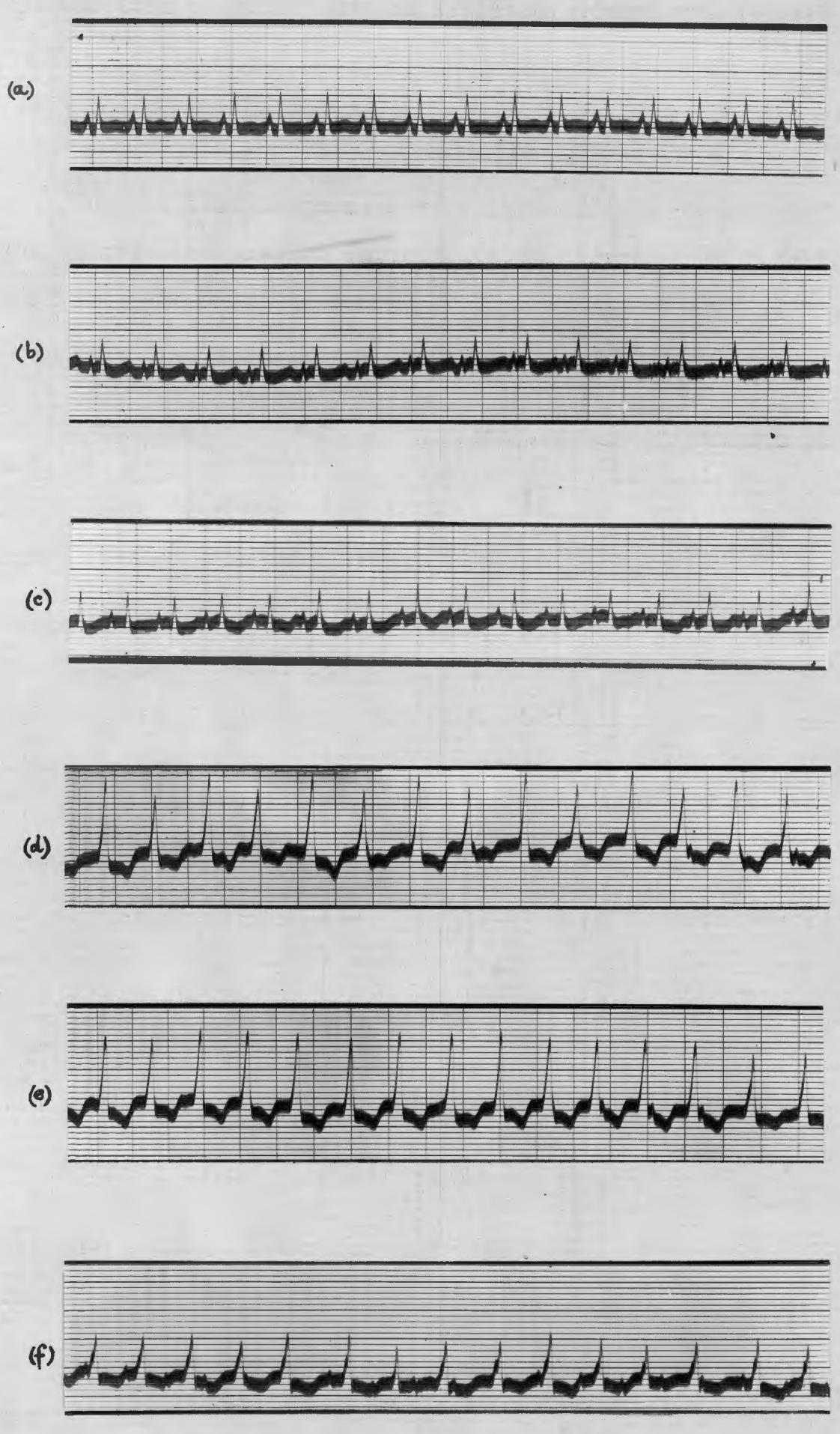
Electrocardiographic studies.

Simple zinc electrodes were fastened to the right forelimb and left hindlimb (Lead II) of cats anaesthetised with paraldehyde administered by stomach tube.

The drug was injected intravenously and the bloodpressure recorded in the usual way to give an indication of changes in the heart. 'Electrical' changes could then be recorded on a moving plate by photographing the deviations of the galvanometer fibre due to auricular and ventricular action.

The photographs opposite (Fig.8) were those taken in one experiment, the drug used being a preparation (50% alc. ext.) of C.Wallichii stems. There are definite changes to be observed in the electrocardiographic tracings which may be due to the drug. The changes may however not be specific since a dying heart may show a change to nodal rhythm, then to bundlebranch disturbance, later to auricular flutter, complete heartblock and ventricular fibrillation (Willius); however in the experiment here recorded photograph f. was taken while the bloodpressure was still at a high level, remaining so even after another 40 mins.

Fig.8.



Ordinates indicate 1/5 sec.

(a) Normal tracing; heartrate 260 per min. PR interval 0.067 sec. T wave positive.

The drug was then inj. intraven. and was followed by a transitory fall of bloodpressure soon recovering to normal level.

(b) T wave more pronounced.

(c) ST interval depressed. PR 0.1 sec.

(d) There is alternation of ventricle, alternate low and high QRS waves. The abnormal rhythm appears to be due to auriculo-ventricular node becoming the "pacemaker" i.e. a change to "nodal rhythm". "Voltage" increased.

(e) Similar to (d); QRS interval also increased to 0.067 sec. Vagi nn. divided.

(f) Notching (slurring) of QRS. "Voltage" has decreased. There appears to be definite auriculo-ventricular dissociation; auricle ? 264-270 per min., ventricle ? 210-228 per min. (Time tracing absent - revolving spoked wheel stopped).

Digitalis produces an increase of conduction time; the T wave may be inverted usually in Lead III at first and may appear within 2 hours of oral administration (36).

Alternation of the ventricle may be found temporarily in patients poisoned by digitalis (37).

Heartblock may arise in some cases from vagal stimulation but in this experiment (vagi divided) is probably a direct effect on the junctional tissues in the heart.

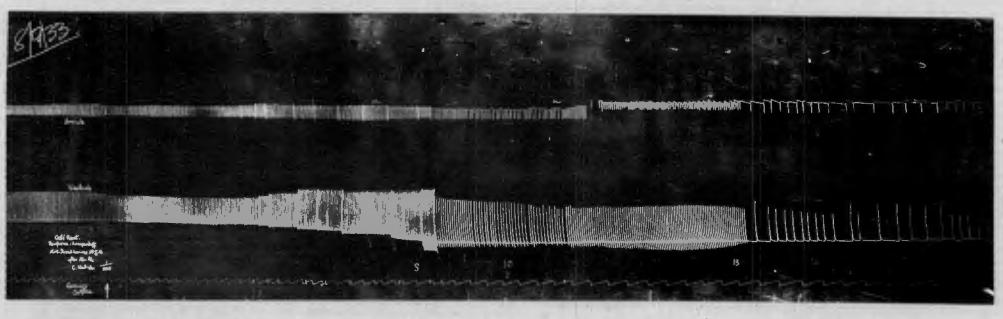


Fig.9. Perfusion of isolated mammalian heart with C.Wallichii leaves.
Increase in ventricular amplitude, terminal heart-block.

ii. Isolated mammalian heart (rabbit, cat).

Perfused through the coronary arteries by the method of Langendorff. The aqueous solutions of drug, neutralised and filtered were perfused through the heart in Locke's solution flowing at constant rate and pressure; the solution was warmed by Gunn's method (28) to a temperature of 37°C. and oxygenated by bubbling through air. Coronary outflow was recorded by Gunn's method (29).

Movements were recorded from right auricle and ventricle by threads attached to Brodie's levers.

C.Wallichii

(C.Paniculata)

At first produced increase of auricular and ventricular beats. The rate was sometimes accelerated; with digitalis preparations this is often the case in perfusion of the isolated mammalian heart and is held to be due to stimulation of the sino-auricular node. The amplitude then diminished with subsequently irregularity of the rhythm and finally cessation of the beat.

The details of a typical experiment are tabulated below.

C.Wallichii 1 in 1000 leaves (50% alc.ext. dried leaves) Fig.9

Time (mins.)	Amplitude in mm. aur.	ventr.	Rate per min.	Coronary outflow per min.	Remarks.
-	9	25	195	24	
2	9	23	180	21	
5	10	34	180	20	
8	10	43	195	18	Maximum increase in ventric. amplitude.
10	9	34	180	14	Marked slowing of heart, then temporary quickening, and marked terminal slowing.
12	6	30	135	12	
14	6-	20-30	27	11	? sinus block.
16	-	-	-	-	No A-V dissociation.

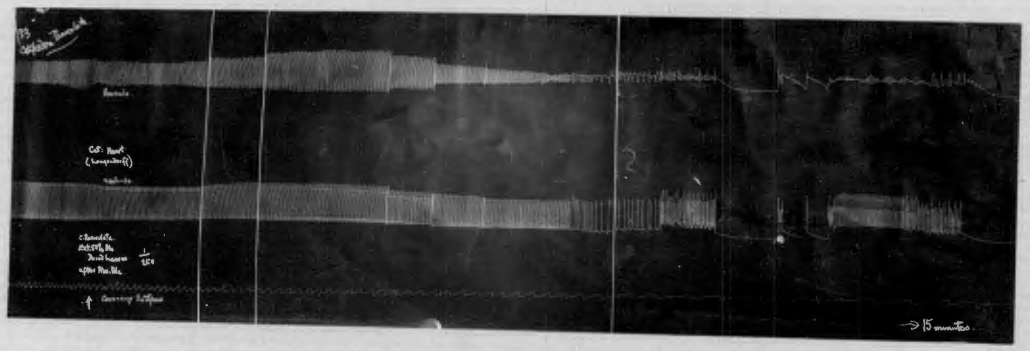


Fig.10. Isolated cat's heart perfused with C.Paniculata leaves.
Increase in auricular and ventricular amplitude, then A-V dissociation.

Similarly,

C.Paniculata 1 in 250 leaves (50% ext. dried leaves) Fig.10

Time (mins.)	Amplitude in mm. aur.	ventr.	Rate/min. Aur.	Ventr.	Coronary Outflow per min.	Remarks.
0	20	30	186	186	45	
1,2	18	25	180	180	35,30	
4,5	22	30	192	192	26	
8	11	22	201	201	20	
9-12	Irreg.	24		90	12	Auric. contrns. irregular.
14	-	24		132	9	Ventricle responding to every 2nd or 3rd impulse.
15	-	11-22		c.222		Ventricle then dissocd. from auricle, ceased, auricle continuing. Both ceased in diastole.

The coronary outflow progressively diminishes, even when the ventricular amplitude is maximal. This would appear to be due either to a direct vasoconstrictor action of the drug, or interference with the coronary circulation by the forcible ventricular contractions. This interference with coronary circulation may account for the cessation of the heart in diastole; the isolated heart perfused with digitalis preparations generally stops in a condition of systole (cf.: intact animals) which is attributed to the absence of accumulation of metabolic poisons (23).

C.Reticulata.

Preparations of stems and leaves did not show the effects described above and produced little change generally; changes in auricle and ventricle may be due to the presence of saponins.

C.Orbiculata.

A 20% tincture of one sample produced the cardiac changes described for C.Wallichii, while a 96% alc. ext. of another sample proved inactive.

Action on Bloodvessels.

1. Vessels of a pithed frog stripped of its skin were perfused from Mariotte bottles, first with perfusing fluid made according to Clark's formula (30), and then when the outflow was constant with different concentrations of drug in the perfusing fluid.

The apparatus used was the same as for perfusion of the isolated frog heart except that the inflow tube was tied into the distal end of the cut aorta, the heart being excised. The fluid after passing through the circulatory system escapes from the cut inferior vena cava, although a certain amount also permeates into the tissues and on to the free surface, the frog becoming increasingly oedematous, and increasing in weight. The number of drops flowing from the animal per minute is reduced if the drug has a vasoconstrictor action and vice versa.

The perfusion pressure in these experiments is much higher than for the isolated heart, but alterations in height do not materially affect the increase in weight (Gunn (31)).

No definite conclusions could be deduced as to the effect of potent preparations on the calibre of the bloodvessels. Though no vasodilatation was observed, the slight vasoconstriction produced was not remarkable.

2. Perfusion of the coronary vessels by Langendorff's method for investigating the isolated mammalian heart shows a diminution in coronary outflow; the coronary vessels are therefore constricted (unless the coronary circulation is impeded). Digitoxin and to a lesser extent strophanthin also constrict the coronary vessels (32) (33).

3. Investigation of the intestinal vessels was done by recording the volume changes of a loop of small intestine (cat)

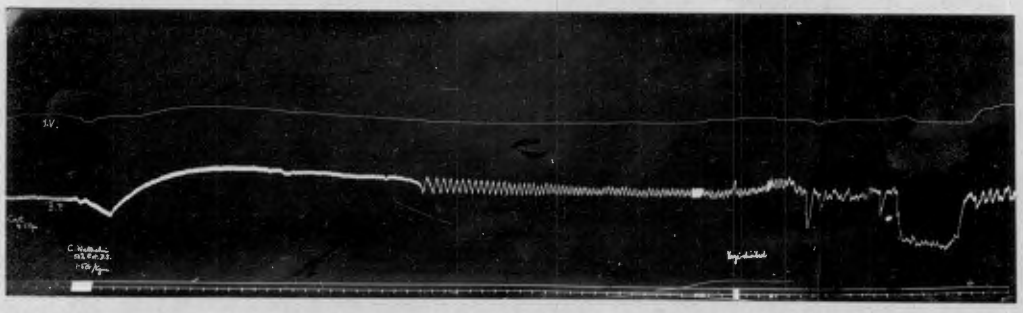


Fig.11. Changes in intestinal volume following alterations in bloodpressure produced by C.Wallichii stems in the cat; vagi divided at 8½ minutes.

enclosed in an airtight plethysmograph connected with tambour and writing lever. Alterations in calibre of the intestinal bloodvessels followed changes in the bloodpressure which were recorded at the same time (Fig.11) i.e. increase with a rise of bloodpressure and vice versa. Any effects of the drug upon the splanchnic vessels by direct action were therefore masked.

The only results which therefore point to a possible vaso-constrictor action are the effects on the coronary vessels; an increase in tone of involuntary muscle is produced by the potent preparations but only in high concentration.

Action on Bloodpressure.

Immediately following the (intravenous) injection of drug into the circulation there may be a fall in bloodpressure which varies with the different preparations used and their concentration. If the injection is made slowly the fall is reduced; this action is due to the presence of depressor substances in the extracts, or is anaphylactoid in nature. The bloodpressure soon returns to normal level or then follows the cardiac alterations produced by the glucosides with digitaloid action.

In the latter case improvement in the ventricular beat with increased output is often accompanied by a slight rise in bloodpressure or the bloodpressure may remain at the normal level. The rise of bloodpressure might be assisted by vasoconstriction from stimulation of the vasomotor centre or from direct action upon certain parts of the arterial tree; no definite evidence for the latter was obtained in these experiments.

Where the stimulation of the vagus nerves produces slowing of the heart and irregularities in the rhythm and force of the beats, the bloodpressure shows corresponding fluctuations tending towards a gradual fall. These fluctuations may take place at a level below the normal, or above it, the latter occurring especially after division of the vagi. The respiratory variations may also contribute to these irregularities.

Finally with the onset of marked ventricular irregularity the bloodpressure falls rapidly to zero. During the stages of extreme cardiac irregularity the bloodpressure might fall practically to zero only to recover to the original level in a short time. (Figs. 7, 11).

The changes described were seen typically with preparations of C.Wallichii, also with C.Paniculata (1933), C.Reticulata peduncles.

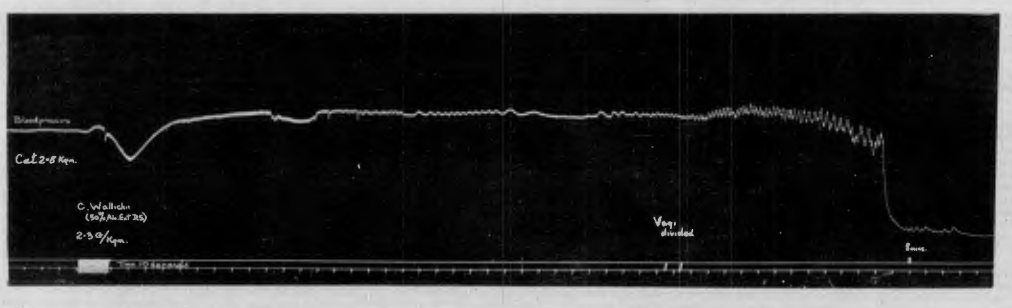


Fig.12. Record of bloodpressure in the cat showing changes following an injection of C.Wallichii stems. Initial transient fall; rise above normal level, then slight variations; division of the vagi nn. followed by rise to a higher level with marked fluctuations. Sudden drop to zero level.

With C.Reticulata leaves and especially stems alterations in bloodpressure were due to marked interference with respiration or to cardiac changes following excessive vagal action arising from medullary stimulation.

The details of a record are shown in Fig.12.

C.Wallichii 2.3 G stems /kgm. cat.

Time (mins.)	Bloodpressure (mm. Hg.)	Remarks.
-	92	Constant.
(10 secs.)	66	Temporary fall following the inj. lasting 30 secs. rapidly recovering to normal level.
1	106	
2-4	110	B.P. rising and remaining high practically to the end.
5	110-112	Onset of slight variations, gradually becoming more marked.
5½		Vagi divided.
6	110-118	Fluctuations in B.P. more marked with cardiac irregularity.
7	98-112	
7½		Sudden marked fall of B.P.
8	4-8	Slight record above zero level for ½ min.

Action on Red Blood Corpuscles.

The red blood corpuscles of a rabbit which were washed free of other blood constituents with Locke's solution and separated by centrifugation or defibrination were incubated with neutral aqueous solutions of various plant preparations at a temperature of 37°C. Dilutions of drug varying from 1 in 20 to 1 in 5120 were used.

The preparations showed little haemolytic activity; haemolysis was never observed with concentrations less than 1 in 80 even after 12 hours.

These aqueous preparations all show a persistent froth on shaking; the haemolytic activity must depend on the presence of saponins the amount present being variable.

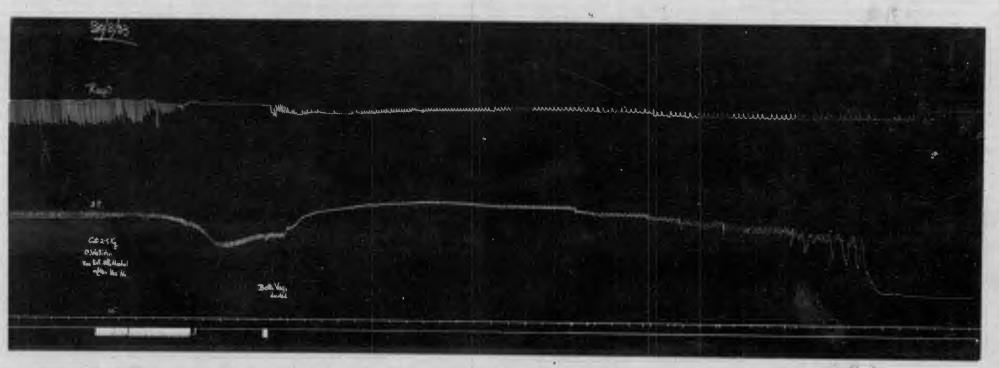


Fig.13. Effect on respirations of cat following inj. of C.Wallichii.
Period of apnoea accompanying fall of B.P. Recovery.
Subsequent respirations shallow and slow.
Respirations continue for a short while after blood-pressure has fallen to zero.

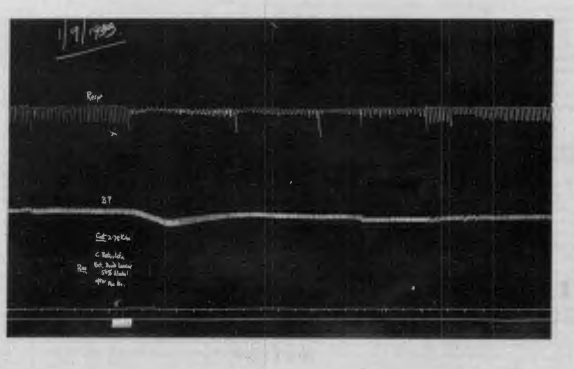


Fig.14. Respirations of cat. C.Reticulata leaves.
Rapid shallow irregular breathing with gradual return to normal.

C. Respiratory System.

The smaller laboratory animals which have ingested toxic members of the Crassulaceae, e.g. C.Wallichii, show signs of respiratory embarrassment - tachypnoea, dyspnoea, as one of the chief signs of poisoning. The animal becomes restless some hours after eating the plant, the breathing becomes rapid and laboured, the animal becomes less and less inclined to move, lies first on its belly then on its side unable to rise.

The respiratory difficulty is due to deficient oxygenation of the blood arising from impaired cardiac action which progressively gets worse as the substances with digitalis-like action cumulate in the heart; and is partly due to the action of the poison on the respiratory centre, this latter being especially the case with C.Reticulata preparations (stems and leaves).

Following the intravenous injection of an active preparation of C.Wallichii there may be an increase in the number of respirations per minute. More often however especially with larger doses the respiration is slowed and the effect of stimulation of the vagus nerves is marked as shown by the effect on the bloodpressure. The respiration becomes shallow and there may even be a period of apnoea with rapid recovery after administration of atropine or section of the vagi, due chiefly to an improvement in the circulation (Fig.13).

The following details of one experiment illustrate the type of change which may be produced (Fig.14).

C.Reticulata leaves 1.7 G/Kgm.

Time (mins.)	Respirations Rate/min.	Respirations Amplitude (mm.)	Heart rate.	Remarks.
-	30	11	166	
½	c.60	½-4	147	Slight fall of bloodpress. Respirations rapid and shallow.
1½	52	4	174	Respirations rapid but of greater depth.
3	36	4	182	B.P. rapidly normal.
5	29	4-5	180	Respirations increased in
6	30	11-12	170	depth to normal amplitude.

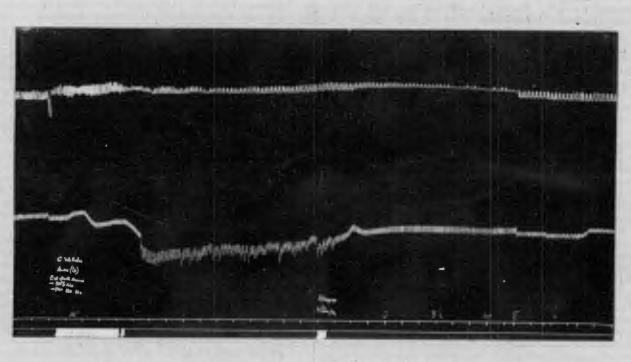


Fig.15. C.Wallichii leaves. Effect on respiration. Marked vagus effect (v. Bloodpressure).

With a dose of 3.4 G/Kgm. of this preparation, respiration ceased immediately, and the bloodpressure fell, but was maintained at normal level with artificial respiration.

In the following experiment the vagus was markedly stimulated by the drug.

C.Wallichii leaves 5 G/Kgm. cat. (Fig.15)

Time (mins.)	Respirations Rate/min	Amp.(mm.)	B.P. mm. Hg.	Heart rate.	Remarks.
-	45	5	112	186	
½	42	8	-60	78	Sudden fall of blood-pressure, heart very slow and beats irregular in force.
1	36	3-4	-48	78	B.P. slight variations Respirations shallow.
2	30	5	-40	96	→ Atropine 1cc.1/1000/Kgm.
3	30	3	-12	174	Recovery of B.P. to normal.
4	30	1-2	-12	156	Irregularity of beats abolished, rate normal.
5	30	5	-10	162	Respirations slowly increased in regularity
8	34	5	-5	186	and amplitude but did not recover normal rate.

D. Central Nervous System.

The term "Krimpsiekte" applied to the syndrome produced in certain animals which have ingested poisonous varieties of the Crassulaceae indicates the characteristic feature of the disease viz. twitchings and spasmodic contractions of the voluntary muscles producing characteristic disturbances in the different animals and often resulting in characteristic postures. Thus in the larger animals, dysphagia, spasm of the neck muscles leading to abnormal positions of the head e.g. torticollis, arching of the back, difficulty in walking. These effects are due to the action of a toxic principle affecting the nervous system.

C.Ventricosa (2) (6), C.Wallichii (6), C.Paniculata (11) (6), C.Orbiculata (5) (12), C.Decussata (11) (7), have been shown to have toxic properties and in this investigation C.Reticulata, especially the stems, has been shown to have neurotoxic properties to a greater and more specific extent than any of the other plants examined. C.Wallichii has marked action on the heart resembling digitalis in its effects though less potent. Many of the neuromuscular effects observed with this and allied preparations are such as would be produced in the later stages of poisoning by toxic doses of any cardiac glucoside. With a failing circulation agonal convulsions may occur.

The members of the digitalis series have a stimulant action on the central nervous system especially on the medulla oblongata which varies and which may extend in some animals to the lower brain (23), but the principle in C.Reticulata has been shown to have a marked and prolonged neurotoxic action when no direct effects on the heart could be demonstrated.

In the case of C.Reticulata the presence of a principle with action on the nervous system is suggested by

- the regularity with which the characteristic persistent movements could be produced in guineapigs (coming on

relatively early and lasting in many instances for several hours); therefore not agonal or terminal convulsions.

- absence of these effects in the decerebrate animal.
- effects on heart and respiration indicative of vagal (medullary) stimulation, abolished by the administration of atropine or section of the vagi.
- those preparations of C.Reticulata which produced these effects had not such powerful direct action on the heart cf: C.Wallichii which has marked action on the heart yet never produced the characteristic convulsions.
- the convulsions following an injection of Picrotoxin more closely resembled the nervous effects than those occurring in the later stages of strophanthin poisoning.

The other poisonous cotyledons no doubt also contain a similar neurotoxic principle in smaller and variable amount, but the effects in these cases are obscured by those produced by the more potent cardiac drugs.

The convulsions differ from those produced by strophanthin and arise from stimulation of the motor areas of the brain; they resemble the "running" movements produced by Picrotoxin which is also analogous to Cotyledontoxin in its stimulant action on the medullary centres.

From the chemical aspect too the toxic principle in crassulaceous plants is found by Kamerman to be a non-nitrogenous nonglucosidal substance related to the picrotoxin group of Kobert and he has called the principle - "Cotyledontoxin" (11).

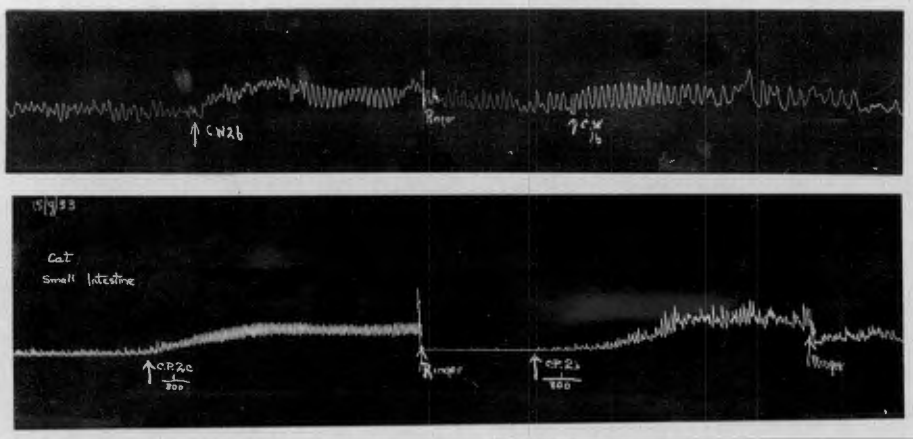


Fig.16. <u>Upper tracing</u> - small intestine of cat - effect of C.Wallichii stems and leaves - rise in tonus, increased contractions. <u>Lower tracing</u> - similar - C.Paniculata stems.

Fig.17. Pregnant cat uterus; marked contractions with C.Wallichii stems.

E. Action on Involuntary Muscle.

(a) Isolated tissues.

Investigated by Magnus' method in which small segments of tissue (intestine, uterus) are suspended in a bath containing warm (37°C.) oxygenated Locke's solution, movements of the strip being recorded by attaching one end to a delicately balanced writing lever, the other end being fixed.

After the normal contractions had been recorded for some time the drug was added in quantities sufficient to make up the requisite concentration.

Intestine.

Certain preparations especially C.Wallichii, C.Paniculata (1933) caused increase in contractions and rise in tonus especially with high concentrations; on replacing the drug solution by Locke's solution the normal contractions reappeared (Fig.16).

Uterus.

More frequent contractions and a rise in tonus was produced (especially with pregnant uterus) Cat, guineapig) when these same preparations were used (Fig.17).

(b) Intestine and uterus in situ.

The movements of the uterus and small intestine were recorded in rabbits and cats, the former anaesthetised with urethane intravenously (4 c.c. 25% per kgm.), the latter with paraldehyde per stomach tube (2 c.c. per kgm.).

Through small midabdominal incisions about ¾ inch of uterine cornu (or of gut) was sewn to the horns of Gunn's holder (34) attached to the side of the bath of warm saline in which the animal was kept immersed.

The head and neck of the animal with the arterial, venous and tracheal cannulae were alone supported above the watersurface.

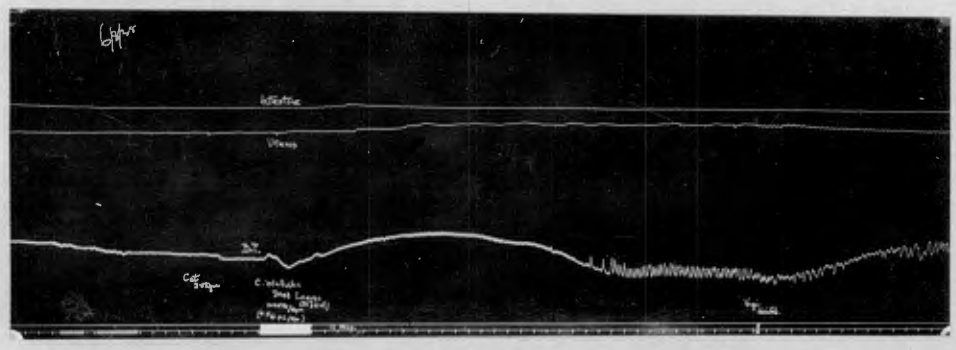


Fig.18. Intestine and uterus in situ, bloodpressure (cat); C.Wallichii leaves.
Slight stimulation of intestine.
Rise in tone and onset of contractions in uterus (nonpregnant).

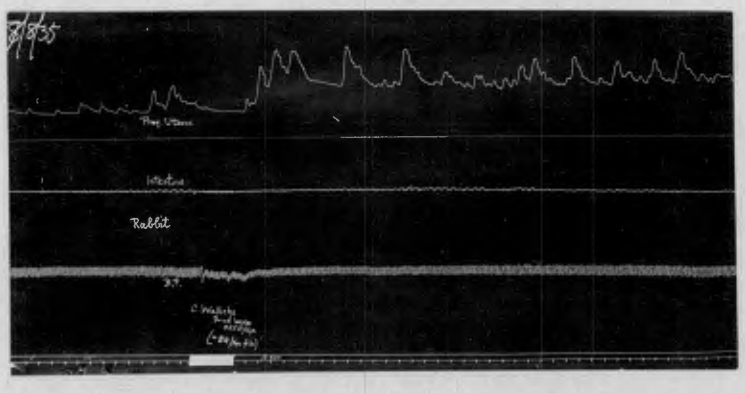


Fig.19. Pregnant uterus and intestine of rabbit; C.Wallichii leaves. Marked stimulation of uterus with rise in tone and larger contractions.

The centre of the segment of plain muscle was connected by thread to delicately adjusted frontal levers (35) writing on a revolving smoked drum.

The uterus (nonpregnant and especially the pregnant) showed a rise in tone and increase of the automatic movements, or the inception of contractions where none had been recorded previous to injection of the drug; the intestine also showed evidence of stimulation by increase in its contractions.

These effects followed intravenous injection of potent preparations e.g. Cotyledon Wallichii leaves or stems (50% ext. dried plant). (Fig.18, 19).

This stimulation of the pregnant uterus probably accounts for the fact that pregnant sheep and goats may abort after eating poisonous varieties in the veld.

F. Chemical Reactions.

Aqueous solutions prepared from the original alcoholic extracts by evaporation of the alcohol and addition of physiological saline solution were in the majority of cases acid to litmus requiring the addition of varying amounts of decinormal NaOH for neutralisation; the addition of alkali often produced brilliant colours - yellow, brown, or intensified that which was already present.

Heating with acids may decompose the glucosides so that there is always the danger of tinctures becoming less active in preparing them in a form suitable for injection. Gentle heat on the waterbath to remove the alcohol and rapid neutralisation on cooling served to reduce this possibility.

The fact that digitalis glucosides show great differences in their solubility in water (and alcohol) is probably another reason in this work for the differences in potency of different extracts of the same plant. The active cotyledon principles are also soluble in water and alcohol. The presence of saponin probably aids the solution of some of the principles in water, in the same way that digitoxin is kept in solution by the saponin (digitonin) and absorption from the alimentary canal similarly facilitated.

The persistent froth which is produced on shaking the majority of aqueous solutions, the fact that they reduce Fehling's solution, the laking of red bloodcells, and the increase in systolic tonus of the frog's heart without other cardiac change indicated the presence of saponins in most plant extracts, the amount present varying greatly from plant to plant, and even in the same plant.

The presence of cardiac glucosides as opposed to the sapo-glucosides is suggested e.g. in C.Wallichii by the fact

that previous boiling (i.e. hydrolysis by the acid already present) gives a slightly better result when subsequently tested with Fehling's reagent and compared with a similar amount of the preparation which was neutralised before boiling.

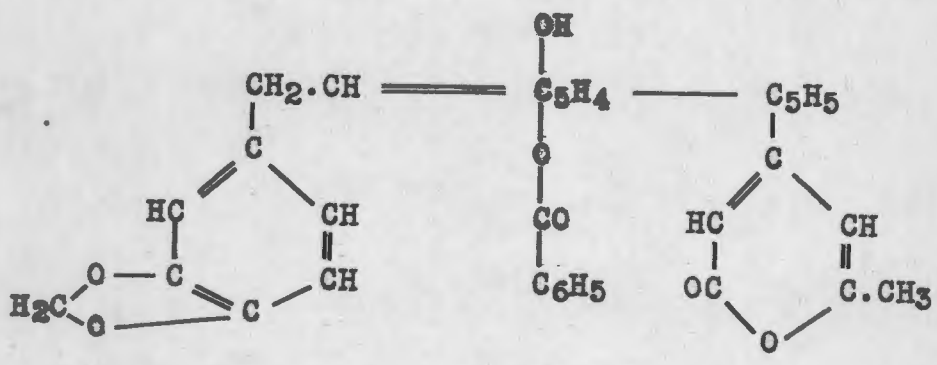
The precipitation tests for alkaloids with picric acid, phosphotungstic acid, Iodine in pot. iod., tannic acid, and Mayer's reagent were all negative.

Tests for strychnine with chromic acid and nitric acid were also negative.

Ferric chloride produced black precipitates of iron tannate with practically all preparations.

Kamerman (11) isolated from several cotyledons a toxic principle whose structural formula relates it to the picrotoxin group; strangely enough he did not find this substance in C.Reticulata which was found to be active in this work. He describes the neurotoxic principle as an amorphous white solid, thermolabile in oxygen, soluble in ether, water, 96% alcohol, insoluble in chloroform. The M.L.D. for guineapigs is about 3 mg./kgm.; subcutaneous injection in goats produces Krimpsiekte.

The formula for Cotyledontoxin is (Kamerman) -



He attributes the toxic action to the methylene dioxybenzene nucleus and a slight local anaesthetic action to the benzoyl group; there is also an α-pyrone group.

CONCLUSIONS.

The members of N.O. Crassulaceae are generally nontoxic; and of the few which have been shown to be active the greater number are not constantly potent. The activity of the cotyledons is very variable, the same species being active at one time yet not at another. Potency depends upon such factors as geographical origin, seasonal variations during the plant development, etc. This has been fully demonstrated by Steyn and others by feeding experiments, and by hypodermic injection in estimating the M.L.D. of different plants; the pharmacological data obtained in the course of this work corroborates these findings.

The method of preparation of extracts is important, e.g. the concentration of alcohol used, degree of division of original plant.

Some dried preparations and potent alcoholic extracts remained active after 2 years.

Cotyledon Wallichii, C.Reticulata, C.Paniculata, C.Orbiculata, C.Decussata, C.Ventricosa are the species which may contain active principles in sufficient amount to be of practical importance. C.Reticulata has been shown to have marked activity though generally considered to be inactive (11) (16) (13). In the case of some plants the leaves may show little activity though an investigation of the stems will demonstrate a high degree of potency; different parts of one plant have been shown to vary in their activity, and e.g. C. Reticulata, actually to contain different principles.

With 96% alcohol extracts prepared as described at the beginning of this work, Crassula Canescens, Crassula Ericoides, Crassula Lycopodoides, Crassula Portulacea, Cotyledon Mammilaris, Cotyledon Glutinosa, were found to be inactive as tested out on frogs, guineapigs and on the mammalian heart in situ.

The toxic plants contain substances, probably glucosidal in nature, which have an action on the heart resembling digitalis and other actions which too are similar to those produced by digitalis glucosides. They may also contain a neurotoxic principle which is a convulsant poison.

C.Wallichii and the peduncles of C.Reticulata belong typically to the first group, as also, to a similar or lesser extent C.Paniculata, C.Orbiculata and C.Ventricosa, while extracts of C.Reticulata stems exert their chief action on the nervous system, stimulating motor areas and the medulla.

Many of the plants no doubt contain principles with both actions (cardiotonic and neurotoxic) but generally the action on the heart predominates and the nervous principle is too small in amount to produce manifestations. Extraction by refined chemical procedures will no doubt demonstrate the presence of cotyledontoxin (Kamerman) in a greater number of varieties of cotyledon than can be demonstrated by the ordinary methods of extraction.

An increasing number of plants are being included in the "Digitalis series" to which the active cotyledons most appropriately belong; the presence of the neurotoxic "cotyledontoxin" demonstrated by the convulsant effects of C.Reticulata stems in guineapigs and by the isolation of the principle from several others by Kamerman (11), places some of the plants in that group of convulsant poisons of which Picrotoxin is a type. The presence of digitalis glucosides, saponin, and substances with action like picrotoxin has been observed in plants belonging to other botanical groups.

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The Action of Atebrin on the Uterus

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THE ACTION OF ATEBRIN ON THE UTERUS

By Norman Sapeika

(From the Department of Pharmacology, University of Cape Town)

Received 16th January, 1934

Quinine has for long been used to stimulate the movements of the uterus, especially when labour pains are imminent or have already commenced. Its use in the treatment of malaria in late pregnancy would therefore seem to be not entirely free from risk. With plasmoquine the action on the uterus is less marked. Epstein,[1] from experiments on animals, concluded that therapeutic doses had probably no action on the human uterus, a conclusion borne out by the clinical use of the drug. As atebrin (an alkylaminoacridine derivative) has recently been introduced for its action on the gametocytes and schizonts of the malarial parasite, it would seem a point of practical importance to determine its action on the uterus to see if it might have any advantage over quinine in the treatment of malaria in the later stages of pregnancy. The author has not been able to find any reference to previous experiments with acridine derivatives on the uterus.

Method

The isolated uterus of rat, guinea-pig, rabbit and cat was investigated by the method of Magnus, by suspension in a bath of warm, oxygenated Locke's solution. Various concentrations of atebrin in Locke's solution were added to the bath and the effects recorded in the usual way. The uterus was also investigated *in situ*; cats were first anaesthetised with paraldehyde administered per stomach tube and a uterine cornu brought out through a low mid-abdominal incision, movements being recorded by Gunn's method.[2] The blood-pressure was recorded from the carotid artery, and the whole animal, except head and neck, kept immersed in a saline bath kept at 37° C. As far as possible pregnant and non-pregnant uteri were investigated for each animal.

Results

In the case of the cat, rabbit and guinea-pig, isolated strips from the virgin, non-pregnant, and pregnant uterus usually showed an increase in tone or the onset of more frequent contractions; this was obtained with concentrations of 1 in 200,000, less frequently with 1 in 500,000. With stronger concentrations, e.g. 1 in 20,000, there is inhibition of all movements with little tendency to relaxation and little or no response to big doses of atropine, pilocarpine or barium chloride.

The non-pregnant rat's uterus showed no response to concentration lower than 1 in 200,000; above this concentration, usually with 1 in 100,000, the result was inhibition of normal

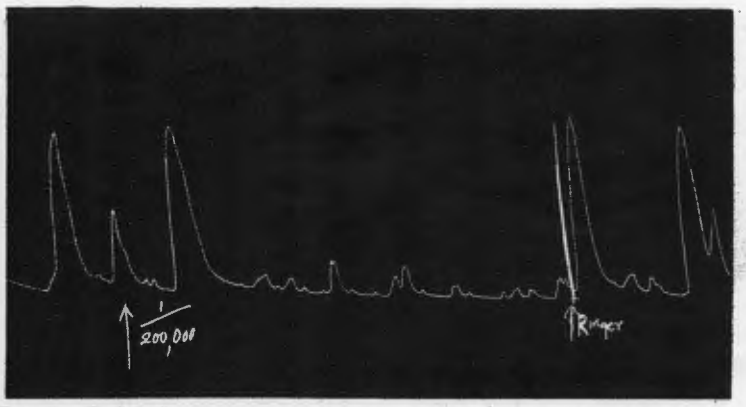

Fig. 1. Isolated uterus of rat: showing inhibition of movements with 1 in 200,000 atebrin.

rhythmical contractions in 15 out of 24 experiments, never increased tone or contractions.

The cat's uterus *in situ* showed a steplike rise in contraction following injections of 1 mgm. per kgm. of atebrin; with larger doses, e.g. 4 mgm. per kgm., similar results were obtained, but

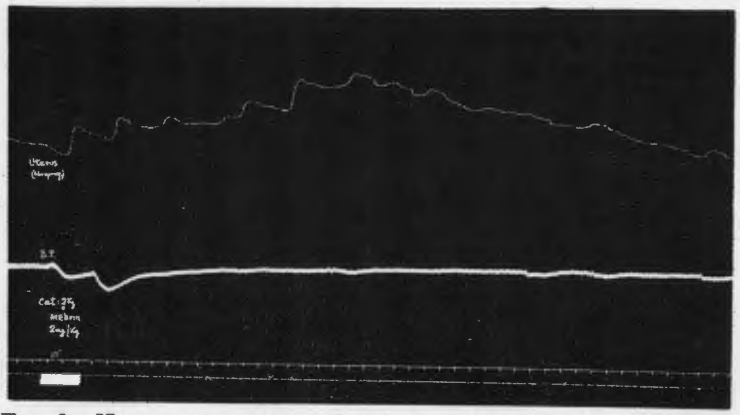

FIG. 2. Non-pregnant uterus of cat *in situ*: showing steplike rise in contraction and fall of blood-pressure, following injection of atebrin (2 mgm. per kgm.)

the drug had to be given slowly and diluted to obviate the fall in blood-pressure; the fall in blood-pressure may be 5 to 10 mm. of mercury with the smaller dosage. The rise in uterine tone came back to normal within a few minutes.

RESULTS

The uterus is stained yellow. When inhibited by atebrin subsequent administration of atropine, adrenaline, pilocarpine and barium chloride have little, if any, effect. After the addition of atebrin the muscle responses are less to subsequent doses.

The non-pregnant and pregnant uterus of the cat have shown increased tonus with doses of 1 mgm. per kgm., a dose corresponding to about 0·1 gm. for a woman weighing 70 kgm. 0·1 gm. is the single dose for atebrin. However, these results follow intravenous injection where the concentration is higher than when the drug is absorbed from the alimentary tract. No atebrin preparation has as yet been recommended for intravenous therapy. Reports from clinicians, May,[3] Hoops[4] and others, have also indicated that atebrin is safe for use in women.

CONCLUSIONS

From these experiments atebrin appears to stimulate the uterus in weak concentrations, e.g. 1 in 400,000, and depress uterine action with 1 in 40,000 or stronger solutions. The rat's uterus, however, appears to be inhibited. Attention has also been drawn by Freund, Guggenheim and others to the anomalous response of the rat's uterus to another drug, namely, histamine.

SUMMARY

1. Atebrin stimulates the uteri of guinea-pig, rabbit and cat in weak concentration and inhibits the isolated strips in strong concentration. The uterus of the rat is inhibited. The action is apparently directly on the muscle.

2. In antimalarial therapy, atebrin is probably safe for women, as evidenced from experimental results and clinical trials.

My thanks are due to Prof. J. W. C. Gunn for helpful criticism and to Mr. J. W. Bates for technical assistance.

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The Action of Acriflavine on the Uterus

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Pharmacodynamie et de Thérapie

VOLUME XLIX, FASCICULE I

EXTRAIT

THE RESPONSES OF THE BATRACHIAN ALIMENTARY CANAL TO AUTONOMIC DRUGS : XENOPUS LAEVIS (South African Clawed Toad) TO ACETYL CHOLINE

THE ACTION OF ACRIFLAVINE ON THE UTERUS

By Norman Sapeika

(From the Department of Pharmacology, University of Cape Town)

Received 16th January, 1934

An investigation into the action of atebrin (an alkylamino-acridine derivative) on the uterus showed this drug to have a stimulant action in weak concentrations and a depressant one with stronger doses. This raised the question as to whether other acridine dyes had this property and a further series of experiments have been done to determine this point, especially as acriflavine is sometimes given intravenously.

Method

Isolated strips of uterus from rat, guinea-pig and cat were set up in a bath containing oxygenated Locke's solution kept at 37° C., the muscle movements being recorded in the usual way.

The uterus was also investigated *in situ* in the cat; paraldehyde 1·5 mils per kgm. being administered per stomach tube, a cornu of the uterus was attached to Gunn's uterine holder through a low mid-abdominal incision and the lower part of the animal kept immersed in a warm saline bath. The blood-pressure was recorded from the carotid artery, and the drug administered in saline through the jugular vein.

Results

Acriflavine was used as such, though acid in reaction, since the dye is so used clinically; the neutral dye was also used.

Isolated segments of uterus from rat, pregnant and non-pregnant guinea-pig and cat showed practically no response to concentrations of the dye varying from 1 in 400,000 to 1 in 20,000; the uterus of the cat did occasionally show increased contractions or rise in tone.

The uterus *in situ* showed increased movements with rise in tone in both pregnant and non-pregnant cats, acriflavine being more potent than neutral trypaflavine, and producing effects with doses of 2 mgm. per kgm.; a slight rise in blood-pressure usually followed injection of the drug.

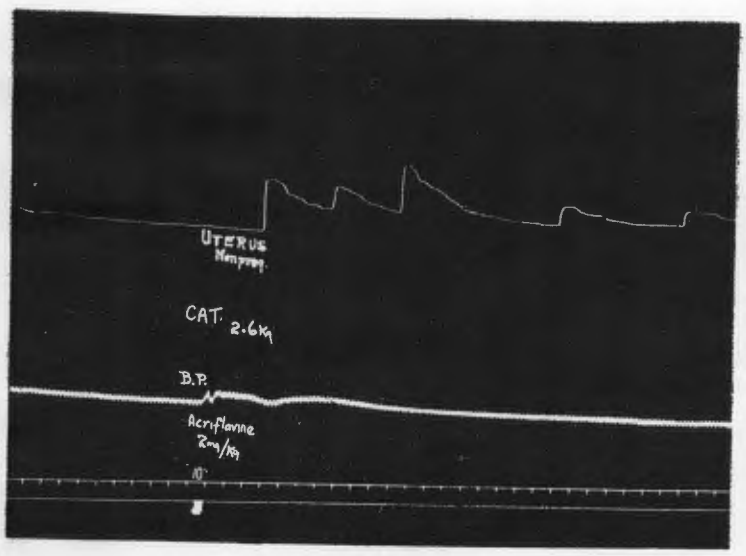

Fig. 1. Non-pregnant uterus of cat *in situ*: showing steplike contractions with increased tone, and slight rise in blood-pressure after acriflavine injection (2 mgm. per kgm.)

Acriflavine has a stimulant action on the cat's uterus, and the use of this drug by intravenous route would therefore appear to be contraindicated in pregnant females.

Summary

Acriflavine increases the movements of the uterus with doses of 2 mgm. per kgm. in the intact cat; the action is directly on the muscle. This fact should be borne in mind where the dye is being administered intravenously to pregnant females.

FROM THE DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF CAPE TOWN

THE RESPONSES OF THE BATRACHIAN ALIMENTARY CANAL TO AUTONOMIC DRUGS: XENOPUS LAEVIS (South African Clawed Toad) TO ACETYL CHOLINE

BY

NORMAN SAPEIKA

(Received for publication 3-9-1934.*)*

The actions of autonomic drugs on the alimentary tract of Xenopus laevis, Rana fuscigula, and Bufo regularis, have been extensively investigated in this laboratory by the late DAVID EPSTEIN. Certain anomalies in his findings, e.g. a negative response or relaxation produced by pilocarpine on the frog's ileum (1) and relaxation of the intestine of Rana with arecoline (2) led him to conclude that pilocarpine in big doses directly depresses the muscular tissue of the thin-walled ileum and arecoline acts on an „ inhibitory " parasympathetic mechanism, since atropine antagonised the relaxation of the small intestine produced by it. Arecoline, however, was found to cause contraction of every portion of the alimentary canal of Xenopus laevis (3). He also showed that physostigmine is without effect on the parasympathetic system of Xenopus (1) but depresses the muscle with large doses.

The action of choline and its derivatives have not been fully investigated. In view of the above findings it seemed of interest to investigate the responses of the frog's alimentary canal to acetyl choline; in particular because it is generally accepted that this ester is the chemical agent which transmits the impulses from parasympathetic and other nerves to muscles.

EPSTEIN (4) showed that choline chloride produces a marked rise in tone in the case of the rectum of Xenopus.

EXPERIMENTAL METHOD

Isolated segments of various portions of the alimentary canal of a pithed frog (X. laevis) were suspended in a bath containing 50 c.c. of Howell-Ringer solution (0.7 % NaCl, 0.03 % KCl, 0.026 % $CaCl_2$) through which air bubbled at constant rate; the movements of these tubular segments were recorded by lightly balanced levers attached by threads to the ends of the tissue, and giving a suitable magnification.

When the temperature of the baths was 14-16° C., the movements of the tissues were sluggish. At a temperature of 22-25° C., initial automatic contractions were more evident, and responses to drugs were more marked and rapid in onset.

Acetyl choline bromide, prepared by The British Drug Houses was used in these experiments.

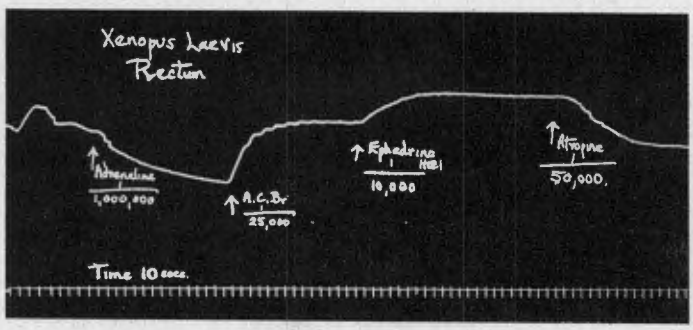

FIG. 2

Isolated Rectum of Xenopus.
Adrenaline (1 : 1.000.000) causes relaxation.
Acetyl choline bromide (1 : 25.000) produces immediate rise in tone, increased by Ephedrine (1 : 10.000).
Atropine (1 : 50.000) causes relaxation.

Experimental Results

The oesophagus, stomach, duodenum, jejunum, and rectum showed an immediate rise in tone on adding acetyl choline bromide in concentrations of 1 in 50,000 or even 1 in 100,000 in many cases, often with the inception of rhythmical waves of contraction where there had been none prior to addition of the drug. Atropine added subsequently produced relaxation, which could be overcome by further addition of acetyl choline. Atropine added before acetyl choline bromide had little effect on the tissue, and the ester might still produce a rise in tone.

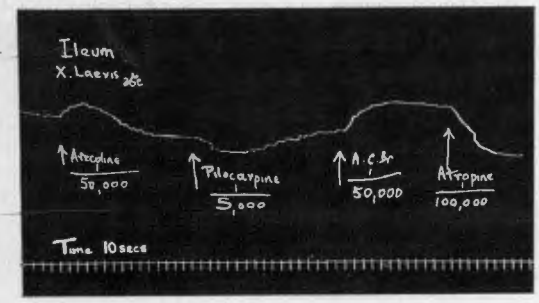

Fig. 1

Isolated Ileum of Xenopus.
Arecoline (1 : 50.000) causes contraction.
Pilocarpine (1 : 5.000) causes relaxation.
Acetyl choline bromide (1 : 50.000) produces rise in tone.
Atropine (1 : 1000.000) causes relaxation.

Acetyl choline 1 in 100,000 or in bigger concentrations produced similar effects on the ileum, even when pilocarpine in large doses had no effect or produced relaxation.

The ester was found to be more active than pilocarpine or arecoline.

Physostigmine had no effect on any portion of the alimentary canal before or after the addition of acetyl choline. This is difficult to explain since physostigmine is regarded as inhibiting the esterase destruction of acetyl choline [Loewi & Navratil, 1926 (6)].

Ephedrine and Acetyl Choline

Ephedrine did not affect the tone or rhythm of the segment either before or after the addition of acetyl choline. Adrenaline produced relaxation even in dilutions of 1 in 1,000,000, and ephedrine as already shown by Epstein (5) reversed this effect if given in concentrations about 1,000 times as great. If relaxation of the tissue were first produced with adrenaline, the addition of acetyl choline produced a rise in tone which was still further increased when ephedrine was added.

Summary

Acetyl choline produces contraction of every portion of the alimentary tract of Xenopus Laevis, atropine antagonising the effect.

The whole alimentary tract of Xenopus therefore has a motor parasympathetic mechanism as already indicated by the experiments with arecoline (3).

Acetyl choline is more active than arecoline or pilocarpine.

Physostigmine produced no effect even in the presence of acetyl choline.

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EXTRAIT

THE TOXICITY OF CERTAIN ALCOHOLS TO XENOPUS LAEVIS

FROM THE DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF CAPE TOWN

THE TOXICITY OF CERTAIN ALCOHOLS TO XENOPUS LAEVIS

BY

NORMAN SAPEIKA

(Received for publication 7-5-1935.)

RICHARDSON in 1869 stated that the toxicity of alcohols increases with their molecular weights, and this observation has been confirmed since by many others.

Some of the recorded results are shown in Table I.

TABLE I

Alcohol	Toxicity			
	Fish (Picaud)	Cat (Macht)	Isolated heart (Kuno)	Isolated heart (Hemmedter)
Methyl	1.0	1.0	1.0	1.0
Ethyl	1.5	1.2	1.3	0.9
Propyl	3.0	2.95	4.4	4.2
Butyl	4.5	19.7	10.0	8.5
Amyl	15.0	39.0	39.0	17.0

In the series of experiments to be recorded here the toxicity of several monatomic alcohols has been determined on Xenopus Laevis (the South Africain clawed toad), to see if any simple relationship could be established between chemical or physical differences in the alcohols and variation in their toxicity.

METHOD

For each alcohol from 20 to 24 frogs were used to find the minimal lethal dose. Doses of the alcohols were calculated in mils per gramme of body weight. The dose was injected through the floor of the mouth into the ventral lymphsac. If symptoms of excessive irritation were seen, the alcohol was diluted with normal saline solution, but the total injection never exceeded 1 ~~mgr.~~ ml.

Results

The effects, except in the case of allyl alcohol, were depression and paralysis, the fore limbs becoming irresponsive to stimuli before the hind limbs. After allyl alcohol the animals were at first hyper-excitable to stimuli. Regurgitation of the stomach contents was also frequent.

The minimal lethal doses are shown in Table II.

Table II

Alcohol	M.L.D. mgs. per gr.	Relative toxicity
Methyl	0.075	1
Ethyl	0.045	1.66
(Iso)propyl	0.025	3
Butyl (secondary)	0.015	5
Butyl (tertiary)	0.015	5
Amyl		
1. Iso-α-ethyl-propyl	0.005	15
2. α-methyl-butyl	0.0045	16.66
3. β-methyl-butyl	0.003	25
4. Normal	0.0025	30
Caprylic	0.0015	50
Allyl	0.00006	1250

Benzyl alcohol, as an example of a phenolic alcohol, has a value of 30 (M.L.D. 0.0025 mgr. per gr.); ether and acetone have values of 3 (0.025 mgr. per gr.) and 2.5 (0.03 mgr. per gr.) respectively.

Comment

There is no apparent relationship between the chemical structure of the alcohols and their toxic action. Though the toxicity increases with the number of C-atoms in the molecule, allyl alcohol is exceptional in this respect. The unsaturated nature of this compound may account for its differences from the other alcohols.

The increasing activity with the increase in molecular weight may be connected with physical properties, e.g. solubility, volatility. Allyl alcohol is exceptional in being soluble in water in all proportions, whereas the solubility of the others shows no definite relationship to the increasing toxic action.

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The Assay of Strophanthus

Mortality Curve for Xenopus Laevis

By
J. W. C. GUNN and NORMAN SAPEIKA

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THE ASSAY OF STROPHANTHUS

Mortality Curve for Xenopus Laevis

By J. W. C. Gunn and Norman Sapeika

(From the Department of Pharmacology, University of Cape Town)

Received 23rd November, 1934

In 1921 it was shown[1] that *Xenopus laevis*, commonly known as the South African clawed toad or frog, is not tolerant to the digitalis series like *Bufo* but may replace *Rana* in the biological assay of these drugs. In 1932 Gunn and Epstein[2] described the mortality curve of *Xenopus* for digitalis and compared it with Trevan's curve[3] for *Rana temporaria*. In a personal communication, C. W. Chapman drew our attention to the close similarity between the mortality curve of *Xenopus* and that obtained by Chapman and Morrell[4] for *Rana pipiens*. Since both these curves differ from that used in the official biological test, and since *Xenopus laevis* is the usual test animal in South Africa, it is important that the mortality curve should be accurately determined for it. For these reasons a further series of experiments has been done.

Experimental

Method

The method used was the usual one of determining the overnight mortality rate. Frogs, obtained in batches of from 150 to 360, were kept in the shade in a tank with an ample supply of water for six to fourteen days before being injected. On the day of the experiment they were taken into the laboratory, the skin was dried with a towel, the urine was expressed, and the animals were weighed.

Strophanthin, obtained from Merck's, was dissolved in absolute alcohol to form a stock solution of 0·1 per cent. Before injection, part of this was further diluted with distilled water, to produce a solution of strophanthin of 1 in 50,000 or 1 in 100,000. The dilution was such that the calculated dose of strophanthin could be given in 0·3 to 0·6 mils of solution. The doses were calculated and the required amount of fluid was drawn into a 1 mil syringe graduated to 0·01 mil, and injected through the floor of the mouth into the ventral lymph sac.

On injection, groups which had the same body weight dose were put in cages which were placed in a white porcelain bath through which water to the depth of 1 to 2 cm. was allowed to run gently during the night. The bath can hold 12 cages each capable of accommodating 20 frogs. Temperature changes of the water during the night were recorded by means of a simple device. A test tube was drawn out at the open end so that a narrow neck was formed. This was connected by a rubber tube to a tambour writing on a slowly rotating drum. The glass bulb containing air was immersed in the water and the temperature changes were shown by the movements of the tambour levers, which can be easily calibrated. The fall of temperature never exceeded 3° C.

The animals were observed sixteen hours after injection and the mortality rate noted. For each consignment of frogs a preliminary test was made on 10 animals, and repeated if necessary, to estimate approximately the average fatal dose. Five or more groups of 20 or 30 frogs were then injected with different doses of strophanthin, one being just above and one just below the expected average fatal dose. On the following day the mortality rate of each group was noted and the results of the experiment plotted on graph paper. If no group had a mortality rate of exactly 50 per cent., the average fatal dose was calculated from the two doses which produced mortalities nearest to 50 per cent. above and below. The following points were noted: actual doses given, mortality, number of frogs in each group, and the actual or estimated average fatal dose. These experiments were repeated, on each day doses being given both to determine the average fatal dose and to try to get mortalities which had not been obtained in previous experiments, so that information on all parts of the mortality curve for *Xenopus* could be obtained. In all, 1180 frogs were used.

Results

Average Fatal Dose

The average fatal dose of Merck's strophanthin was found to be $0{\cdot}00025 \pm 0{\cdot}00002$ mgm. per gm. For any one consignment of frogs there was little variation from day to day. Variations may be due to slight diurnal differences in sensitivity or possibly to slight errors in the dilution of the stock solution. This latter experimental error was diminished as far as possible by always using the same pipettes and burettes in preparing the dilutions. In different consignments there was a difference of sensitivity and we can confirm the observation of Chapman and Morrell that this difference may persist for some months.

In most cases the variation was not great, but the extremes were 0·00022 mgm. and 0·00029 mgm. The latter figure was seen in a consignment received in April, the autumn in Cape Town. We have not, however, done sufficient estimations on batches received throughout the year to be able to say that *Xenopus* is most tolerant to these drugs in April or that the maximal and minimal doses found by us are the limits of the average fatal dose.

The Mortality Curve

When the data from any one day's experiments were sufficient for plotting a mortality curve, the ratio of mortalities 0, 50 per cent., and 100 per cent., was approximately 3, 4, 5. Individual curves varied only slightly from this. An attempt was made to obtain an average curve by plotting all the curves on a graph in which the abscissa was represented by actual units (0·00001 mgm. of strophanthin per gm. of body weight), and then superimposing the curves. A curve showing the above mortalities resulting from doses varying as 3 : 4 : 5 becomes less steep as the average dose increases. With average fatal doses of 0·00022 mgm. and 0·00028 mgm., the curves would occupy 11 and 14 divisions of the abscissa respectively. If superimposed, they do not coincide, and a mean of the two curves would be correct only for an average fatal dose of 0·00025 mgm. per gm.

It was thought more accurate, therefore, to obtain the curve in the following way. In each experiment the actual dose which was found or calculated to produce a 50 per cent. mortality was given the arbitrary value of 4, as used by Trevan. The value of the abscissa for a dose producing another mortality was then calculated as the proportion of 4 which the actual dose bore to the average fatal dose. These figures were noted and the number of frogs injected with each particular dose. When, as was usual, the same mortality rate was seen in frogs from several experiments an average was taken. In three experiments, for instance, mortalities of 30 per cent. were seen. The number of frogs and the abscissae (as calculated) were 30, 30, 20, and 3·68, 3·72 and 3·86 respectively. This gives an average for 80 frogs of 3·74 (± ·07). This has been calculated for the different mortalities, and is shown in Table I.

These points have been plotted and are shown by the continuous line, A, in Fig. 1. For comparison Trevan's curve for *Rana* is shown by the interrupted line B.

The mortality curve for *Xenopus* to strophanthin, like that already published for digitalis,[2] is considerably steeper than that described by Trevan for *Rana temporaria*. In this respect it agrees closely with Chapman and Morrell's curve for *Rana*

TABLE I

Mortality per cent.	Abscissa	No. of frogs	Mortality per cent.	Abscissa	No. of frogs
3·3	3·1	30	55	4·05	60
5	3·1	40	60	4·12	80
10	3·4	70	66	4·20	30
15	3·51	40	70	4·20	50
20	3·68	30	75	4·28	80
25	3·70	40	80	4·42	30
30	3·74	80	85	4·50	60
40	3·90	60	90	4·62	60
45	3·93	80	95	4·80	40
50	4·00	110	—	—	—

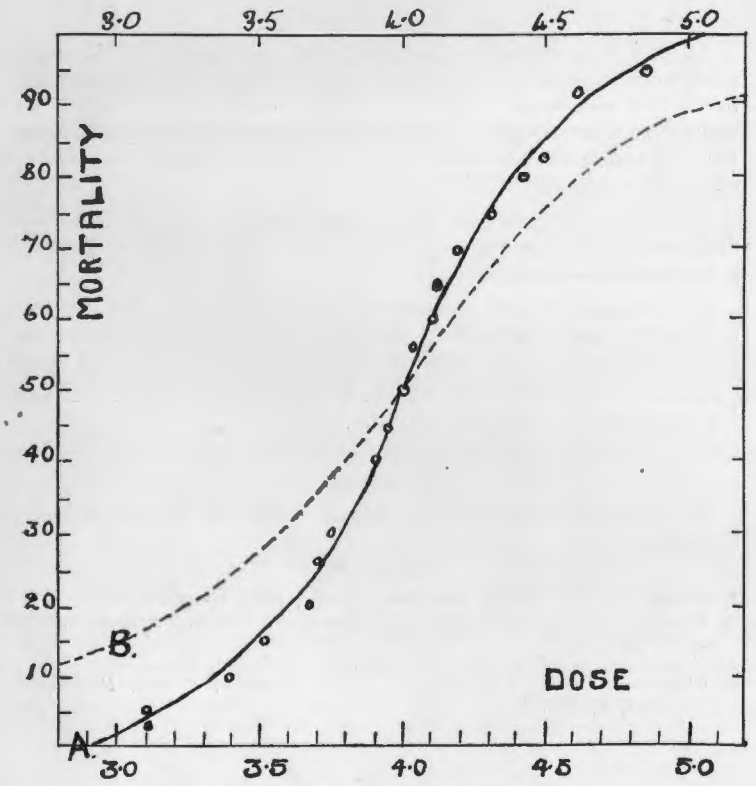

Fig. 1.—A. Curve for *Xenopus laevis*.
B. Trevan's curve for *Rana temporaria*.

pipiens.[4] From the plotted curve and from the experiments in which too small or too large a dose was given, it would seem that the ordinary limits of mortality curve from 0 to 100 per cent. are from about 3·9 to about 5·1 on the abscissa.

The Use of the Curve in Assays

We have used this curve in the assay of solutions of strophanthin of known dilution (made from the stock solution) and the results agreed closely. Known dilutions of 91 per cent., 80 per cent., and 60 per cent., gave by assay readings of 89 per cent., 81 per cent. and 56 per cent. respectively. We received two specimens of digitalis tested in Ottawa of which 0·85 gm. and 1·02 gm. were said to be equivalent to 1 gm. of International digitalis powder. Tested on *Xenopus* 0·87 gm. and 1·00 gm. were found equivalent to 1 gm. of International standard powder.

Conclusions

In the experiments described the specimens of *Xenopus* used were from 20 to 32 gm. in weight. Males and females were used, but not large females distended with eggs, in which the weight is over 40 gm. The animals were received in different batches and kept separate. Under these conditions the mortality curve is as described.

If several batches are mixed and there is any considerable difference in the average mortality rate in the different batches, a flatter curve would result.

In animals which recovered from injection, the average mortality dose returned to its previous level in three weeks. Frogs used after that time give similar results to fresh frogs.

We are indebted to Dr. C. W. Chapman and Dr. J. H. Burn for specimens of assayed digitalis.

Summary

A mortality curve for *Xenopus laevis* to strophanthin is described.

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