separated. To remove this material and obtain any additional material which might not have separated, the extract was washed several times with petroleum ether. This removed the oily substance.

The concentrated alcoholic extract was slowly poured into a 1 per cent hydrochloric acid solution which had been cooled to 10°. The precipitate thus obtained was washed three times by decantation, placed on a force filter and sucked dry, finally washed well with cold water. The product was allowed to dry at room temperature. It was noted that the dried product prepared in this manner did not agglutinate when allowed to stand in a warm room. Inasmuch as fatty material as well as the resin is precipitated when the concentrated alcoholic extract is poured into acidulated water, it seems desirable to remove the fat previous to the precipitation of the resin. The resin thus prepared answered all the requirements of the U. S. P. XI, and of the several batches prepared at various times, not once did coalescence or agglutination appear.

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(2) J. U. Lloyd (*Am. J. Pharm.*, 62, pages 242, 386, 605) has pointed out that the substance obtained by Hodgson (*Am. J. Pharm.*, 3, page 273) from the dried root by the use of hot water, cannot be the same substance that is now obtained by the extraction with alcohol. He gives King the credit for being the first to obtain and recognize the true resin in 1835. (See King's letter to Lloyd, above reference.) Power (PROC. A. PH. A., 25, 420 (1877)) decided from his own researches that the substance obtained by Hodgson could have been none other than the resin as we now know it. He considers the inactivity of Hodgson's product to be due to the observed effect of alkalies upon the resin in diminishing the activity. Abenthy (*Am. J. Pharm.*, 33, 299 (1861)) followed in detail the method of Hodgson, and showed that the product contained a certain amount of the active property of the drug.

(3) *Am. J. Pharm.*, 46, 231 (1874).

PHARMACODYNAMICS OF THE CARDIOACTIVE PRINCIPLES OF URGINEA MARITIMA (SQUILL).*

BY DAVID ROBERT CLIMENKO, M.D., PH.D.

Squill (Urginea maritima) has played a peculiar rôle in the therapeutic armamentarium. The ancients regarded it as one of their most thoroughly understood drugs and accorded it a high place among their therapeutic agents. It was familiar to the Egyptian, the Arabian and the Greek schools of medicine, and descriptions of it are to be found in the Ebers papyrus, in the works of Epimenedes, Dioscorides, Hippocrates and Galen. The drug did not survive the classical period and fell into complete desuetude during the middle ages. But it is not the purpose of this study to discuss the history of the use of this drug, accounts of which may be found in the works of Joz (1), Chamberlin and Levy (2) and Scheer and Sigerist (3).

It is not difficult to account for the almost complete abandonment of this once highly respected drug. The crude substance contains an extremely variable concentration of therapeutically active principle together with a mass of highly irritating resinous substances. The presence of these irritants makes it almost impossible to administer a therapeutically effective dose without inducing nausea, diarrhoea and irritation of the upper respiratory tract. The isolation of the cardioactive principles from squill started with the pioneer work of Vogel (4) who, in 1812, described "scillitine" and Thomson (5) who described "scillitite" in 1831. It was

* From the Pharmacological Laboratory, Calco Chemical Company, Inc., Bound Brook, N. J.

not until Stoll (6) isolated scillaren and Dyas (7) described a method for the extraction of crystalline "Urginin A" and amorphous "Urginin B"[1] from the crude drug that a practical method was made available for the isolation and preparation of the cardioactive principles of squill.

It is with the pharmacodynamic action of a mixture of Urginin A and Urginin B that this study is primarily concerned, and the work reported here has been carried out on a preparation known as Urginin.[2]

Urginin was obtained in the form of compressed lactose tablets containing 0.5 mg. of the active principle in each tablet. For intravenous administration, for assay purposes or for perfusion purposes, the active principle was prepared in the following manner. Three hundred tablets were weighed in batches of twenty in order to establish the average tablet weight. The tablets were then ground in a mortar until all of the powdered material passed through a 60-mesh sieve. Powder equivalent in weight to 40 tablets (20 mg. Urginin) was placed in a glass-stoppered Erlenmeyer flask to which 40 cc. of alcohol and 20 cc. of glycerine were added. After agitation in a mechanical shaker for about twelve hours the contents of the flask was transferred to a 1000-cc. volumetric flask and made up to volume with normal saline solution. This suspension was well shaken and centrifuged. The supernatant liquid was decanted and the lactose residue discarded.[3] This slightly opalescent supernatant fluid which contained 0.002% of active principle formed the basis for all solutions used in experimental work or in assaying the drug. Fresh solutions were always employed and watery solutions were always discarded after twelve hours.

The other drugs used in this study were official samples of ouabain obtained from the Bureau of Food and Drugs of the U. S. Department of Agriculture, a preparation of the active principles of Digitalis lanta (digoxin, Burroughs Wellcome), a sample of digitoxin (Merck) and a Tincture of Digitalis (Lilly). All solutions for experimental administration were made up to contain the same percentage of alcohol and glycerine as the final Urginin solution.

All assays were carried out on cats, the rate of injection being adjusted so that death occurred from 55 to 65 minutes after the initiation of the intravenous administration. Ten animals were used in each assay.

Uniformity and Stability.—One of the most desirable features of a cardiac glucoside for therapeutic use is uniformity of potency. In actual practice, all samples of cardiac glucosides designed for therapeutic purposes are subjected to

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[1] Originally known as "Scillonin A" and "Scillonin B."

[2] Urginin is described in N. N. R. as a mixture of two water-insoluble glucosides, derived from squill, in the proportions in which they exist in the drug; namely, about equal parts. The drug was supplied by the Calco Chemical Co., Inc., Bound Brook, N. J.

[3] The completeness of this extraction process was once questioned. To clear up this point, the residue from 200 Gm. of tablet material was obtained after following the procedure outlined above. This residue was transferred to a Soxhlet and extracted with ethyl acetate for twelve hours. The ethyl acetate was removed by evaporation in a current of warm air and the extracted material taken up in 5 cc. of absolute alcohol and glycerine. This alcoholic solution was made up to 200 cc. with normal saline and examined for the presence of pharmacologically active substances on the isolated turtle heart, with a resultant demonstration of the presence of traces of a cardioactive principle. The amount, however, was so small that it was not amenable to mensuration by the cat method.

bio-assay and those which fail to fall within certain arbitrary limits are either discarded or adjusted so that they will meet these requirements. During the course of the last two years, thirty-nine samples of Urginin have been assayed by the Hatcher cat method. The results of these assays are compared with a series of twenty-eight assays carried out by the same method on official samples of ouabain.

TABLE I.

Drug.	Number of Assays.	C. U. per Mg.	Lower Limit.	Upper Limit.	Standard Deviation.	Probable Error of the Mean.
Urginin	39	4.16	3.47	4.83	±0.2	±0.026
Ouabain	28	9.48	8.47	11.11	0.32	0.035

This uniformity of potency is even more striking when it is realized that the above comparison is made between an extended series of manufacturer's batches, and a number of official ouabain samples with assays carried out during every month of the year. It should be pointed out that the assay value for ouabain falls slightly below the value usually given.

A second requirement, almost as important as uniformity, is stability. None of the cardiac glucosides are stable substances, but the degree of instability varies greatly, particularly with regard to the conditions under which the glucosides are kept. The cardioactive principles of urginin are very susceptible to hydrolytic change, and aqueous suspensions are very unstable. The lactose tablets, or anhydrous alcoholic solutions, however, are stable. The following table shows the results of a series of assays made on three samples of the drug at intervals of six months. The tablets were kept in ordinary glass containers fitted with screw tops. No attempt was made to exclude air.

TABLE II.

Sample No.	Date Assayed.	C. U. per Mg.	Standard Deviation.	Probable Error of the Mean.
1117	7/2/36	4.22	±0.092	±0.011
(Tablets)	12/8/36	4.14	0.032	0.007
	6/5/37	4.02	0.026	0.007
	12/2/37	4.12	0.045	0.010
1101	2/20/36	4.01	0.024	0.005
(Solution)	9/5/36	3.85	0.029	0.006
	3/11/37	3.80	0.065	0.014
1111	2/5/36	4.38	0.026	0.0071
(Tablets)	8/14/36	4.10	0.053	0.012
	3/12/37	4.18	0.065	0.015
	7/14/38	4.14	0.024	0.0053

Pharmacodynamic Action.—It has been pointed out by Cushing (8), by Clark (9), by Jenny (10) and by Mendel (11) that the general pharmacological action of the cardioactive principles of squill is fundamentally the same as that of digitalis. The end result of the slow intravenous administration of a fatal dose of the two substances to test animals is so similar that it is almost impossible to distinguish them from one another. These effects have been described in detail by Joz and Wallace and Van Dyke (13). The therapeutic effects in cardiac disease are similar, as has been shown by Markwalder (14), Massini (15), Carr and Mayer (16), Chamberlin and Levy, and Chauncey and Maher (12).

There are, however, a number of differences between the action of Digitalis and Urginin that are not brought out by a study of the effects of the slow intravenous administration of a fatal dose of the drug. The principal difference, both from a pharmacodynamic and therapeutic point of view is to be found in the cumulative effect of the two drugs.

Cumulation: The rate of excretion or inactivation of the cardioactive principles of squill is much more rapid than that of digitalis or strophanthus. Wallace and Van Dyke (13) have shown that the drug is only half as potent a cumulative poison as ouabain and less than one-third as potent as digitoxin when tested on dogs. Kwanichira-Okushima (17) has described similar results for the cat. Kingisepp (18), on the other hand, has described scillaren as having a much greater cumulative effect than digitoxin or adonis glucosides when tested on the frog.

This point has been investigated experimentally, using the principles laid down by Hatcher (19). Three samples of urginin, three samples of ouabain and four samples of digitoxin were assayed by the cat method. After establishing the potency of each preparation in terms of cat units, each of a large series of cats received an intravenous dose equivalent to 50% of the M. L. D. of the substance being tested. At intervals of 24, 48 and 72 hours after this initial administration, groups of animals were used for the assay of the drug. If all of the drug originally injected was still effectively present in the animal at the time of the assay, then it would be expected that 50% of the M. L. D. would be required to produce death. Therefore, the difference between the quantity of the drug required to produce systolic arrest of the heart and the expected 50% may be regarded as an index of the quantity of drug excreted or inactivated during the interval between the original administration and the subsequent assay. Thus, 72 hours after the administration of 50% of the M. L. D. of urginin, the effect of only 3.6% remains; after the same interval of time, the effect of 14% of ouabain and 28% of digitoxin remain. These facts are brought out in the following table.

TABLE III.

Drug.	Assay Value (C. U. per Mg.)	Per Cent Lethal Dose Administered.	No. of Animals.	Per Cent Lethal Dose to Produce Systolic Arrest after 24 Hours.	No. of Animals.	Per Cent Lethal Dose to Produce Systolic Arrest after 48 Hours.	No. of Animals.	Per Cent Lethal Dose to Produce Systolic Arrest after 72 Hours.	No. of Animals.
Urginin									
A	0.244	50%	15	69%	5	90%	5	96%	5
B	0.229	50%	15	83%	5	92%	5	97%	5
C	0.210	50%	15	77%	5	89%	5	94%	5
Ouabain									
D	0.109	50%	10	67%	4	78%	3	90%	3
E	0.106	50%	08	62%	2	80%	3	84%	3
F	0.110	50%	08	70%	3	84%	2	84%	3
Digitoxin									
G	0.301	50%	10	52%	4	55%	3	70%	3
H	0.314	50%	19	51%	3	57%	3	74%	3
I	0.308	50%	10	54%	3	54%	4	72%	3
J	0.310	50%	10	56%	3	59%	4	71%	3

Thus, at 72 hours, one might describe urginin as having one-fourth the cumulative effect of ouabain and one-seventh the cumulative effect of digitoxin. Unfortunately, different values are found for different time intervals. It would be very desirable to express this ratio in a quantitative manner. If the logarithm of the concentration of the drug remaining in the animal is plotted against time (see Fig. 1) it will be seen that an exponential relationship exists.

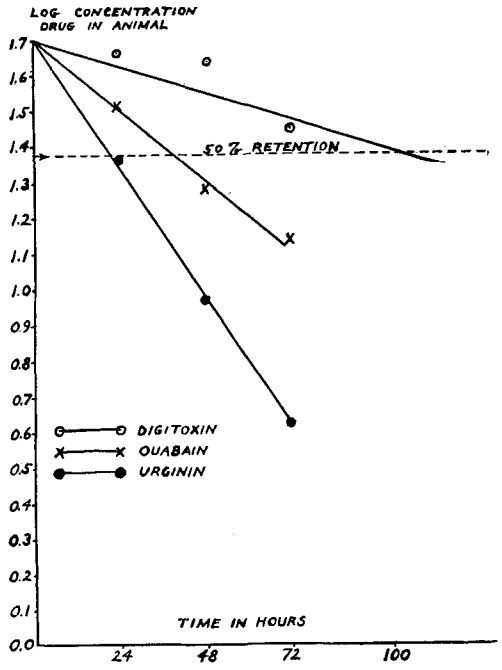

Fig. 1.

This relationship may be expressed in the following manner:

$$\log c = c_0 \left(\frac{1 - T}{B}\right)$$

Where c = concentration remaining in the animal
c_0 = log. of initial concentration
T = time
B = cumulative index.

In the three cases illustrated, the cumulative indices are:

Urginin	111
Ouabain	204
Digitoxin	595

A simpler method for quantitatively expressing the relative cumulative effects of these three drugs is to describe it in terms of time required for 50% of the amount of drug initially administered to disappear. Using this method, the following values are obtained for the relationship of the cumulative action of these three drugs:

Urginin	20
Ouabain	40
Digitoxin	100

Reversibility of Action.—Closely allied with the phenomenon of cumulation is the question of drug fixation and reversibility of action. Cumulation depends primarily on the rate of excretion or the rate of inactivation of a drug. This in turn is dependent on the degree of fixation which takes place between the drug and the physiological structure with which it is reacting. In the case of the cardioactive glucosides of strophanthus and digitalis, the myocardial fixation is very potent, much more so than is the case with the active principles of squill. This point has been demonstrated by Rothlin (20) and Graf (21), but Kingisepp (22) states that the reversibility of the squill reaction is about of the same order as ouabain.

If ouabain or digitoxin is applied to an isolated turtle heart until systolic arrest occurs it is practically impossible to reëstablish rhythmic contractions by washing with Ringer-Locke solution. On the other hand, systolic arrest resulting

from the application of the cardioactive principles of squill is readily reversible; the spontaneous rhythm may be reëstablished by washing with Ringer-Locke. Under these circumstances, subsequent applications of Urginin will produce systolic arrest in successively shorter periods of time and diminishing quantities of the drug will be required, while increasing periods of time will be required for Ringer-Locke washing to reëstablish spontaneous rhythm. These facts are brought out in the following table which shows the comparative effects of urginin, digoxin, digitoxin and ouabain on the isolated turtle heart.

TABLE IV.

Drug and Concentration.	Time Required to Produce Initial Systolic Arrest.	Time to Reëstablish Rhythm.	Time Required to Produce Second Systolic Arrest.	Time to Reëstablish Rhythm.	Time Required to Produce Third Systolic Arrest.	Time to Reëstablish Rhythm.
Urginin						
(1:100,000) a	21.00 min.	10.50 min.	12.00 min.	18.50 min.	7.50 min.	42.0 min.
b	20.25	12.25	11.50	24.00	1.50	...
c	22.50	10.30	14.25	15.50	6.75	46.0
d	24.50	11.50	9.00	18.75	5.00	48.0
e	21.00	10.50	7.50	35.00	0.50	...
f	19.25	12.50	11.00	24.00	1.75	54.0
g	20.00	11.00	10.25	18.50	3.00	42.0
h	22.50	10.50	8.50	15.25	6.50	38.0
Digoxin						
(1:50,000) a	90.00	90.00	12.00	...	...	...
b	94.00	...	...	...	...	...
c	82.00	75.00	10.00	...	...	...
d	105.00	...	...	...	...	...
e	98.00	68.00	16.00	...	...	...
Ouabain						
(1:60,000) a	32.00	20.00	9.00	65.00	4.50	...
b	35.00	...	...	...	...	...
c	35.00	18.00	7.00	90.00	2.00	...
d	28.00	24.00	5.00	...	...	...
e	26.00	21.00	11.00	...	...	...
Digitoxin						
(1:60,000) a	32.00	68.00	12.00	...	...	...
b	38.00	...	...	...	...	...
c	42.00	...	...	...	...	...
d	31.00	...	...	...	...	...
e	30.00	...	...	...	...	...

It will be noted that the concentration of urginin in the above table is approximately one-half that of the other glucosides employed. This has been done in order to produce systolic arrest in approximately the same time interval. A 1:100,000 solution of Urginin produces systolic arrest in about twenty minutes, while a 1:60,000 solution of ouabain or digitoxin required about thirty minutes to produce the same effect. 1:50,000 Digoxin requires ninety minutes, but more concentrated solutions produce an irreversible change.

Emetic Action.—The emetic action of the cardioactive glucosides of digitalis and strophanthus has been the subject of a comprehensive investigation by Hatcher

and his co-workers (23), who have shown that this action is primarily the result of reflex stimuli arising from the neighborhood of the heart. It is common knowledge that during the course of the assay of the digitalis bodies by the intravenous cat method hypersalivation and vomiting occur.

It is, however, not equally well known that it is difficult to induce the vomiting mechanism with an animal in the supine position, the common position of an experimental animal used for slow intravenous injection. It has been our experience that vomiting occurs in about 75% of cases where such supine animals are receiving ouabain, and in 60% of the cases where they are receiving digitalis. In a series of 460 animals that have received urginin, our records show that vomiting occurred in six animals, or less than 2%. This discrepancy between the production of emesis by the cardioactive principles of squill and those of digitalis and strophanthus bear out the clinical experiences of the large number of investigators who have shown that therapeutic doses of the cardioactive principles of squill may be administered without the production of nausea or emesis in patients who always become nauseated or vomit after the administration of digitalis bodies.

The intravenous administration of 50% of the M. L. D. of urginin to a series of twenty cats in an unrestrained position produced emesis in only 40% of animals. The remaining 60% showed marked hypersalivation. Electrocardiographic records taken immediately before and 30 minutes after administration showed a marked bradycardia, with an increase in AV conduction time in all animals, and occasional ventricular extrasystoles in 10% of the animals.

The administration of 50% of the M. L. D. of ouabain to a series of twenty animals was followed by nausea and vomiting in every case, while electrocardiographic records taken thirty minutes after administration showed marked bradycardia, increased AV conduction time, and occasional ventricular extra-systoles.

The intravenous administration of 50% of the lethal dose of Tincture of Digitalis produced vomiting in 100% of cases, showing the same type of electrocardiographic changes as described above. In this series the bradycardia was not so marked as it was in the two preceding series.

After the administration of ouabain or digitalis vomiting occurred within three minutes of the completion of the intravenous administration. In those cases where emesis occurred after the administration of Urginin there was a delay of ten to eighteen minutes before the onset.

CONCLUSIONS.

1. Urginin is a mixture of two water-insoluble cardioactive glucosides of Squill, possessing a uniform biological potency and demonstrating a high degree of stability.

2. A method is described for the quantitative expression of the relative cumulative effects of cardioactive glucosides.

3. The cumulative effect of Urginin is half as great as that of ouabain and one-fifth as great as that of digitoxin.

4. The reversibility of the action of a series of cardioactive glucosides on the isolated turtle heart may be expressed in the following way: Urginin is more readily reversible than ouabain, which is more readily reversible than digoxin, which is more readily reversible than digitoxin.

5. The emetic action of Urginin on the intact cat is less than that of ouabain or Tincture of Digitalis.

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NOTE ON UROTROPIN MANDELATE.*

PREPARATION AND TOXICITY OF A NEW URINARY ANTISEPTIC.

H. G. KOLLOFF AND J. W. NELSON.

The recent discovery that mandelic acid (1) and certain of its salts (2), (3), (4) are efficacious in the treatment of urinary infections makes it desirable to study new salts and combinations of this acid. In this preliminary paper, we wish to report the preparation and toxicity of the hexamethylenetetramine salt which appears to offer possibilities as a superior mandelic acid preparation.

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