THE ACTIONS OF RUBELLIN AND PRORUBELLIDIN (in comparison with Transvaalin)

THESIS

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C O N T E N T S

INTRODUCTION

Squill is one of the oldest drugs known to man, and is also one of the oldest heart remedies used to-day. It is a bulbous plant with white or blue flowers, which grows abundantly in the sandy soil in countries on the Mediterranean coast, and also in Malta. It is mentioned in the Ebers papyrus (about 1500 B.C.), and in the works of the great physicians of the ancient world such as Dioscorides, Celsus, Theophrastus, and Galen. Hippocrates used it.

Preparations of the bulb of the sea onion (Squill, Scilla maritima L., Urginea maritima Baker) were used therapeutically by the ancient Egyptians, Greeks, and Romans, i.e. by peoples of the Mediterranean region where the plant grows.

With the passing of the centuries it assumed an important place in therapeutics especially for the dropsy. The drug was held in such high esteem at one time that a temple was erected to it in Pelusium. However, it fell into disfavour because of its tendency to produce nausea and vomiting.

Squill was introduced again into clinical practice in the middle of the 18th century but, with the publication by William Withering in 1785 of his classical monograph on digitalis, squill was again relegated to a period of relative obscurity. During the latter part of the last century and in recent years the position of squill as a

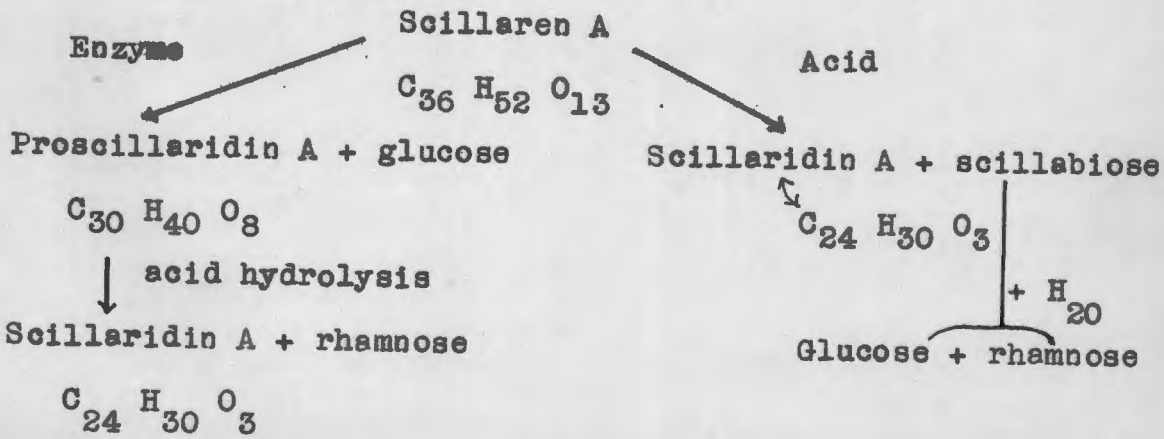
cardioactive drug was recognised and established by experimental work in animals and in patients with cardiac failure. However, at the present day the use of squill or any of its official galenical preparations is practically obsolete.

The reasons why squill has been generally unacceptable in therapeutics are : the unreliability of crude galenical preparations (inconstant potency and tendency to decomposition), clinical experience that the absorption of squill from the alimentary canal is uncertain, and the liability to cause nausea and diarrhoea from the action of irritant resinous principles in the crude substance.

In 1921 Stoll and Willstatter succeeded in isolating pure active principles from fresh squill bulb, which they named Scillaren, and which presents the advantages of accuracy of dosage, stability, and constancy of action. Special protective methods were used to isolate the unstable natural substances free from inactive accessory substances. Dyas (1934) developed methods for the extraction of two water insoluble glycosides scillonin A and scillonin B, now known as urginin A and B respectively; the commercial 'Urginin' is a combination of crystalline and amorphous scillonins. With the isolation of these principles squill therapy has found some use in modern

clinical practice.

Scillaren is a mixture of two closely united fractions differing somewhat chemically but having the same fundamental actions. One component forming about two-thirds of the natural mixture, is a crystalline powder, slightly soluble in water and designated Scillaren A. The remaining one-third is amorphous, more soluble and more active, and called Scillaren B; it is used for the manufacture of an injectable preparation. For oral administration a mixture of both scillarens is available. Scillaren A ($C_{36}\ H_{52}\ O_{13}$) can be transformed by the action of an enzyme scillarenase present in squill substance into proscillaridin A ($C_{30}\ H_{42}\ O_{8}$) which is a crystalline product having one molecule of glucose less than scillaren A. Acids hydrolyse this new glycoside proscillaridin A into scillaridin A ($C_{24}\ H_{30}\ O_{3}$) and rhamnose. Acid hydrolysis of scillaren A produces the aglycone scillaridin A and a hitherto unknown disaccharide scillabiose which on further hydrolysis yields glucose and rhamnose.



Both Scillaren and Scillaren B are recognised in New and Nonofficial Remedies (1947) and are marketed for therapeutic use. Experimental studies with scillaren on animals have been reported by Premankur De (1928), Rothlin (1927), Dubinsku (1928), Fahrenkamp and Nocke (1928), Stehle, Ross and Dreyer (1931), Peters and Visscher (1936), Chen et al. (1936), and reviews on the clinical investigation of squill preparations have been written by Movitt (1946) and Isaacson (1949).

Squill glycosides increase cardiac 'tone', improve the force of systole, and lengthen the diastole of the heart in failure; the effect on the junctional tissues is similar to that produced by the digitalis glycosides.

The activity of Scillaren A in animals has been found by Rothlin to be as follows according to Stoll (1938).

	Frog (medium lethal dose; subcutaneous injection, timeless method)	Cat (intravenous infusion; Hatcher's method)
	Frog units per mg.	Cat units = mg. per kgm.
Scillaren A	1200	0.145
Ouabain	2400	0.100
Digoxin	650	0.280

The toxicity of the different squill glycosides varies in experimental animals (Rothlin, 1927), e.g.,

scillaren A and B show different but corresponding toxicities for cold blooded and warm blooded animals.

Scillaren A and B produce the following effects: rapid action of short duration due to rapid excretion, and hence slight tendency to cumulative action. In terms of "cat units" these glycosides are much more active than digitoxin and other "digitalis" glycosides, except for ouabain and strophanthin, on the basis of weight. However, the marked differences in the degree of absorption from the intestine, the rate of fixation, and the rate of excretion, must be considered in the proper comparison of the various glycosides. Scillaren A and scillaren B are actually well absorbed from the intestine.

The constant chemical composition of Scillaren allows the dose to be expressed by weight; for example, each oral dose contains 0.0008 gramme Scillaren and each dose for injection 0.0005 gramme.

Scillaren acts on the heart like the digitalis glycosides but it has its own special features. It is recommended for oral or rectal administration if digitalis fails or is not tolerated and when it is not desired to give injections. The squill glycosides have a cumulative effect but to a lesser extent than the glycosides of digitalis. According to Koerner (1924) scillaren, when injected intravenously, stands between the native digitalis

glycosides and the strophanthus glycosides, but more closely to the latter, in its power of fixation and rapidity of action. The rapidity with which it is eliminated (Bach and Beer, 1935) makes it useful in patients who are hypersensitive to other glycosides and in patients in whom cumulative action should be at a minimum. The persistence of active glycoside within the heart muscle of mammals has been shown by the greater susceptibility of heart preparations from animals which had previously been treated with these drugs; with scillaren A this could be demonstrated for two hours, with ouabain for fifty-four hours, with digitoxin for some weeks.

Fahrenkamp (1924) concluded that it is a mistake to regard squill as merely a substitute for digitalis, believing it is relatively more potent in its vagal action and less potent in its muscular effects. It is more rapid and less persistent than digitalis in its effects.

In the past century squill was used as a diuretic and nauseant-expectorant; these effects were generally attributed to local irritation of the kidneys and stomach respectively. To-day, however, it is classed with the digitalis group and it is generally believed that its diuretic and emetic effects are secondary to its cardiac actions.

The diuretic action of scillaren is nowadays attributed to its action on the heart, but French physicians believe it is due also to a direct action on the renal tubule epithelium increasing the excretion of water, salt, and nitrogenous bodies. The diuretic action has also been demonstrated in human beings in whom there is no circulatory disturbance, and there is an increased excretion of uric acid and creatinine. The diuretic action is equal to that of theobromine. A prerenal action has been demonstrated in the reduction of the sodium and chloride content of rabbit muscle after the administration of scillaren. A diuretic action of scillaren was noted in experimental animals by Premankur De (1927) and by Rothlin (1927).

Diarrhoea is more frequent with squill (and strophanthus) than with digitalis. The nauseant and irritating substances in squill are not identical with the glycosidal principles responsible for the cardiorenal activity. With intravenous injection Eggleston and Hatcher (1915) found squill less emetic than the other glycosides investigated; in ascending order - squill and apocynum; digitoxin and amorphous strophanthin; strophanthus, ouabain, digitalis, digipuratum, digalen and adonis.

The official squill (Scilla; Urginea scilla; U. maritima) which was commonly employed in therapeutics

is collected from countries on the northern shore of the Mediterranean and is also known as White Squill. Though official in the British Pharmacopoeia, it is no longer official in the United States Pharmacopoeia. It has actions resembling those produced by digitalis, but is used as tincture, vinegar, syrup, and oxymel, mainly as an expectorant. Urginea indica (B.P.) is a distinct species with the same effects as the White Squill and used in India in the same dosage.

Another variety of squill which grows in Algeria and is known as Red Squill was used widely at one time as a rat poison. It has been stated to contain the same cardiac glycosides and in about the same quantity as in White Squill, and also scilliroside, a glycoside with "digitalis" actions that is toxic to rats, causing death after some days of alternating convulsions and paralysis. From the difference in behaviour of white and red squill after oral administration in rats Winton (1927) concluded that the cardiac glycosides and the rodenticidal principles are not the same, and this was also reported by Wokes and Willimott (1934). Most of the comparisons of red with white squill in the rat were based on oral administrations. Gold and his co-workers (1947) obtained results with amorphous scilliroside which indicate that the convulsant action of red squill is not due to a special factor

but is a function of its cardiac glycosides or their genins. Another material obtained from red squill, urginin, was found to be half as potent as amorphous scilliroside.

Several squill plants have been used by the natives in South Africa as medicinal agents. Scilla natalensis has been used as an application to sprains, boils, veld sores, and fractures, as an aperient, and as an enema. Scilla rigidifolia has also been used as an aperient, enema, and in the treatment of rheumatic fever; it has been stated to cause loss of stock (Marloth, 1913). Scilla inandensis, Scilla cooperi, and Scilla galpini have been used as medicines for women in the fourth month of pregnancy. Scilla lanceafolia is stated to be used as expectorant, diuretic, and as an irritant to the gastro-intestinal tract; also as a local irritant for lumbago, skin eruptions, wounds and sores. Scilla cooperi, Scilla lanceaefolia, and Scilla rogersii have been shown to have a "digitalis" action by Gunn et al. (1925).

The author has been especially interested in the genus Urginea which belongs to the natural order Liliaceae and is closely related to the genus Scilla. It comprises a group of bulbous rooted plants, of which more than twenty-five species have been recorded in South Africa. A few have proved toxic to stock; the features of poisoning

in animals are described by Watt and Breyer-Brandwyk (1932) and by Steyn (1934, 1949). The active principles in these plants have not been adequately studied and there is little information about them. Some of the facts about the toxic Urginea plants occurring in Southern Africa are given below, and an experimental investigation is reported later in this work on two new glycosides, viz: (a) Rubellin (from Urginea rubella Baker) which was isolated from the plant bulbs by Dr P.G.J. Louw of the Department of Toxicology, Onderstepoort, Pretoria. Some of the chemical features of this glycoside were reported by Louw (1949). (b) Prorubellidin, a derivative of rubellin, later became available for investigation. A crystalline product (from Urginea Burkei Baker) was studied at the same time.

The following table indicates the better known toxic Urginea plants in Southern Africa.

Plant	Active Principle	Author
Urginea altissima	Scillitoxin, scillipiain	Bernhard-Smith
	"glycoside"	Epstein
Urginea Burkei Baker	"glycoside"	Gunn, George, Watt
	Transvaalin	Louw
(Urginea sanguinea is probably U. Burkei)		
Urginea capitata Baker	unknown	
Urginea macrocentra Baker	"alkaloid" (?)	Juritz
Urginea rubella Baker	Rubellin	Louw

Urginea altissima: The toxic features produced in stock by this plant resemble those produced by Urginea Burkei. Marloth (1913) stated that the bulb produces effects resembling those caused by Scilla maritima. According to Bernhard-Smith (1923) the plant contains scillitoxin and scillipiain. Extracts of the plant, the active principle of which appears to be a glycoside, were shown by Epstein (1933) to have a digitalis-like action in animals, and he stated that the tincture of Urginea altissima is equal in activity to the official tincture of squill. Amongst other features he noted that the activity was greatly diminished when the preparation was administered by mouth, owing to poor absorbability, and that auriculo-ventricular block was a marked feature of the action on the heart. Slowing of the heart due to central vagus action was not found to be marked. Vomiting was due to local irritation in the stomach.

Urginea Burkei Baker (Transvaal slangkop): This plant has long been known to be poisonous to sheep and other stock in certain parts of Africa. The features of poisoning in animals are described by Watt and Breyer-Brandwyk (1932) and by Steyn (1934, 1949). All parts of the plant may be toxic: bulb, leaf and flowering top. Poisoning is usually due to animals eating the young shoots

and leaves. Similar effects have been recorded when animals are fed experimentally with the bulb.

The Sutos use an aqueous preparation of the bulb as an abortifacient and also in the treatment of circulatory disorders.

Gunn (1921, 1925) found that alcoholic extracts have a digitalis-like action in laboratory animals, and suggested that Urginea Burkei might replace squill (Scilla maritima) in South Africa and might even be found to be superior.

George (1925) isolated a colourless and a red amorphous glycoside, neither of which is pure and crystalline. Watt (1927) found the colourless glycoside poisonous to cats, causing paralysis of the central nervous system and respiration; the red glycoside had a digitalis-like action. Previous investigators have not succeeded in preparing crystalline compounds. A crystalline material was isolated from the bulb and its chemical properties described by Louw (1949); it would appear on further analysis to be a mixture, with the main constituent scillaren A (personal communication). It cannot be stated at present whether this is the only active principle in Urginea Burkei.

Urginea capitata Baker is toxic to sheep. Its active principle is unknown. Urginea delagoensis Bkr.

and Urginea lydenburgensis R.A. Dyer are also mentioned by Steyn (1949).

Urginea macrocentra (Natal slangkop):

This plant has been stated by Juritz (1923) to contain a toxic "alkaloidal" substance. However, as Steyn (1934) points out, plant extracts which do not contain alkaloids as active principles may give precipitation tests for alkaloids due to the presence of non-poisonous alkaloids or other chemical substances.

Urginea rubella Baker: From this species of Urginea an active principle named rubellin was isolated some time ago but only recently reported by Louw (1949). A preliminary account of its pharmacological properties, especially its potency on frogs and comparison with certain other cardiac glycosides, has been reported by the present author (Sapeika, 1944, 1946) while a clinical study of the glycosides administered intravenously has been made by Isaacson (1949). More detailed studies on this very potent glycoside are reported in the present work. Another new glycoside prorubellidin has also been investigated more recently, and is reported in the present work.

The investigations on Urginea species have been mainly concerned with extracts of the plants. This is

unsatisfactory as chemically pure compounds are desirable for proper identification and characterisation of the active principles of plants, as well as for pharmacological experiments. The main difficulties in the study of the "digitalis" glycosides have been the fact that in many cases more than one active principle has been present; small quantities may only be present: all have a very complex chemical structure and vary in their actions in the body according to degree of absorption, fixation in the heart muscle, and rate of elimination; unless special extraction processes are used the enzymes in the plants alter the compounds naturally present ("native glycosides"). On hydrolysis with acids the glycosides yield a noncarbohydrate moiety known as "aglycones" or "genins", and sugars which in some cases are unique in character. Many of the aglycones are related and contain 23 carbon atoms; scillaridin A, however, contains 24 C atoms. It is the aglycones which produce the characteristic cardiac action, the sugar portion affecting absorption, transport, and penetration of the glycosides.

URGINEA RUBELLA Baker

PRESENT INVESTIGATION

In the present study the chemically pure glycoside Rubellin (from U. rubella) has been investigated, and also to a limited extent the glycoside prorubellidin which became available more recently. The investigation of new glycosides which have an action on the heart is of practical importance. Cardiac disorders are so diverse and the reaction of the patient so individual that each drug and its dose must be chosen carefully for each case.

(a) Rubellin, which is soluble in water, alcohol, and chloroform, was found on analysis to have a mol. wt. of 656; calculated for $C_{36}H_{48}O_{16}$ the mol. wt. is 736.7. These chemical details are given by Louw (1949).

(b) Prorubellidin is a derivative obtained by acid hydrolysis of rubellin; the sugar part hydrolysed off is a dicarboxylic sugar acid, e.g. like saccharic acid, which is something quite new in cardiac glycoside chemistry. The acid has as yet not been identified. Prorubellidin still contains a sugar molecule and is therefore a new cardiac glycoside (personal communication from Louw). It is not the true aglycone of rubellin. It is slightly soluble in water, but fairly soluble in alcohol.

It cannot be stated at the present time whether these active glycosides represent the "genuine glycosides" from the respective plant sources, i.e. the unchanged

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initial glycosides as they exist in the fresh plant, or products decomposed by enzymes. As Stoll (1937, 1938) has pointed out, hydrolytic enzymes which in the living plant cell synthesise glycosides and other substances may participate in their decomposition or transformation after the death of the cell.

Rubellin may be compared with Scillaren A ($C_{36}H_{52}O_{13}$), the "genuine" glycoside obtained as a crystalline product from Scilla maritima, and with amorphous scilliroside obtained from a red squill powder by Stoll and Renz (1942). The latter glycoside has an ultraviolet absorption spectrum very similar to and practically coincident with that of rubellin. It acts like a typical digitalis glycoside in the frog, rat, cat and man (Gold et al, 1947).

Transvaalin, a crystalline material obtained from Urginea Burkei Baker, a plant closely related to Urginea rubella, was also examined in the present study. It recently became apparent that it is a mixture of closely united fractions, consisting mainly of scillaren A, with presumably a trace of scilliroside, or a related glycoside.

EXPERIMENTAL

LOCAL ACTION.

Placed on the human tongue both rubellin and transvaalin have a bitter taste, but no local irritation or local anaesthesia was produced. When administered by mouth to normal subjects neither drug (rubellin 0.25 mg; transvaalin 0.25 to 1 mg) produced gastrointestinal disturbances. Rubellin when given intravenously in man was found to cause pain and discomfort if any of the drug was placed in the perivenous tissues; this local irritation occurs with most cardiac glycosides.

In animals no obvious local action was observed. Injection into the ventral lymph sac of frogs produced no signs of irritation. When instilled into the conjunctival sacs of rabbits no evidence of local irritation or anaesthesia was observed even with high concentration of the drugs. When given to cats or rats through a stomach tube no gastro-intestinal irritation was observed.

The powdered bulb of Urginea Burkei is very irritating, small quantities causing sneezing, lachrimation and coughing when inhaled. An extract applied to the frog's mesentery produces marked inflammation in a few minutes. When given orally to cats vomiting and diarrhoea occur (Gunn, 1921). These results must be due to the fact that the plant contains other principles apart from transvaalin.

The local irritant or anaesthetic action of the "digitalis" glycosides is not equally developed in all members of the series; digitoxin is regarded as most irritant, whereas ouabain and strophanthin are practically non-irritant. The irritant action of those glycosides which produce anaesthesia precludes their use in man as local anaesthetic agents.

Guevara et al. (1949) recently examined the local emetic action of four pure glycosides whose structure is known and which are poorly absorbed from the gastro-intestinal tract. These glycosides, ouabain, scillaren A, scilliroside and lanatoside C were administered to cats through a stomach tube, and were found to show the following order of increasing approximate potencies: ouabain - 1, scillaren A - 4, lanatoside C - 10, scilliroside - 10. The local irritant action is thus developed in widely different degrees in the molecular structure of these cardioactive glycosides. There was no relation between the cardiac potency and the local emetic action.

GENERAL ACTION.

Frog: Transvaalin, rubellin, and prorubellidin all caused death in frogs (Xenopus laevis) when adequate doses were injected into the ventral lymph sac. The movements of the animals became less frequent with later little or no response to stimuli. There were no

signs of stimulation or convulsions. At autopsy the heart had ceased with the ventricle in "systolic stand-still". With these glycosides too, as is well known for digitalis, the selective cardiac action is shown by the fact that spontaneous active movements of the frog still occur after the heart had been arrested.

The potency of the glycosides as determined by the frog assay method is discussed later.

Rat: Rodents have long been known to be resistant to the digitalis glycosides, though the animals are not immune. This congenital tolerance has been attributed to resistance of the rat heart to the action of these poisons, but is also due to the ability of the rat to eliminate large quantities of these drugs, due either to their destruction in the blood (Straub, 1919), or to fixation in the liver and excretion via the bile into the intestine (Farah, 1946). The fatal dose of digitalis for rats is nearly a thousand times greater (per kilogram) than that for cats even by subcutaneous injection (Hatcher, 1909). Gunn (1913) showed that the M.L.D. of strophanthin for the white rat is thirty times greater than that for the rabbit, and the isolated hearts of these animals showed the same degree of difference. In the present study the rat was found to be resistant to transvaalin and prorubellidin but not so markedly to rubellin.

(a) Transvaalin: By subcutaneous injection this complex produced effects when doses of 0.04 mg. per gramme body weight were given. Within half an hour the rats first showed disinclination to move, the respiration became slower and irregular; paresis (dragging) of the hind limbs occurred (but no sensory paralysis) from which the animal slowly recovered in a few hours. With this dose death did not occur. Large doses could not be given because of the alcohol required as solvent.

The digitalis bodies are known to have a stimulant action on the central nervous system, e.g. the medullary centres, but they may also act on other parts of the nervous system. This action is particularly well demonstrated in an animal such as the rat in which the heart is resistant to certain glycosides. Hatcher (1912) has stated that digitoxin produces strychnine-like convulsions in the rat, while Epstein (1931) described similar effects in the toad Bufo regularis, which were followed by paralysis of the nervous system. Transvaalin produced no convulsions (clonic or tonic) in the albino rat, but definite temporary paralysis of the hind limbs. The mechanism of this is not clear.

(b) Rubellin: An aqueous solution injected subcutaneously produced rapid death, without preliminary convulsions, due to the action on the heart. Thus

rubellin shows a marked difference in action and toxicity from transvaalin in its action on the rat; 0.001 mg. per gramme body weight caused death.

Dose mg. per gramme	No. of rats injected	Dead	Time of death in minutes
0.0003	3	0	
0.0005	3	0	
0.001	3	3	35, 40, 45
0.002	3	3	28, 33, 44

This lethal dose may be compared with the "hour dose" of rubellin on the frog (Table 3), showing that the rat is not very resistant to this cardiac glycoside. These results may also be compared with those obtained with amorphous scilliroside (Gold et al, 1947) who found the intravenous LD_{50} in the rat to be about 0.0025 mg. per gramme by the cardiac action.

When administered orally to rats through a stomach tube the drug failed to cause death with doses (0.006 mg. per gramme) six times greater than that given by subcutaneous injection. The reason for this is not known, but it may be due to some alteration of the glycoside in the alimentary canal, or to slow or incomplete

absorption. With scilliroside a dose of 0.01 mg. per gramme produces effects in an hour.

(c) Prorubellidin: The small amount of this glycoside available made it possible to conduct only a limited investigation. A dose of 0.002 mg. per gramme by subcutaneous injection, and 0.006 mg. per gramme by oral administration did not cause death (cf. rubellin).

Cat: Limited supplies of the glycosides and of animals have prevented assay procedures on cats. It may be pointed out that the cat assay method is not satisfactory. Active drugs showing exactly the same strength by this method may show great variation in their clinical effectiveness. This is due to the fact that in the assay the material is injected intravenously whereas the cardiac patient is given the drugs by mouth. The method fails to indicate one of the primary differences in the various active glycosides, namely the degree to which they are absorbed from the intestinal tract. Some idea of the potency of the glycosides in the cat may be gathered from the doses causing death in the various experiments described later in this investigation.

(a) Transvaalin: The following table shows the effect of intravenous doses in certain experiments conducted under anaesthesia.

Cat weight Kgm.	Anaesthetic	Dose mg./kgm.	Death in minutes
2.6	ether	0.1	-
2.75	pentobarbitone	0.1	-
2.1	ether	0.15	15
2.75	pentobarbitone	0.15	-
2.75	pentobarbitone	0.18	-
1.1	pentobarbitone	0.2	9
3.5	ether	0.2	9

(- alive at end of the experiment).

The effect of transvaalin administered orally on an empty stomach by means of the stomach tube was as follows:

Cat	Dose	Effect
3.0 Kgm.	0.3 mg./Kgm.	survived
2.25 "	0.5 " "	survived
3.5 "	0.75 " "	survived
2.0 "	1.0 " ""	death in 2 hr. 20'

Thus a larger dose had to be given by mouth to produce death. This may be due to (a) imperfect absorption

of the drug, (b) possibly some destruction of the glycoside by the digestive enzymes, or (c) rapid elimination rather than poor absorption, as with a rapidly eliminated drug the excretion of some of the active principle may occur before all of it is absorbed from the gastro-intestinal tract.

With extracts of Urginea Burkei given by mouth Gunn (1921) observed local effects in the alimentary canal and attributed the comparative inability of the extract to produce general symptoms to vomiting, destruction of the active principle in the canal, or to slow absorption. The crude extract no doubt contains principles, irritant and cardiotoxic, other than transvaalin itself.

(b) Rubellin: By intravenous injection this glycoside produced death in various experiments, as shown in the following table.

Cat weight Kgm.	Anaesthetic	Rubellin mg./Kgm.	Death minutes
2.8	ether	0.07	20
1.8	ether; (dibenamine)	0.07	14
2.4	ether	0.073	35
3.25	pentobarbitone; ether	0.08	55
3.0	(decerebrate)	0.08	9
2.3	ether	0.086	25
2.75	pentobarbitone; ether	0.09	70
2.1	ether; (dibenamine)	0.09	7
4.0	pentobarbitone; ether	0.1	30
2.0	pentobarbitone	0.1	30
2.0	pentobarbitone; ether	0.12	$4\frac{1}{2}$
2.4	(decerebrate)	0.12	6
2.3	pentobarbitone	0.13	12
2.75	ether	0.14	16

This table gives some idea of the potency of rubellin and demonstrates the well-known fact that animals even of the same species show different sensitivity to a particular cardiac glycoside.

Rubellin was administered by mouth

through a stomach tube to several cats, but even a dose of 1.0 mg. per kgm. body weight, produced no obvious disturbances. No signs of a local (gastrointestinal) or a general (heart) action were noticed with this dose and electrocardiographic records made at frequent intervals for a number of days also failed to reveal any effect on the heart. A dose of 2 mg. per kgm. produced diarrhoea, but no other disturbance was noticed.

The drug is therefore not so effective orally as by parenteral administration, where (as shown in the above table) 0.07 mg. per kg. body weight causes rapid death, i.e. the ratio of oral to intravenous dose in the cat is more than 14 to 1.

(c) Prorubellidin: The effect of this glycoside by intravenous injection in anaesthetised cats was observed in animals which received the drug for studies of the changes produced in the electrocardiogram. In one cat which received 0.12 mg. per kgm. and in which marked ECG changes were recorded the animal was still alive at the end of 70 minutes. In another cat 0.15 mg. per kgm. produced death in about twenty minutes, respirations persisting for about two minutes after the heart had stopped.

One cat which received 0.5 mg. per kgm. body weight by mouth showed no ill effects, but in two others a dose of 2 mg. per kgm. produced diarrhoea, but no other obvious disturbance.

ASSAY OF THE GLYCOSIDES in comparison with ouabain.

In order to determine the potency of the cardiac glycosides, assays were performed on frogs (Xenopus laevis) by the 16 hour (overnight) mortality method, ouabain (international anhydrous) being used as the standard of comparison. A number of animals are injected with a suitable dose of the glycoside being assayed. The animals are examined the next morning and the mortality rate noted. Preliminary tests are first made to estimate the approximate average fatal dose. This method of assay allows a long time for absorption of the drug. The results were calculated by means of the table of potency prepared by Gunn and Sapeika (1934, 1936). This table was prepared from the mortality curve for Xenopus laevis obtained with strophanthin, the curve being similar to that obtained by Gunn and Epstein (1932) with digitalis; both these curves were almost identical with the mortality curve determined by Chapman and Morrell (1931) in Toronto for Rana pipiens, and differed from the mortality curve obtained by Trevan (1927) who used Rana temporaria in his investigation. Rana pipiens is more susceptible than R. temporaria (Gaddum, 1932). We have found that these curves for Xenopus laevis for the comparison of preparations of digitalis and of strophanthin are remarkably accurate. The reaction of Xenopus laevis to cardiac

glycosides is remarkably constant. There is ordinarily no great variation from day to day, and it is rare to find individual animals displaying either unusual tolerance or susceptibility. Observations made at different times of the year have been almost identical. The frogs, if kept in a cool place in a large shaded tank with frequent changes of water, even after six months react to the glycosides quantitatively like the freshly caught animal.

Stock solutions of the glycosides were prepared in ethyl alcohol (50 per cent by volume), part of the stock solution used in each experiment being diluted with distilled water to produce the final solution for injection. Care was taken to use the minimal amount of alcohol. The dilutions used are indicated in the tables. Injections were made through the floor of the buccal cavity into the ventral lymph sac. The compounds were compared by simultaneous assays on frogs from the same stock.

(a) Transvaalin.

The experiments with transvaalin were done in February, 1949. The results are given in table 1. Transvaalin has a potency 25.6 per cent that of ouabain.

Table 1

Assay by 16 - hour method

	Dose (mg.) per gramme	No. of frogs	Mortality	Potency	Average Potency
Ouabain international anhydrous 1 in 100,000	0.000128	10	25		
	0.000132	20	70		
	0.000136	10	80		
	0.000136	10	80		
Transvaalin 1 in 10,000	0.0005	10	60	26	
	0.0005	10	60	25.3	
	0.0006	10	90	26.3	
	0.0006	20	75	24.8	25.6
Rubellin 1 in 100,000	0.0001	10	70	132	
	0.00012	10	90	120	
	0.00013	16	93	125	126
	0.00015	10	100		

(b) Rubellin.

This glycoside was assayed simultaneously with transvaalin as shown in table 1; its potency in this particular assay is 126 compared with ouabain (100), transvaalin having a potency of 25.6. Thus in the frog, as also in the rat and the cat, rubellin is more active than transvaalin. This is of interest in view of the origin and the close chemical relationship of these two glycosides.

Rubellin has also previously been shown in preliminary studies to be more potent than ouabain (Sapeika, 1944). This is of interest because ouabain has for a long time been regarded as the most potent of all cardiac glycosides.

The potency of various chemically pure glycosides on the frog Xenopus laevis may be gauged from tables 1 and 4, and from the tables 2 and 3 taken from work previously published by Sapeika (1946).

Table 2

Assay by 16 - hour method

	Dose (mg.) per gramme	Mortality %	Potency	Average Potency
Ouabain international anhydrous 1 in 100,000	0.00016	29		
	0.000176	70		
	0.000176	80		
	0.0002	100		
Rubellin 1 in 100,000	0.00013	16	109	
	0.00014	33	111	
	0.00015	50	108	109
Strophanthin 1 in 50,000	0.00035	50	46	
	0.0004	67	42	44
	0.00045	100		
Lanatoside C 1 in 20,000	0.0005	40	32.5	
	0.0007	88	30	31
	0.0009	100		
Digoxin 1 in 10,000	0.0009	38	19	
	0.001	75	19	19
Digitoxin 1 in 5,000	0.005	63	3.6	
	0.005	75	3.7	3.6

Table 3

Assay by 1 - hour method

Compound	Dose (mg.) per gramme	No. of frogs in systole / No. of frogs used
Rubellin 1 in 50,000	0.0003	2/5
	0.00035	4/5
	0.0004	4/5
	0.0005	5/5
Ouabain international anhydrous 1 in 50,000	0.0004	0/5
	0.0004	1/5
	0.00042	1/5
	0.00044	2/5
Strophanthin 1 in 40,000	0.00085	1/5
	0.0009	1/5
	0.00095	2/5
	0.001	2/5
Lanatoside C 1 in 50,000	0.004	1/5
	0.0042	2/5
	0.0045	4/5
	0.005	5/5
Digoxin 1 in 5,000	0.004	1/5
	0.0042	1/5
	0.0045	3/5
	0.005	3/5
Digitoxin 1 in 4,000	0.0085	2/5
	0.009	2/5
	0.009	2/5
	0.01	3/5

Rubellin is the most active cardiac glycoside of those examined by the frog assay method.

(c) Prorubellidin

This glycoside which only recently became available for study was assayed simultaneously with ouabain (as standard) and with rubellin to which it is very closely related.

Table 4

	Dose mg./gm.	No.of frogs	Mortality %	Potency	Average potency
Ouabain international anhydrous 1 in 100,000	0.000136	10	30		
	0.000144	10	50		
	0.000144	10	70		
Rubellin 1 in 100,000	0.0001	10	20	129	
	0,00012	10	40	117	123
Prorubellidin 1 in 100,000	0.0002	10	10	60	
	0.00025	10	10	48.4	50.5
	0.0003	10	30	43.2	

In summary, the following results have been obtained.

By the 16 - hour frog assay method, which is official in the British Pharmacopoeia, but using Xenopus laevis as test animal and the table of potency prepared by Gunn and Sapeika (1936) for the calculations, the

relative potency of the cardiac glycosides at present under investigation is, in descending order: rubellin, ouabain (international anhydrous, used as standard), prorubellidin, transvaalin.

By this method too the relative potency of the following glycosides as determined from various assays performed in this investigation is, in descending order; rubellin (average of several assays) (121), ouabain (international anhydrous (100), prorubellidin (50.5), strophanthin (44), transvaalin (25.6), lanatoside C (31), digoxin (19), digitoxin (3.6).

It is of interest that though these steroid compounds are so closely related chemically they differ in their pharmacological activity. There are many other examples of this difference between chemical constitution and biological activity in the field of pharmacology. In the case of these cardiac glycosides the sugar part of the molecule may be one important factor in determining the degree of action.

There is still controversy as to which method of biological assay of cardiac glycoside preparations shall be used. The frog method used in the present investigation is official in the British Pharmacopoeia. In recent years assay on human beings has been advocated to overcome the discrepancy between the potencies of

preparations obtained by animal methods and the actual activity in man especially by oral administration (Gold et al, 1942), but this method also presents many difficulties; the test subjects must first be carefully selected from a large number of individuals, several months are required for the calibration, the estimation of the effect is not strictly quantitative but depends on the judgment of the electrocardiographer, and no correction is made for fluctuations in rate. Chemical methods are also a complicated matter (Canback, 1949).

No standard for squill has been adopted by the Permanent Commission on Biological Standardisation. The U.S.P. required that squill be assayed by the one-hour frog method formerly employed for the assay of digitalis. Squill is no longer included in the U.S.P., and neither the B.P. nor the N.F. requires any assay process. Considerable work has been done with different methods of bio-assay of squill products (Burn, 1927; Rosen, 1934) but since the isolation of the pure active principles which can be assayed gravimetrically, as with rubellin and transvaalin, bio-assay is no longer so essential. The availability of these purified drugs makes galenical preparations such as tinctures no longer necessary. It may be mentioned in passing that biological assay in the cat (Hatcher-Brody method) has shown that 1 mg. of urginin

has a potency of 4.26 cat units, one cat unit thus containing approximately 0.2 mg. of the drug (N.N.R. 1936). In the case of scillaren A and B one cat unit represents 0.18 mg., and 0.14 mg. of the active principle respectively. In terms of cat units these glycosides are more active than digitoxin and the other digitalis glycosides, except for ouabain and strophanthin, on the basis of weight. Rubellin is also a very potent glycoside as shown in the assays on frogs and in cats which received the drug intravenously. The values derived from animal assay cannot however serve as an accurate basis for therapeutic dosage nor for comparison of the therapeutic value of different glycosides, as they only give figures for the lethal doses. Marked differences in the degree of absorption from the intestine, the rate of elimination, and other factors, and the effects in man must however be considered in the final comparison of the efficiency of these substances.

POTENCY OF RUBELLIN AS INFLUENCED BY ENVIRONMENTAL FACTORS.

Repeated investigations have been made to determine the influence of season and environmental temperature on the suceptibility of frogs to digitalis glycosides and allied compounds. The older workers have reported that summer frogs are more resistant than winter frogs, while others believed that the season of the year made little difference to the suceptibility of frogs. It has been shown that the higher the environmental temperature the greater the potency or toxicity of the cardiac glycosides on intact frogs or isolated frog hearts, especially over certain ranges of temperature.

The results obtained with rubellin in comparison with ouabain at room temperature at various times during the course of work are shown below.

	Date of assay	Mean diurnal temperature Degrees Centigrade	Potency
Spring	29.10.1943	19.5	128
	1.11.1943	16.3	120
Summer	17. 2.1949	21.0	125
	18. 2.1949	21.9	120
	21. 2.1949	22.4	132
Winter	6. 6.1949	17.3	129
	7. 6.1949	16.4	117

The results obtained are generally close enough and within the experimental error. Environmental factors may account for variability in the results, but animal variation (chiefly inherent in the heart) is more important. The difficulty with regard to varying susceptibility, particularly great in certain species of frogs, is minimised by using a considerable number of animals and by treating the results statistically. Control tests with a standard preparation are also performed in each assay. The overnight (16 - hour) mortality method gives more consistent results than the short time methods, as enough time is given for the drug to be absorbed.

ACTION ON THE HEART

ON THE FROG HEART

The South African clawed toad or frog (Xenopus laevis) is not tolerant to the "digitalis" series like Bufo and in this country is used instead of Rana in the biological assay of these drugs.

The reaction of the frog's heart to the glycosides transvaalin, rubellin and prorubellidin is characteristically the same as to other glycosides with the typical "digitalis" action. The drugs have a direct effect on the heart, producing slowing of the rate especially towards the end when arrest of the ventricle occurs eventually in systole; the auricles which are less sensitive eventually cease in diastole. While most of the changes can be followed in the exposed heart in situ without any recording apparatus, details were best studied in the isolated heart perfused with Ringer's solution to which one of the glycosides was added.

A. Perfusion of the isolated heart of Xenopus laevis:

The effect of the cardiac glycosides on the isolated suspended heart was studied by the perfusion method of Clark (1913).

(a) With transvaalin there was at first an increase in the size of the beat. The heart rate became

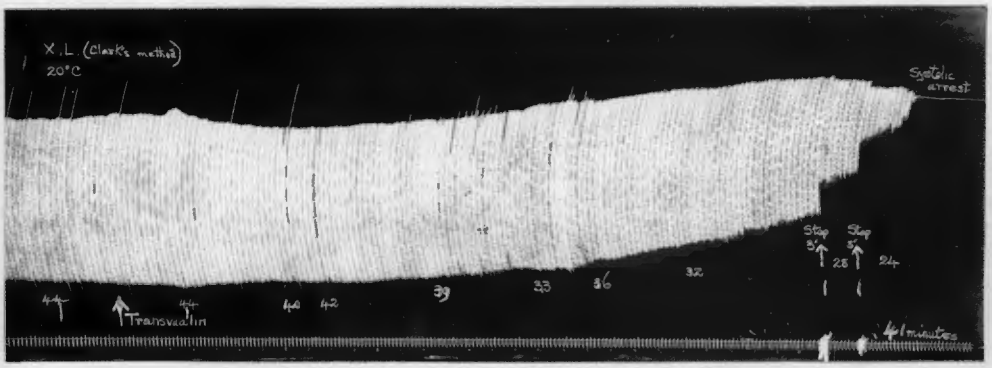


Fig. 1. Effect of transvaalin 1 in 6 million on perfused frog heart. Note reduction in ventricular rate and systolic arrest.

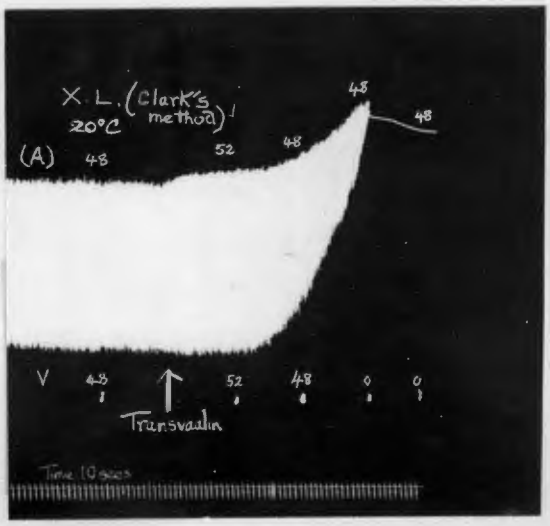


Fig. 2. Effect of transvaalin 1 in 3 million; auricular rate (A) above, ventricular rate (V) below, as counted during the actual experiment.

progressively slower in some experiments. The ventricle relaxed less completely during diastole, the line of diastoles of the kymographic record rising progressively from the base line, while systole was increased. Finally the ventricle ceased in tight contraction (systolic standstill). The auricles continued to contract for some time, ceasing in diastole later. These effects are illustrated in figures 1 and 2.

With a concentration of transvaalin 1 in 3 million the heart was arrested within ten minutes, and similar effects were sometimes produced with a concentration of 1 in 6 million; the sinus venosus continued to beat in some experiments after the rest of the heart stood still.

It is of interest that with these dilutions and with stronger solutions, e.g. 1 in 750,000 disturbance in conduction was seldom noted. This would suggest that transvaalin acts mainly on the myocardium, with less effect on auriculo-ventricular conduction. Similar results were noted by Gunn (1921) on the perfused frog heart with 1 in 5,000 of an extract of Urginea Burkei; no mention was made of conduction disturbances. However, in the isolated mammalian heart auriculo-ventricular block was observed.

Of closely related drugs it may be noted that

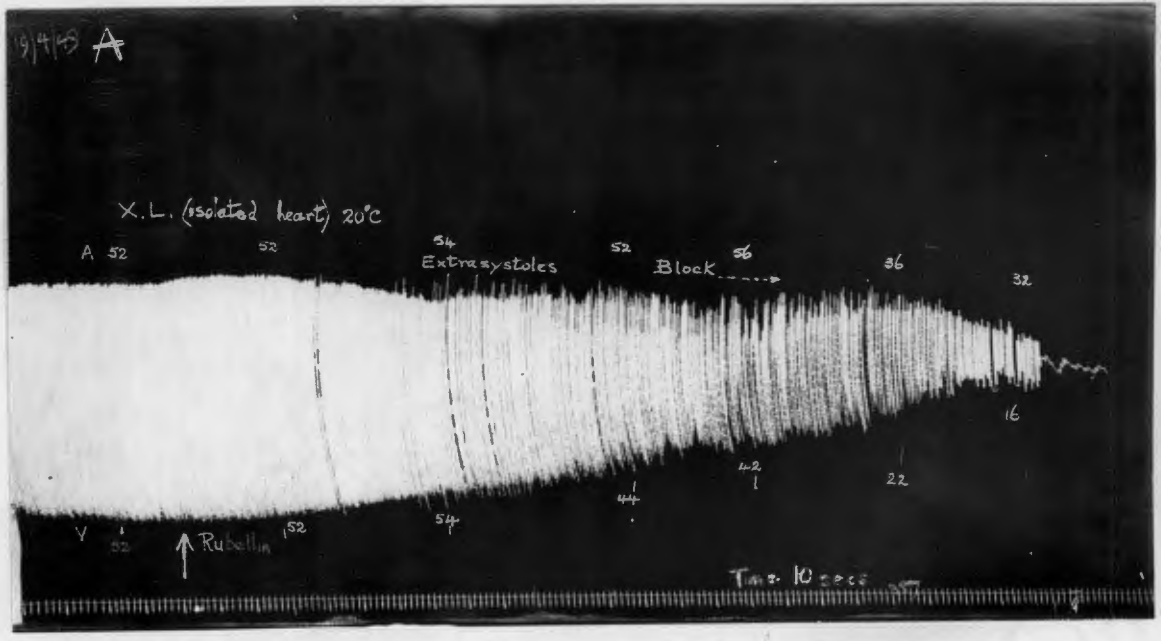


Fig. 3. Effect of rubellin 1 in 3 million on isolated frog heart. Note extrasystoles and auriculoventricular block, and arrest in systole.

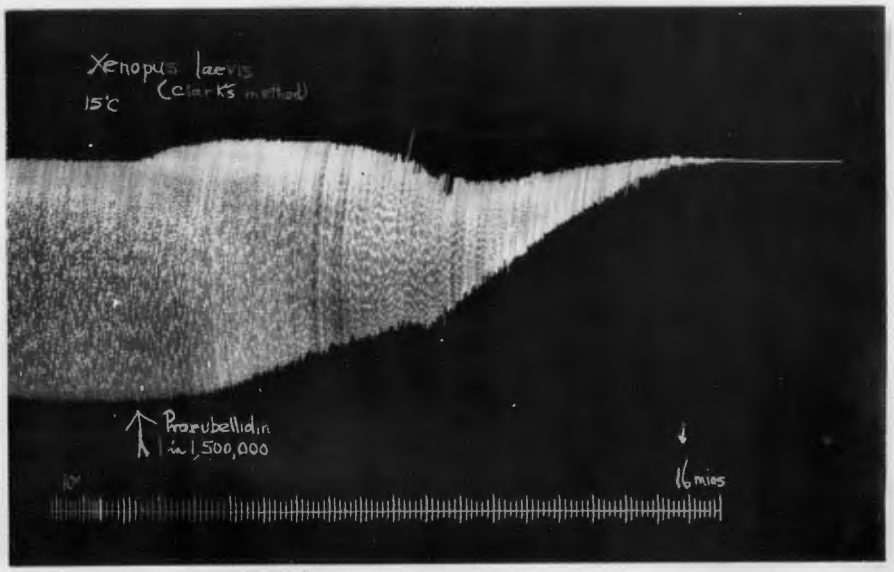


Fig. 4. Effect of prorubellidin 1 in 1½ million. Note increase in ventricular amplitude, followed by systolic tendency and arrest.

with extracts of Urginea altissima Epstein (1933) recorded marked auriculoventricular block, and Premankur De (1927) demonstrated that scillaren produces partial a-v block in the frog's heart.

(b) Rubellin, the glycoside from Urginea rubella, produced some improvement in the heart beat, slowing of the heart rate, conduction disturbances, and less and less relaxation of the ventricle, until it finally ceased in systole. In one experiment perfusion with a concentration of 1 in 6 million produced 2 : 1 block and sino-auricular block. A typical tracing is illustrated in figure 3.

In certain experiments performed on the isolated frog ventricle which was kept beating by regular electric shocks, rubellin 1 in 2 million produced contracture, but in the unstimulated ventricle complete systole also resulted without any regular beat having occurred. The heart takes up the glycoside and the action is a direct one on the muscle.

(c) Prorubellidin acted in a similar manner on the frog heart; a concentration of 1 in 6 million produced typical effects with 2 : 1 block a short while before terminal systolic arrest of the ventricle. A concentration of 1 in 1½ million stopped the heart in 16 minutes (figure 4).

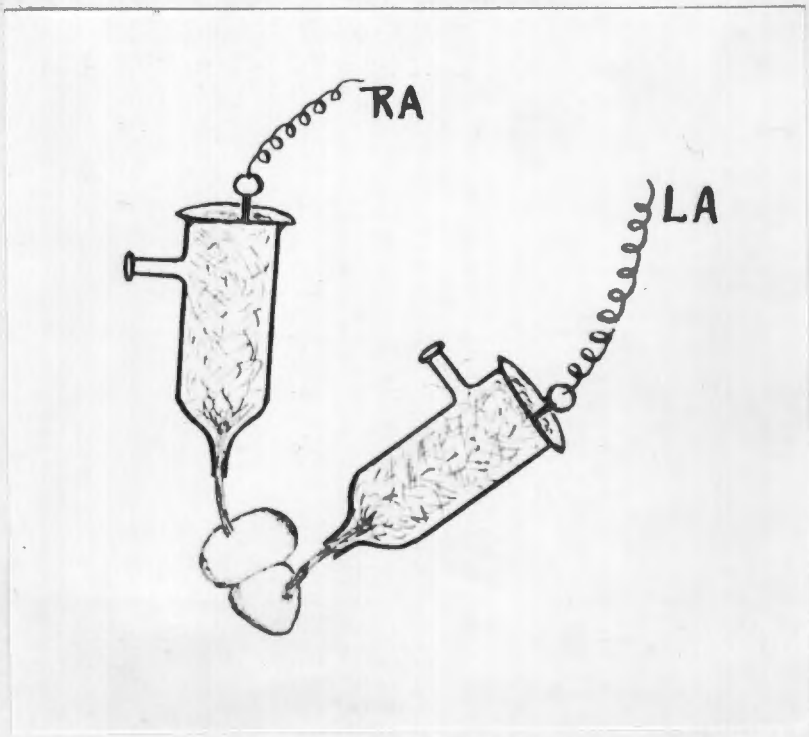


Fig. 5. Electrodes used in electrocardiography of isolated frog heart (glass cannula containing cotton wool and wick wet with saline).

B. Perfused isolated frog hearts - electrocardiographic records.

The effect of the glycosides on the electrocardiogram of the isolated perfused frog heart (Xenopus laevis) was recorded with the Cossor-Robertson cathode ray tube. The electrodes used were made from two arterial cannulae packed with cottonwool and with a wick projecting from the pointed ends (figure 5). The electrodes were soaked in frog Ringer's solution before use. One lead (RA) was lightly placed in contact with the upper surface of the auricle, the other (LA) was fixed so as to touch the apex of the ventricle, while the third (LL) lead of the instrument was earthed to the supporting stand of the apparatus.

The chief changes recorded when the heart was perfused with the glycosides were (figures 6, 8, 10): widening of the R wave, and depression of the ST segment; in some cases the T wave became lost in the downward limb of the R wave, in others the T wave became inverted.

There are very few reports on electrocardiography in the frog. The changes produced by the cardiac glycosides may be very striking, and are of interest for further investigation on the mechanism of production of the waves, especially the RT segment of the complex. The shortening of RT interval is the electrocardiographic

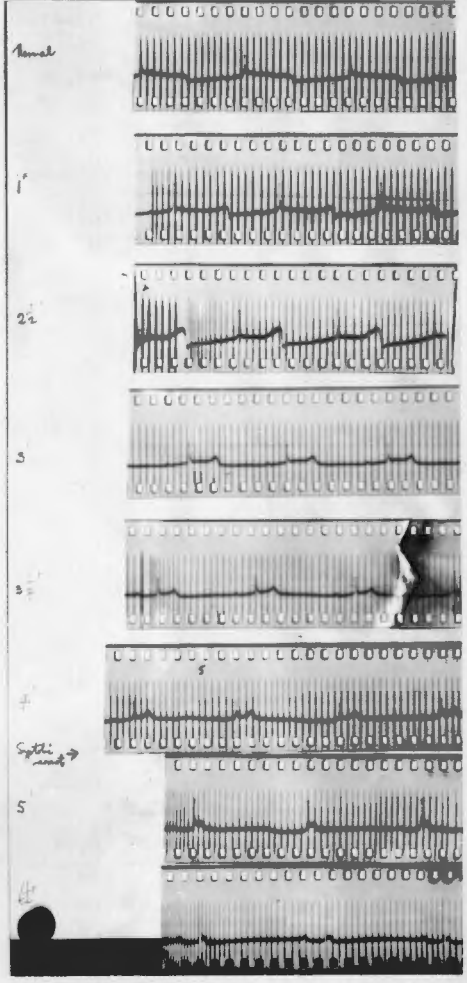
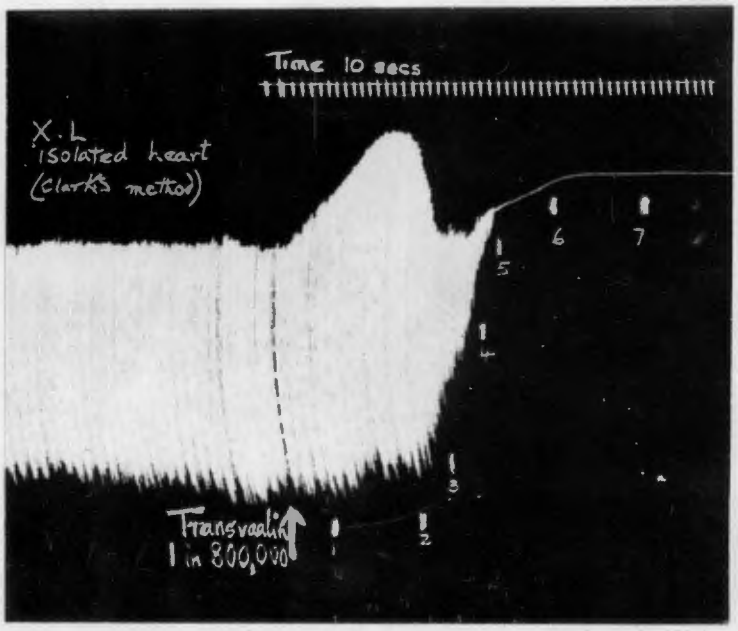


Fig. 6. Electrocardiograms taken (first tracing normal) at points indicated in figure 7.
Fig. 7. below: kymogram of isolated frog heart perfused with transvaalin 1 in 800,000.



equivalent of the shorter duration of ventricular systole. It is due to the direct action of the glycosides on the myocardium, and this electrocardiographic manifestation supports the theory of those workers who believe that myocardial effect represents the most important therapeutic mode of action of the cardiac glycosides.

C. Kymographic tracings were also made with electrocardiographic recordings taken simultaneously at intervals.

The Cossor-Robertson cathode ray tube was used to obtain the photographic electrocardiogram records. This instrument which allows one to see the electrocardiographic complexes made it more convenient to study the electrocardiographic changes occurring contemporaneously with the kymographic tracing. A typical experiment is illustrated in figure 6 - 7, and in figure 8 - 9.

As demonstrated in the records the electrocardiograms showed changes before marked changes occurred in the kymogram and tracings could still be obtained for some time after kymographic recording indicated the heart had stopped. Electrical impulses are still present when the ventricle is in systolic contracture.

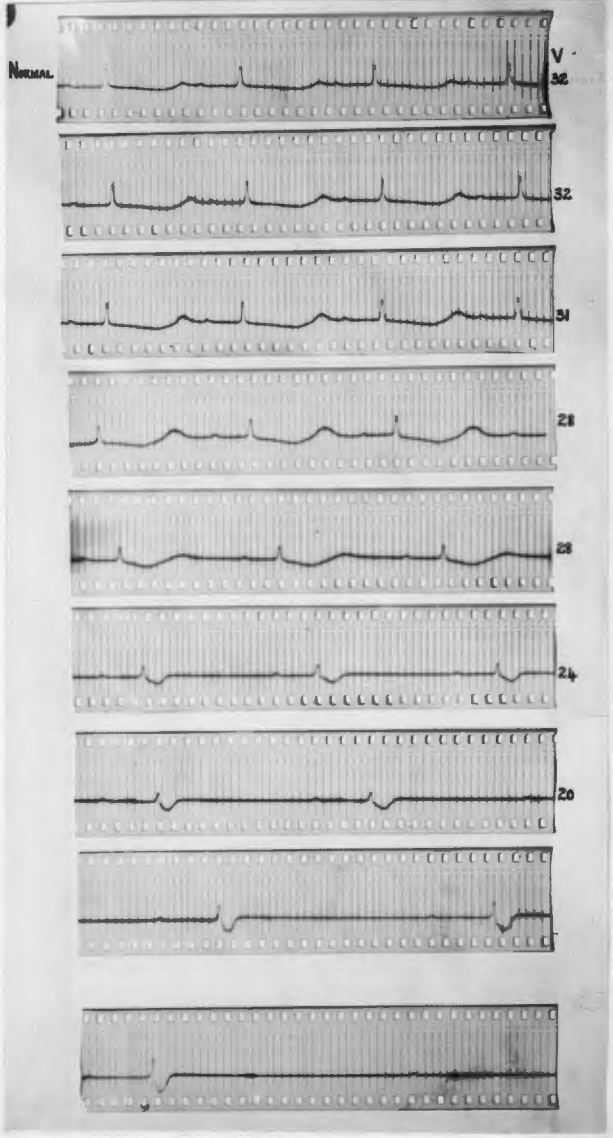
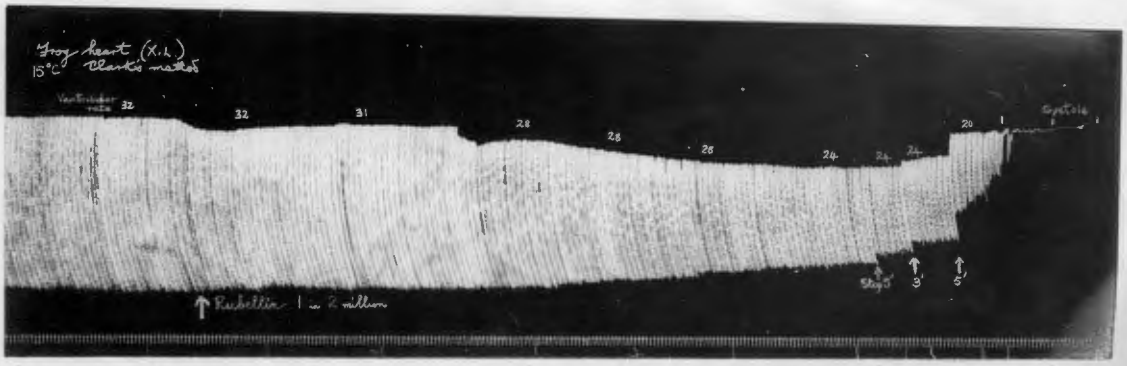


Fig. 8. Electrocardiograms taken at points indicating ventricular rate in figure 9. Note change in RT and deep wide S wave at systolic arrest in the kymogram.
Fig. 9. Effect of rubellin 1 in 2 million on perfused isolated frog heart. Note slow progressive reduction of ventricular rate and systolic arrest.



The effect of digoxin on rhythmically stimulated strips of frog auricle (Rana pipiens) and of turtle heart was studied by Wedd, Blair, and Dwyer (1941). They found that the drug invariably shortened systole, both electrical and mechanical, an effect that has also been observed in man. The shortening of the QT interval, the refractory period, and mechanical systole, which they observed may indicate (a) that recovery is more rapid, or (b) that the shortened systole contributes to recovery by permitting a longer diastole. The final action of the cardiac glycosides may therefore be related to the lengthened diastole produced, with reduction in the maintenance energy requirement and longer time for recovery. Such action is at times improved by a certain degree of slowing of the rate of contraction.

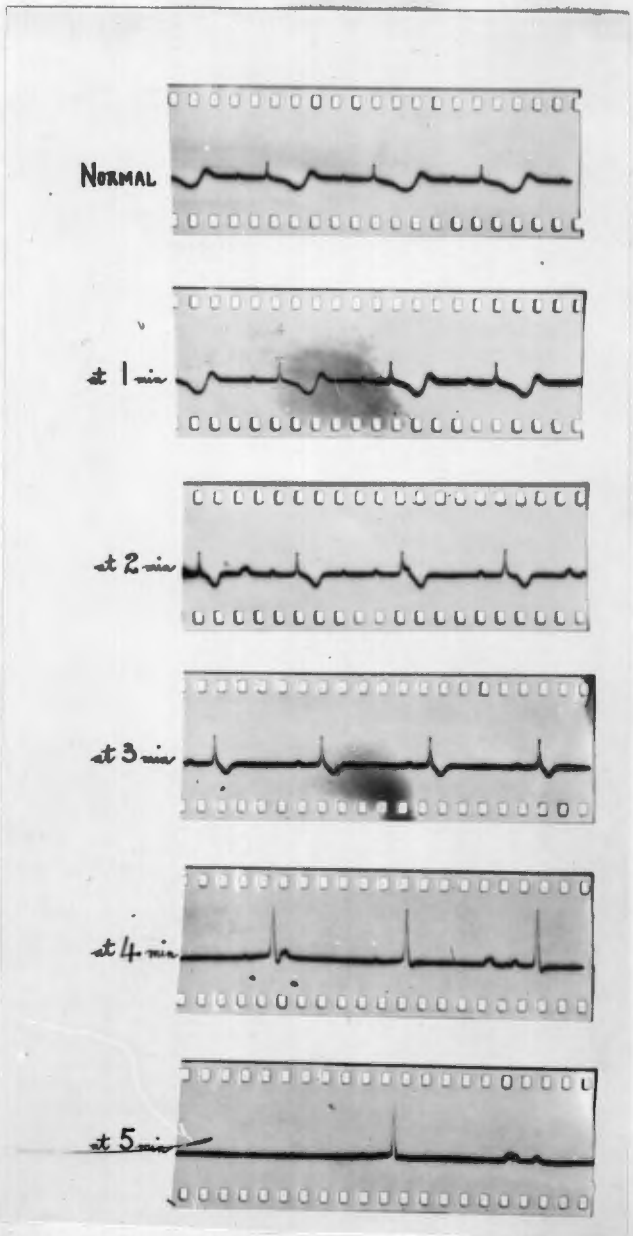


Fig. 10. Effect of transmaalin 1 in 800,000 on electrocardiogram of frog.
Note block at 2 minutes and RT changes.

ACTION OF OTHER DRUGS WITH DIGITALIS-LIKE EFFECT

The striking changes produced in the frog's electrocardiogram by adequate doses of the cardiac glycosides suggested an investigation of the effect produced by other substances known to have a digitalis-like action.

(a) Saponins: These substances and drugs of the digitalis series have many similar actions. They arrest the frog's heart in systole. Saponins are believed to affect cells by their power of reducing surface tension.

It was therefore of interest to determine whether the action of saponins resembles that of the cardiac glycosides used in this study as revealed by electrocardiographic records.

Also, on account of the difficulty that may occur occasionally in deciding whether a glycoside is a saponin or a "digitalis" body it would be useful to find some readily detectable difference in action (apart from reduction of surface tension and haemolysis) between these bodies. In this connection it may be mentioned that Gunn and Epstein (1933) from perfusion experiments suggested that the high tolerance of the toad heart (Bufo regularis) to the digitalis bodies and the absence of tolerance to sapotoxin be used as a biological test for distinguishing the action of these bodies. The method is however laborious. In any case,

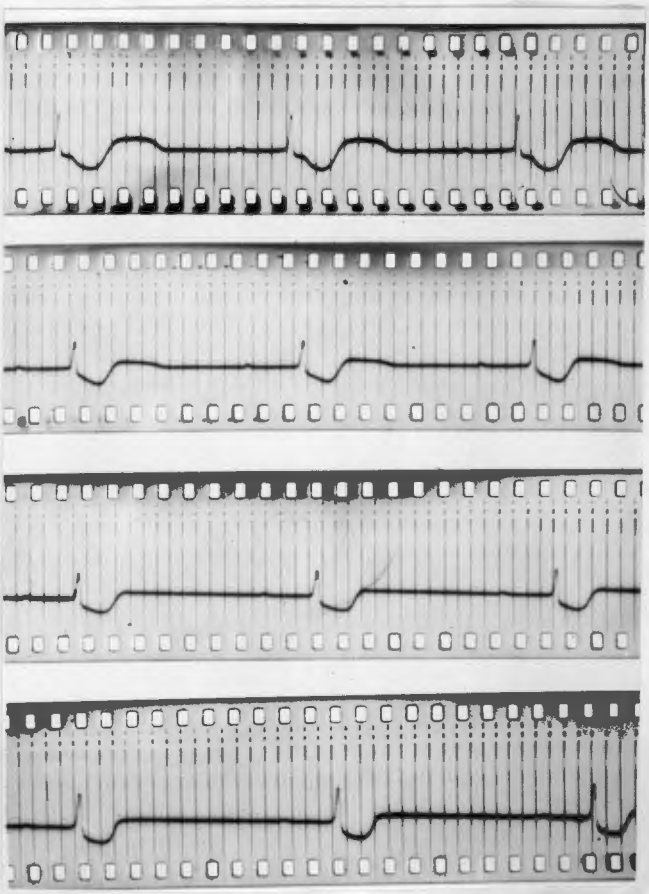


Fig. 11. Electrocardiographic changes in frog heart perfused with sapotoxin 1 in 6,000. Tracings taken at first four points indicated on kymogram below.

Fig. 12. Effect of sapotoxin 1 in 6,000 on frog heart; note digitalis-like action.

perfusion of the frog heart of Xenopus laevis (which animal is more readily available) cannot be used for this differentiation of the drugs as they found no marked difference in the action (of sapotoxin and strophanthin) in this animal.

Results: In several experiments where the frog heart (Xenopus laevis) was perfused with sapotoxin in concentrations varying from 1 in 6,000 to 1 in 12,000 and the effect recorded kymographically it was noted that differences in the action of the cardiac glycosides (transvaalin and rubellin) and sapotoxin could not be detected in any one experiment but could be noted in a series. With the saponin the systolic arrest of the ventricle was seldom so firm or complete as with the cardiac glycosides. Electrocardiographic studies revealed no detectable difference in the action of the drugs. The effect of sapotoxin 1 in 6,000 on the frog heart is shown in the kymographic and electrocardiographic tracings in figures 11 and 12. The typical ECG change observed is again shortening of the RT segment, disappearance of the T wave, and finally a deep S wave.

Sapotoxin is certainly less potent on the heart as shown by the big differences in concentration required to produce the effects described.

(b) Methyl violet: This dye, which induces systolic

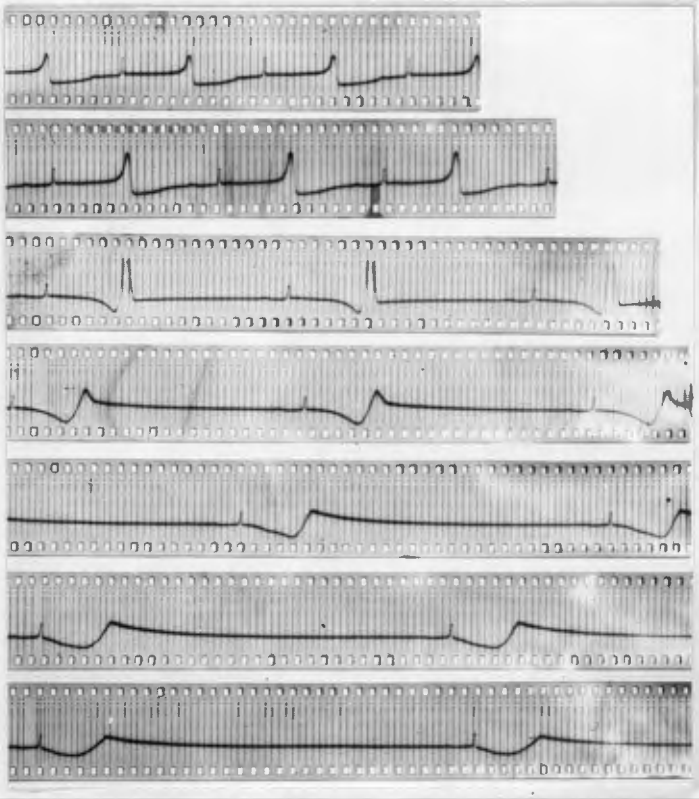


Fig. 13. Effect of methyl violet on the electrocardiogram of the frog. Note slowing and RT changes. Tracings taken at points indicated on kymogram below.

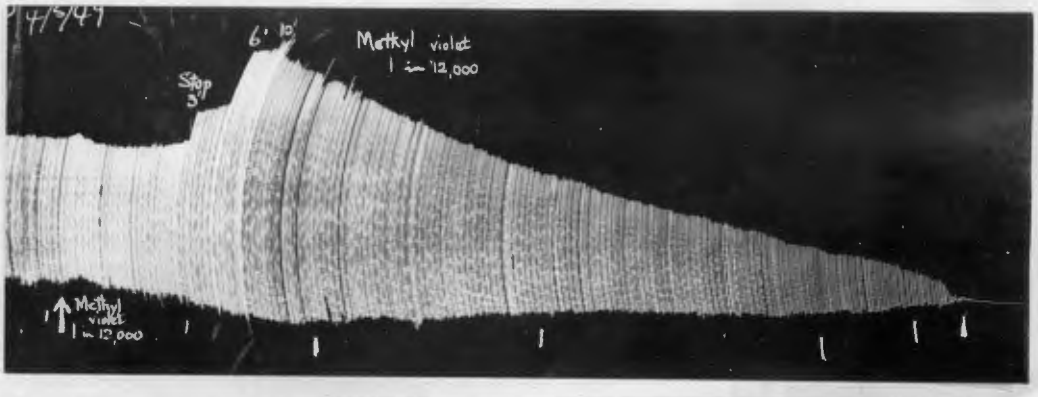


Fig. 14. Effect of methyl violet 1 in 12,000 On frog heart. Note improvement in the ventricular contraction and slowing of the rate.

arrest like strophanthin, is withdrawn rapidly from the perfusing fluid (Ringer's solution) which becomes colourless, the heart muscle combining with and taking on the colour of the dye. The effect of this substance on the heart and the electrocardiogram is shown in figures 13 and 14.

(c) Barium chloride produces an action on the heart which resembles the action of digitalis, but only superficially. More complete systole, diminished diastole (figure 16) and heart block may occur. The electrocardiograms revealed increased and widened R waves, but no striking changes in the T segment (figure 15). This suggests that the mode of action of the barium salt is different from that of the cardiac glycosides.

The effects of barium have been attributed to the precipitation of colloidal barium sulphate which would modify the permeability of cells analogous to the change in anaphylaxis. Another suggestion that has been made is that deprivation of sulphate ions occurs but this view has been disproved.

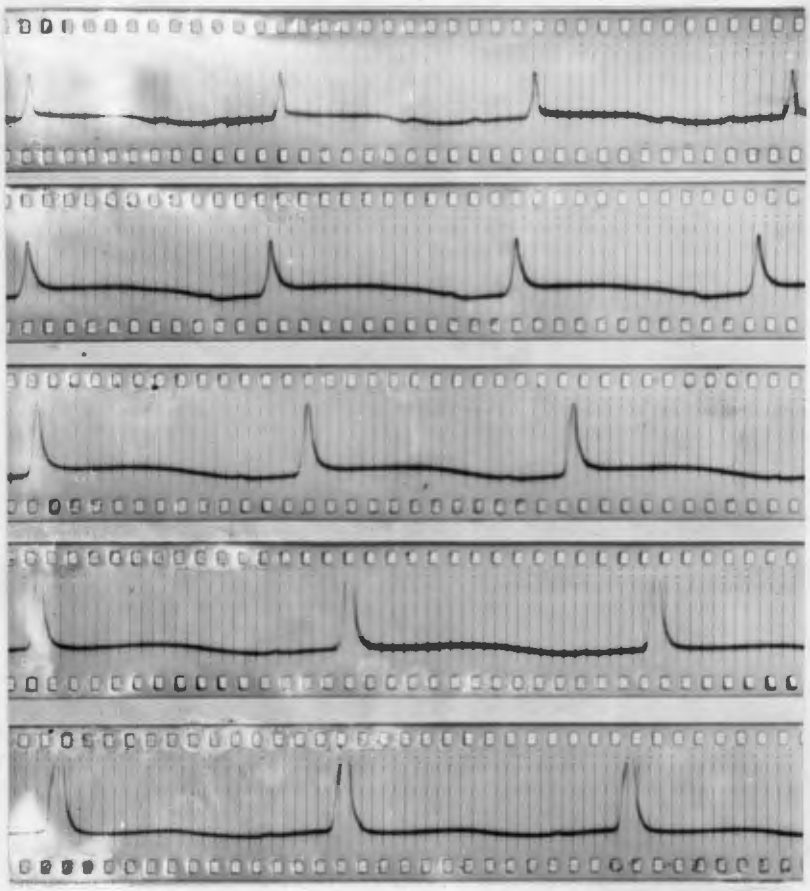
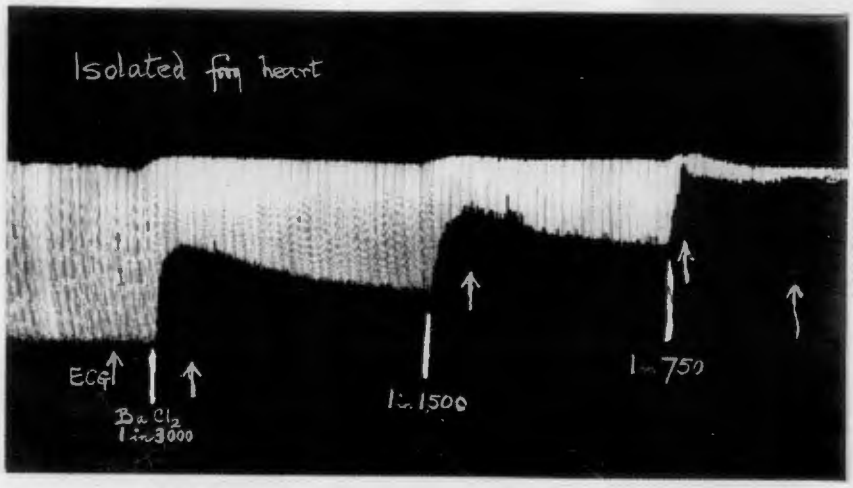


Fig. 15. Effect of barium chloride on electrocardiographic tracings taken at points indicated in figure 16. Note increased R wave, and slowing of ventricular rate.
Fig. 16. Effect of barium chloride 1 in 3,000, 1 in 1,500, and 1 in 750 on isolated frog heart.



EFFECT OF DIBENAMINE ON THE TOXICITY OF CARDIAC GLYCOSIDES IN FROGS

It was of interest to determine whether dibenamine, one of the more recently discovered adrenergic blocking agents, would protect the frog heart against the toxic effects of rubellin. Both the benzodioxanes 933F and 883F have been found ineffective in digitalis overdosage in cats (Emerson, 1943), although they are effective against heterotopic rhythms produced by electrical stimulation and barium chloride (Dongen, 1939) apparently on the basis of direct myocardial depression. Barium chloride produces effects resembling those of the digitalis group.

Spühler (1936), according to Kisch (1944), "tried to prove that strophanthin has a general influence on the whole sympathetic nervous system, e.g. by increase of the supra-renal production", and states that the experiments challenge re-examination.

Dibenamine, like other adrenergic blocking agents, does not inhibit the chronotropic and inotropic actions of adrenaline, and reflex and direct sympathetic nerve stimulation, on the mammalian myocardium, but the chronotropic response of the amphibian heart to adrenaline is blocked and even reversed by dibenamine and related compounds. Dibenamine does however prevent the

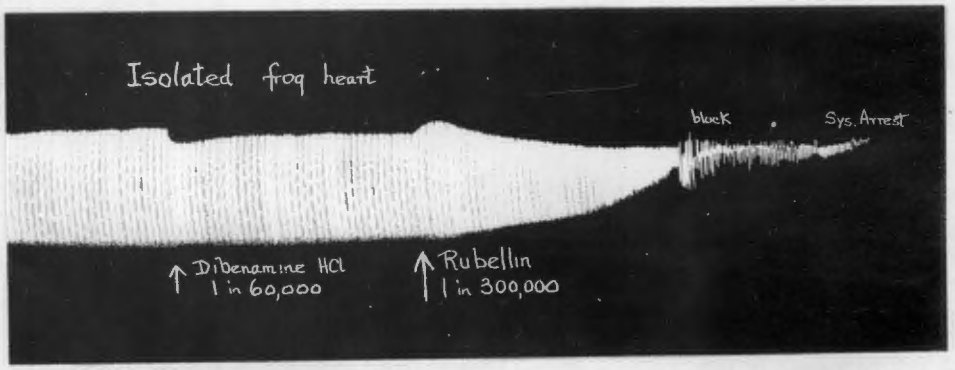


Fig. 17. Failure of dibenamine 1 in 60,000 to prevent the action of rubellin 1 in 300,000 on perfused frog heart.

cardiac arrythmias induced in the mammalian heart by adrenaline alone in unanaesthetised animals and man or after myocardial sensitisation by volatile hydrocarbons, affording protection apparently by virtue of its adrenergic blocking activity

In isolated perfused frog hearts (Xenopus laevis) dibenamine did not prevent the effects of the glycosides e.g. after ten minutes perfusion with dibenamine hydrochloride 1 in 60,000 a concentration of rubellin 1 in 300,000 produced auriculoventricular dissociation and systolic arrest (figure 17). With prorubellidin and transvaalin too no protection was given by dibenamine. In intact frogs dibenamine also did not prevent the action of rubellin from producing systolic arrest.

THE EXCITABILITY OF THE MYOCARDIUM

The effect of rubellin on the excitability of the frog heart was studied on the isolated ventricle, which was made to contract by stimulation with rhythmical electrical shocks at 5 second intervals. Minimal effective stimuli were used.

A typical result is illustrated in figure 18. The numerals refer to the distance (in millimetres) between the coils of the Du Bois-Reymond induction apparatus and show that excitability was at first increased, but was decreased later, stronger electric shocks being required to produce contractions; eventually stimuli were ineffective ~~as~~ when the ventricle passed into permanent systolic standstill (a state of prolonged contraction).

That toxic doses of digitalis diminish or abolish the irritability of the heart to electric shocks has been reported by other workers. Gies (1927) found that strophanthin shortened but then prolonged the refractory period in the frog's heart. Kisch (1944) made similar observations, but suggests that the heart muscle may be irritable to naturally conducted stimuli, which are of longer duration, even if it does not respond to highly intensive galvanic, faradic, and mechanical stimuli.

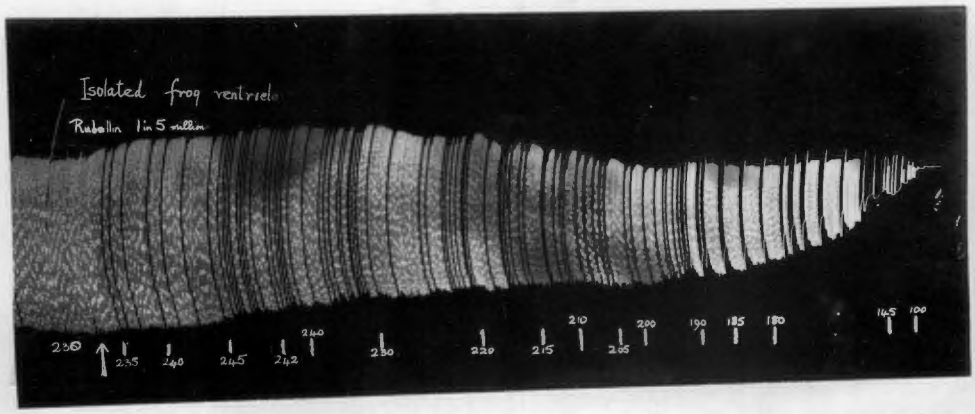


Fig. 18. Effect of rubellin 1 in 5 million on excitability of isolated frog ventricle. Numerals show temporary increase followed by decrease in excitability of the muscle.

Irregularities in rhythm, intermissions and half-rhythm, in the poisoned frog's heart, have been ascribed to failure of excitability of the ventricle, but may be due to depressed conduction rather than failing excitability by the natural impulses.

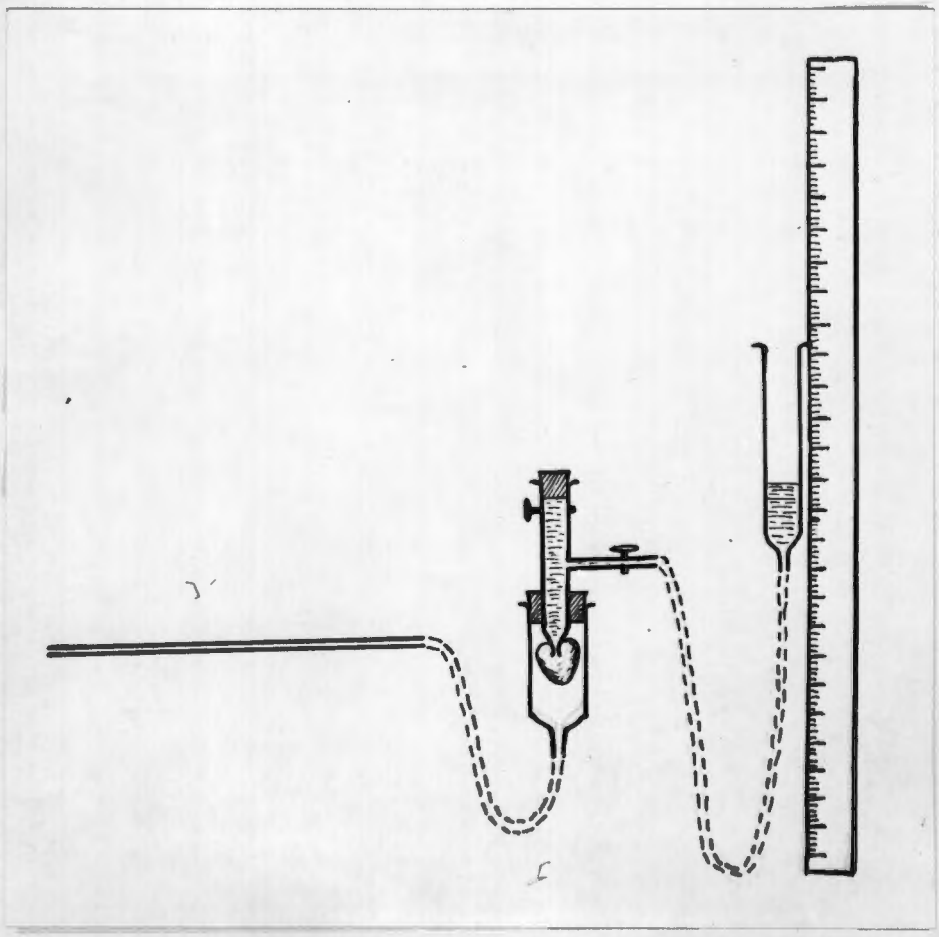


Fig. 19. Apparatus used to measure coefficient of elasticity of frog ventricle in contracture.

THE CONTRACTURE

There have been many investigations to elucidate the mechanism of action of "digitalis" glycosides in the heart. As is well known these drugs produce an increase in the amplitude of contraction and later cardiac arrest. With higher (toxic) concentrations early systolic arrest occurs, indicating an increase in the coefficient of elasticity which impedes filling.

In the following experiment the effect of the water soluble glycoside rubellin on the coefficient of elasticity of the frog ventricle in contracture was studied.

The frog heart (Xenopus laevis) was excised. A cannula was passed through the cut aorta into the ventricle, and a ligature tied round the heart at the auriculo-ventricular junction. By means of a reservoir of frog Ringer's solution attached to the cannula the pressure in the ventricle was raised from the level of the heart to 30 cm. above it. The volume of the ventricle was read on a horizontal tube (1 ml. pipette as scale) connected to the cardiometer in which the ventricle was fixed (figure 19).

The pressure was first increased in stages, and then decreased. The reservoir was raised each time from the heart level to the desired pressure.

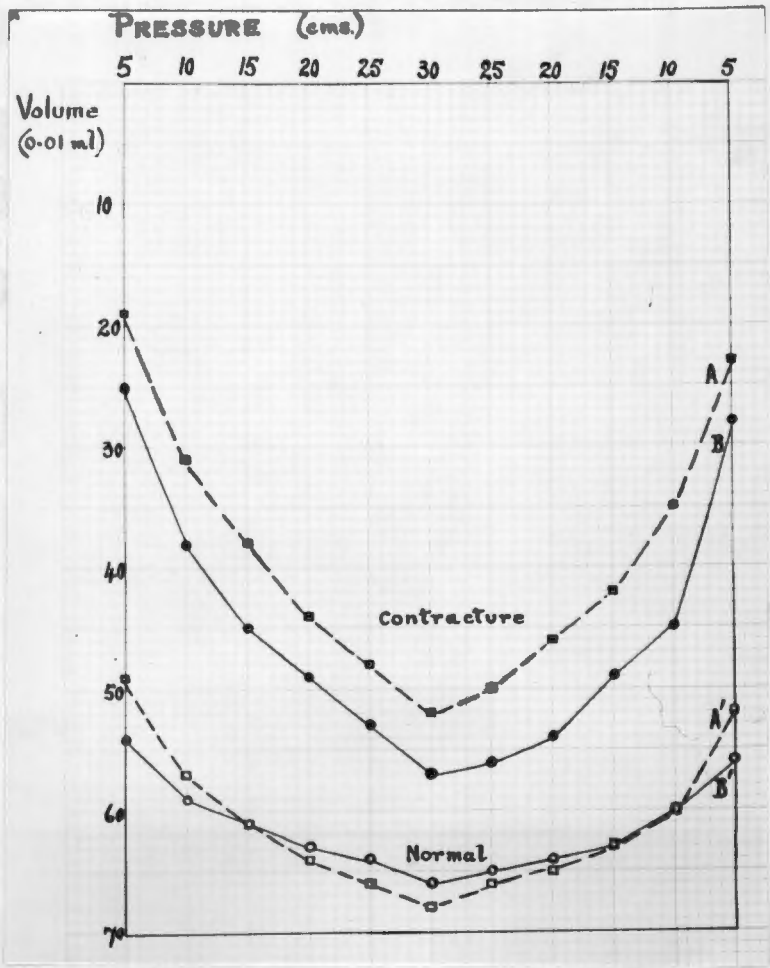


Fig. 20. Graph showing volume changes of ventricle in contracture more marked and at a higher level than that for the normal.

Contracture of the ventricle was produced by introducing the rubellin solution into the cannula, and the changes in volume than observed under the same series of pressures as before. On drawing graphs of the volume changes the curve in contracture is shown to be at a higher level than that for the normal heart, the two curves running parallel throughout (figure 20).

The results show a large increase in the coefficient of elasticity of the heart in contracture.

The state of contracture is however of little practical importance because the doses producing it exceed the range of possible clinical usefulness.

With moderate doses of certain cardiac glycosides, it has been shown that diastolic filling becomes faster and greater, indicating a lowered elasticity with therapeutic doses of the drug. Thus the "tone" of the heart muscle does not appear to be improved by the glycosides; they produce essentially an increase in mechanical efficiency (Moe and Visscher, 1938). According to the latter workers Cushny (1925) is probably incorrect in believing that the "tone" of the heart muscle is improved by digitalis, unless this term is regarded as synonymous with mechanical efficiency as was done by Starling and Visscher (1927).

ON THE MAMMALIAN HEART

A. MOVEMENTS OF THE HEART:

Cats were used in these experiments. The animals were anaesthetised with pentobarbitone sodium given by mouth (1 gr. per kgm. body weight), or with ether. The movements of the heart were then recorded with Cushny's myocardiograph. Blood pressure was recorded from the carotid artery. Injections were made into the external jugular vein. As with other cardiac glycosides the changes in the heart's action could be demonstrated slowly even after intravenous administration of the drugs.

Following the injection there may be an increase in the amplitude of the auricular and ventricular beat with slight rise in blood pressure. The heart then becomes slowed and irregular to a variable degree in different animals. Auricles and ventricles may soon contract independently, the ventricles beating with faster or slower rhythm than the auricles. Later, the excitability of the heart muscle increases and the ventricle beats rapidly. Periodic grouped beats with variations in the strength of both auricles and ventricles may occur. Premature contractions may be produced, many of these not expelling blood into the aorta. The blood pressure shows marked fluctuations at this stage; with

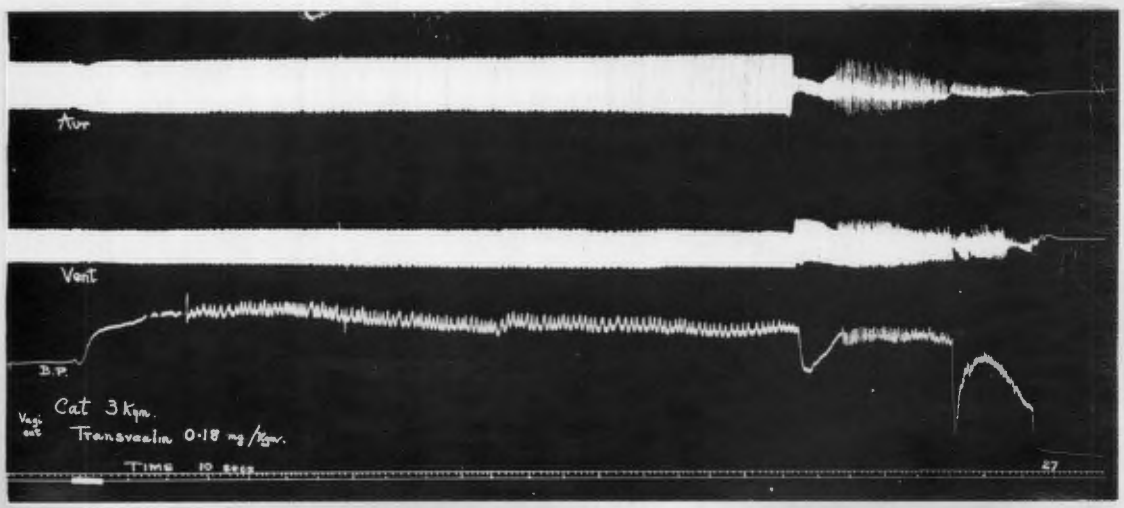


Fig. 21. Effect of transvaalin 0.18 mg. per kgm. body weight on auricular and ventricular contractions and blood pressure of the cat. Note increased excitability, A-V dissociation, and fluctuations in blood pressure.

terminal fibrillation the blood pressure falls to zero.

(a) Transvaalin in suitably large doses produced typical changes in the ventricular and auricular contractions as described above. The three stages originally described by Cushny (1925) were observed. The heart was finally arrested. The blood pressure also showed the characteristic changes with fluctuations in the level and finally a fall to zero. The administration of atropine or division of the vagus nn. did not prevent the effects. A typical tracing is illustrated in figure 21.

(b) Rubellin was also very active, the heart being affected by smaller doses than with transvaalin (see table of fatal doses). Thus, for example (figure 22) with 0.1 mg. per kgm., increase in the amplitude of auricular and ventricular contractions, and extrasystoles were produced, with little change at the end of thirty minutes; another intravenous dose of 0.1 mg. per kgm. then produced marked irritability, smaller and irregular contractions, fibrillation, rapid fall of blood pressure, and death within five minutes. In another experiment a single dose of 0.13 mg. per kgm. produced an increase in the amplitude of ventricular contractions, then irregularity, auriculo-ventricular dissociation, auricular

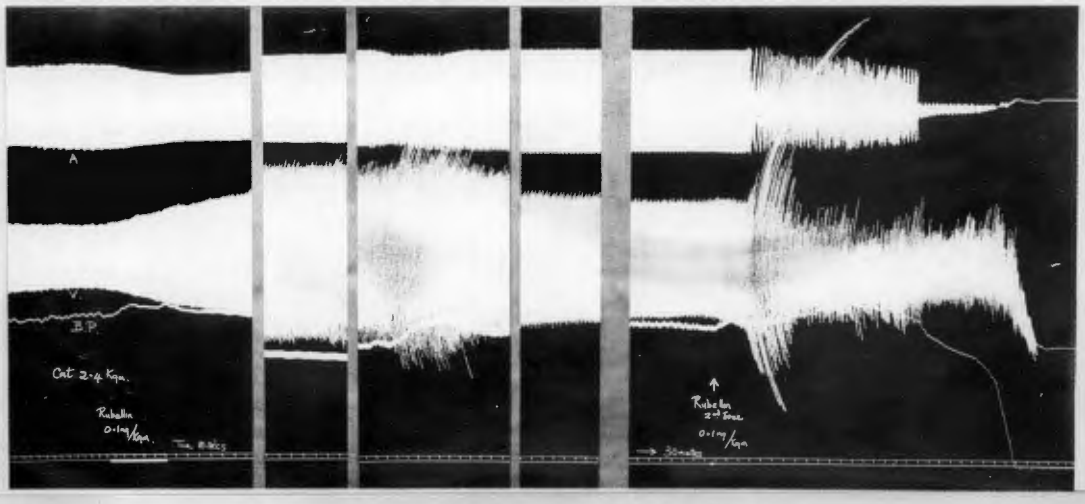


Fig. 22. Effect of rubellin on auricular contractions, ventricular contractions and blood pressure of the cat (two doses, each 0.1 mg. per kgm.). Note increased amplitude after first dose, and marked disturbance after second dose given 30 minutes after first injection.

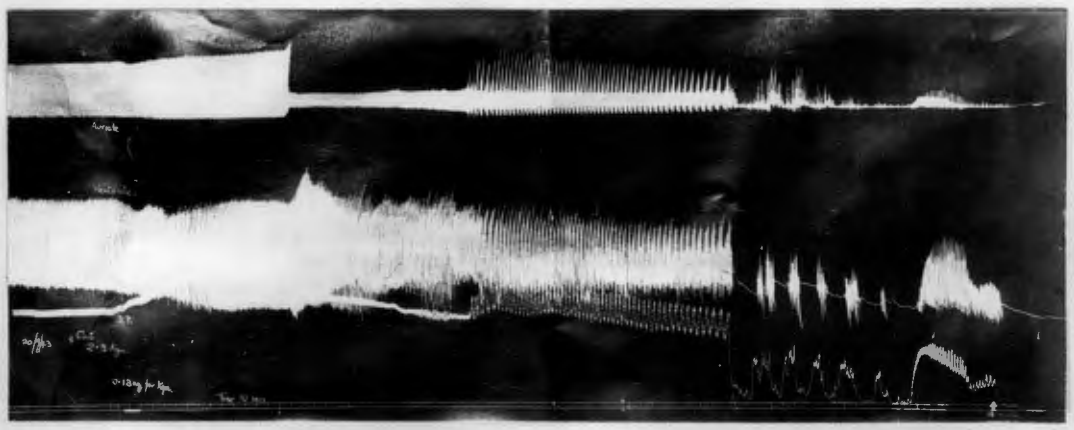


Fig. 23. Effect of rubellin on myocardiogram of cat which received 0.13 mg. per kgm. Note increased contractions followed by A-V dissociation, auricular flutter and fibrillation, periodic beats and irregularities in blood pressure.

flutter and fibrillation; periodic fluctuations and a progressive fall in blood pressure; ventricular fibrillation and sudden arrest of the heart in twelve minutes (figure 23).

Slowing of the ventricular rate noted by Isaacson (1949) in cases of auricular fibrillation treated with rubellin was no doubt attributable in part to the effect of the glycoside on the junctional tissue. Slowing of the heart beating with a regular rhythm was observed both in the animal experiments and in patients with congestive cardiac failure with regular rhythm and tachycardia.

With the Cushny myocardiograph Stehle, Ross and Dreyer (1931) found that scillaren B increases the amplitude of the ventricular beat. Peters and Visscher (1936) demonstrated an increase in the efficiency of heart muscle at constant external diastolic volume after the administration of scillaren. In experimental animals scillaren also produces heart block, premature contractions, and ventricular fibrillation, when large doses are administered.

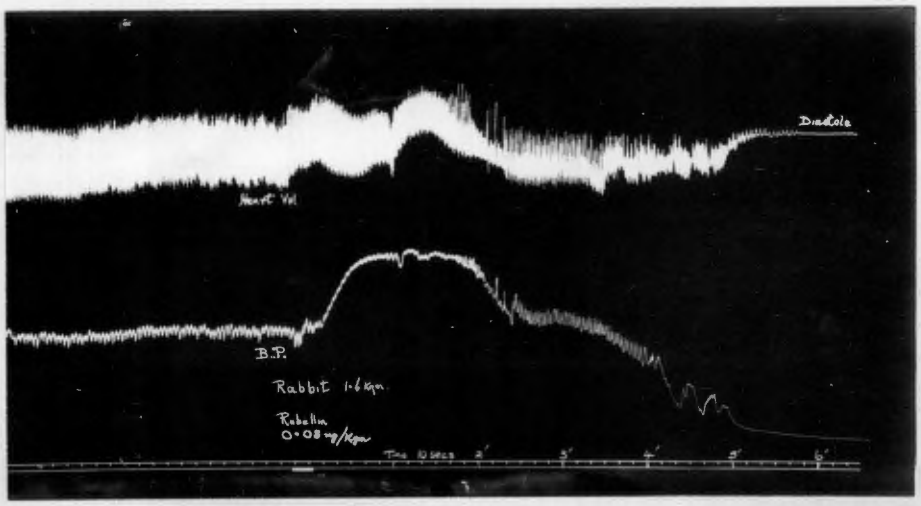


Fig. 24. Effect of rubellin on volume changes in rabbit heart; dose 0.08 mg. per kgm. Note diminution in volume and cessation in diastole; marked rise in blood pressure at first.

B. CARDIOMETER EXPERIMENTS.

These were performed on cats anaesthetised with pentobarbitone or ether, or on rabbits anaesthetised with urethane. Respiration was maintained by artificial respiration once the thorax was opened. A thistle funnel of suitable size placed over the heart and within the pericardial sac was connected to the recording tambour which gives a record of the heart beat. With small "therapeutic" doses of the glycosides no marked increase in the output of the heart was observed, but with large (toxic) doses, the output rapidly became diminished as the auricles began to fibrillate (figure 24).

In cardiometer experiments made on cats Premankur De (1927) demonstrated an increase in systolic contraction with slight increase in the stroke volume after the intravenous injection of scillaren.

Comments on the effect on cardiac output following the administration of cardiac glycosides will be found later in this work.

C. ON THE ELECTROCARDIOGRAM:

Convincing evidence of the digitalis-like action of the glycosides was recorded on the electrocardiograms of cats which were lightly anaesthetised with ether. The drugs were injected into the right femoral vein at a slow steady rate of 1 ml. per minute. The tracings were taken from lead II at different stages of the experiment. The effect of various doses, lethal and sublethal, was investigated.

Typical changes in the electrocardiogram produced by large doses of the glycosides are shown in figures 25 and 26.

The chief changes in the electrocardiogram produced by the glycosides were slowing of the rate, prolongation of the PR interval, ectopic rhythm, secondary tachycardia, and finally ventricular tachycardia.

Toxic doses produced impure auricular flutter, ventricular paroxysmal tachycardia and widening of the QRS complex, while doses considered "therapeutic" produced a decrease of the cardiac rate without significant changes in the configuration of the complexes.

Rubellin given intravenously to cats in small doses e.g. 0.003 mg./kgm. body weight or 0.006 mg./kgm. produced slowing of the heart rate; the complexes were unaltered. With 0.012 mg./kgm. (a) slowing of the

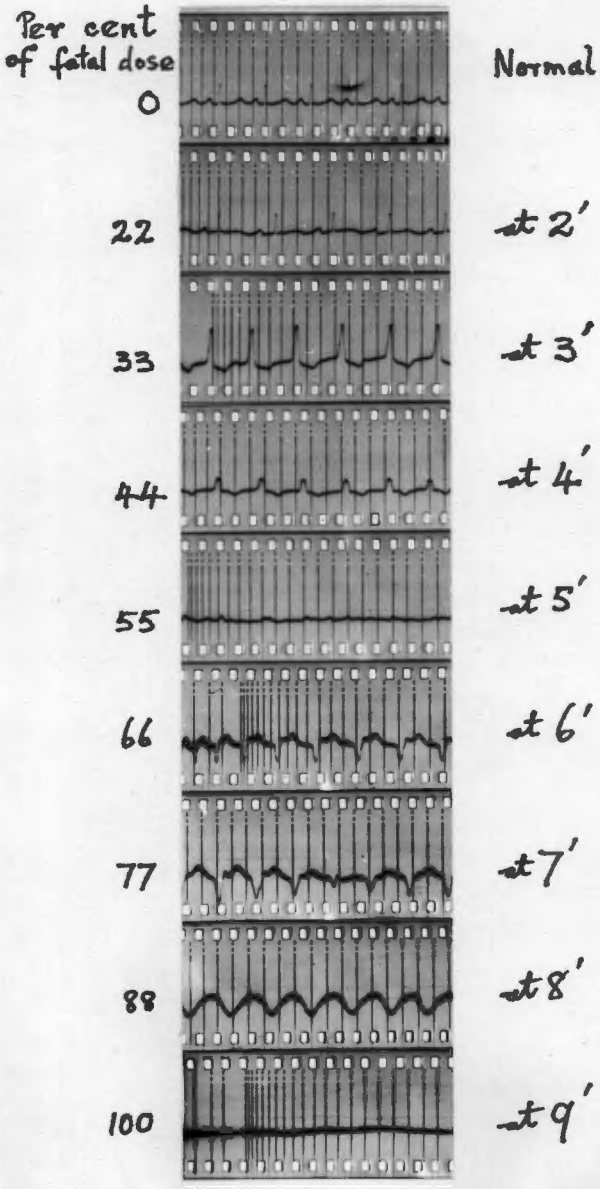


Fig. 25. Electrocardiographic changes produced in the cat by transvaalin 0.2 mg. per kgm. 1 cm. 1 mv. Normal tracing followed by tracings taken at 2,3,4,5,6,7,8,9, minutes after intravenous injection, at 0, 22,33,44,55,66,77,88, and 100 per cent of fatal dose.

rate, and impure auricular flutter were noted, and alternation of the QRS complexes; (b) paroxysmal ventricular tachycardia was also observed in another animal in which death occurred two days later; (c) widening of QRS (intraventricular block) and temporary acceleration of the heart occurred in another animal which died six hours after the experiment.

Assuming the human heart would react like the cat heart the dose used for investigation in man was calculated on the basis of the dose 0.003 mg. per kgm. body weight, i.e. for an average man of 70 kgm. body weight the dose would be 0.21 mg. This dose was actually used by intravenous injection in studies on patients by Isaacson (1949).

Thus, in experimental animals (and in man with rubellin) the urginea glycosides under investigation produced alterations in the electrocardiogram resembling those produced by digitalis.

The changes in the electrocardiogram following the administration of squill glycosides resemble those produced by digitalis, particularly with regard to the RS-T segments. Van Dyke and Wallace (1933) noted an increase in the PR interval, dropped beats, sinoauricular block, and ventricular extrasystoles in cats and dogs which received scillaren. With urginin

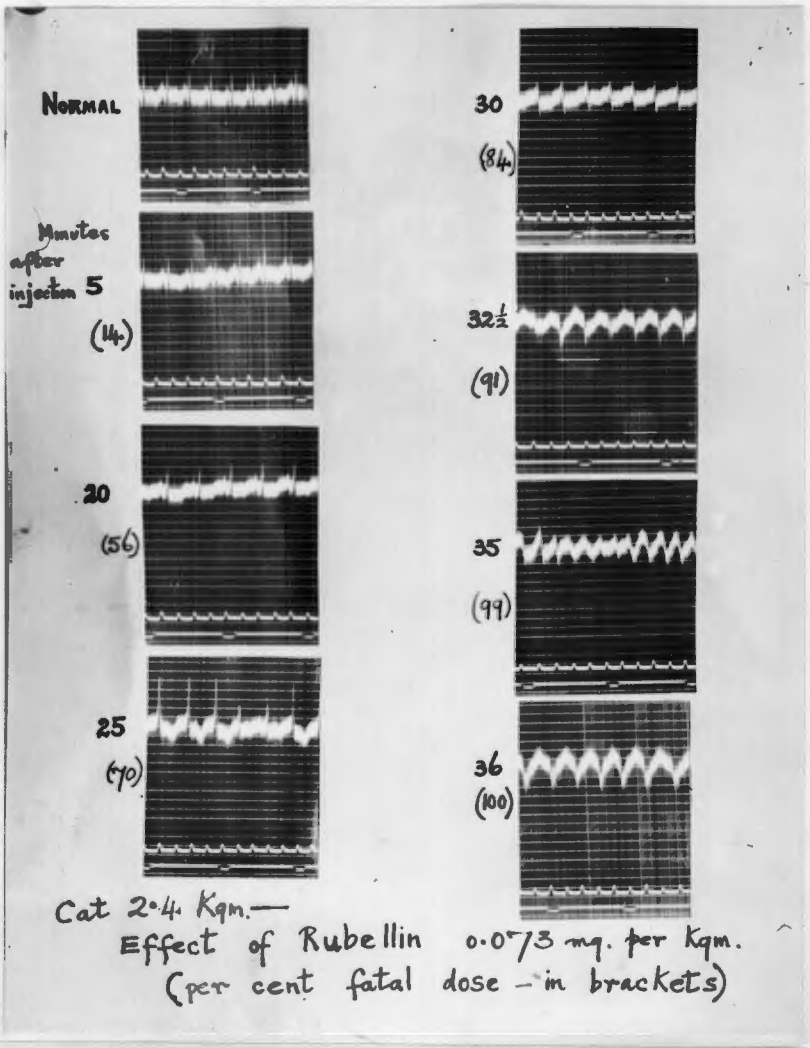


Fig. 26. Electrocardiographic changes produced in the cat by rubellin 0.073 mg. per kgm. The drug 1 in 200,000 solution was injected into the right femoral vein at a rate of 1 ml. per minute. Eight tracings taken from lead 2 at different stages of the experiment are shown. Time 0.2 and 1.0 second; ordinates represent 0.0002 volt.

the changes produced in cardiac patients are the same as with digitalis when equal amounts of the drugs are given over equal periods of time (Maher and Sittler, 1936). Larger doses produced extrasystoles, prolongation of the PR interval, and auricular fibrillation. Turnbull (1910), Windle (1911), White and others (1920) have also reported the effects of overdosage with squill.

In normal subjects as well as in those suffering from cardiac failure alterations in the ST segment of the electrocardiogram may be produced by digitalis. They are frequently the earliest effect observed, especially depression of ST, and inversion of T_3 but in some cases also of T_1 and T_2. The T waves may sometimes become diphasic. These changes are due to an action on the heart muscle, and are an important sign, appearing before nausea and vomiting, or even before changes in rhythm or in conduction time. Transvaalin and rubellin given to man by mouth produced no gastrointestinal disturbance, and do not appear to be satisfactorily absorbed. Rubellin given intravenously did not produce nausea and vomiting, but marked electrocardiographic changes were recorded. Similar observations have been made with regard to squill glycosides. These points will be discussed again later.

Numerous reports have been made on the

electrocardiographic changes produced in the experimental animal by cardio-active glycosides administered intravenously. Gold et al. (1930) compared the effects of seven varieties of digitalis which were given intravenously at five minute intervals in the cat, and found the qualitative changes to be similar but individual variations were extreme; no electrocardiographic sign was found which was as accurate as the minimum lethal dose for evaluating the toxicity of the drug (Gold et al., 1931). These experiments were performed on cats without the use of general anaesthesia. Scott and Wheeler (1946) made a statistical study of the electrocardiographic changes during the bioassay of digitalis in 218 cats by the U.S.P. $\overline{XII}$ method. They found that under ether anaesthesia coupling is more frequent before than after the infusion of digitalis, that the PR interval for a given heart rate is smaller under the influence of digitalis, that depression of the ST segment was more constant than changes in the T wave alone, and that the interval between A-V dissociation and the lethal dose tends to be constant. This last observation implies that the chief variable is the point at which complete A-V dissociation occurs, the resistance to a toxic level of digitalis beyond this point being fairly constant from one individual to another.

(Extrasystoles have been reported by Kurtz, Bennett, and Shapiro (1936) to occur in twenty per cent of human cases when ether is used as an anaesthetic, and Frommel (quoted by Scott and Wheeler (1946)) has reported the occurrence of coupling in guinea pigs under ether anaesthesia).

D. EFFECT OF DIBENAMINE ON THE TOXICITY OF THE CARDIAC GLYCOSIDES IN CATS.

The introduction of potent cardiac glycosides and the method of rapid digitalisation sometimes used increases the necessity for finding a drug which will inhibit their toxic effects on the heart.

Adrenergic blocking agents such as dibenamine, priscol, yohimbine, ergot alkaloids and benzodioxanes inhibit certain cardiac arrythmias in animals such as those induced by anaesthetics and other sensitising hydrocarbons. Both the benzodioxanes 933F and 883F have been found completely ineffective in digitalis overdosage in cats (Emerson, 1943) but according to Dongen (1939) they are effective against ectopic rhythms produced by barium chloride and adrenaline.

It was of interest to determine whether the disturbances of the heart's action produced by rubellin would be suppressed or inhibited by adrenergic blocking agents. For this purpose dibenamine hydrochloride was used, this being a prototype of the beta - haloalkamines which represent the most recently discovered series of adrenergic blocking agents. The blockade produced by this group of compounds is apparently more complete and more specific than that produced by members of other series, and is not significantly altered by anaesthetic

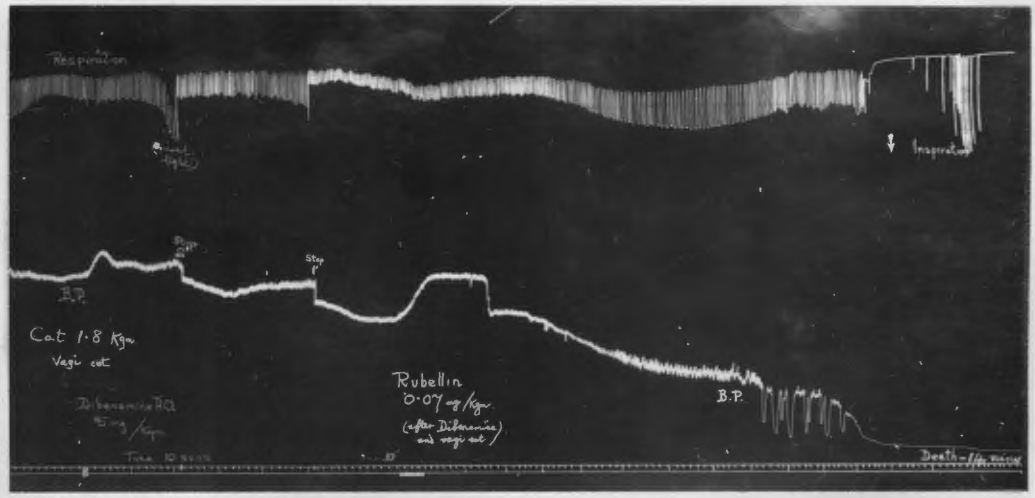


Fig. 27. Failure of dibenamine to prevent the action of rubellin 0.07 mg. per kgm. on the cat heart (with vagi nn divided).
Note the changes in respiration, increasing depth with the progressive fall of blood pressure.

agents (Nickerson, 1949).

In cats lightly anaesthetised with ether the blood pressure was recorded from the carotid artery and the drugs were given intravenously. Dibenamine hydrochloride 5 mg. per kgm. body weight was administered five or ten minutes before the rubellin solution, but failed to prevent the increasing tachycardia, arrythmia, and fibrillation produced by the glycoside (figure 27). (In intact frogs and the perfused frog heart dibenamine also failed to prevent the action of rubellin, prorubellidin, and transvaalin).

It has been shown by Wegria et al. (1941) by measuring the strength of a short D.C. stimulus applied in late systole just sufficient to induce fibrillation, that ventricular fibrillation produced by toxic doses of digitalis in the dog is different from that produced by electrical stimuli or coronary occlusion. The onset and development of ventricular fibrillation produced by digitalis and ouabain may depend, according to these workers, on the favourable development of localised blocks and changes in myocardial conductivity and does not require the advent of an effective stimulus during the vulnerable period as in electrocution and coronary occlusion. Emerson (1943) showed that the "sympatholytic" (adrenergic blocking) agents 933F and 883F,

procaine, and quinidine do not protect cats against lethal doses of digitalis.

The type of ventricular fibrillation produced by cardiac glycosides appears therefore to differ from the type caused by adrenaline, chloroform, or other adrenergic agents which is inhibited by the blocking agents.

In man Isaacson (1949) has observed prolongation of the P-R interval up to 0.32 second, the Wenkebach phenomenon, auricular fibrillation, depression of ST and "digitalis-like" T waves, extrasystoles, and pulsus bigeminus; these are all toxic manifestations, but were of transient nature, disappearing soon after the injections were stopped. The effects produced by rubellin closely resemble those of squill glycosides.

ON THE ISOLATED MAMMALIAN HEART

The freshly isolated heart of the guinea pig was perfused through the coronary arteries by the method of Langendorff, the oxygenated perfusing solution being warmed and the coronary outflow measured (with syphon outflow recorder) by Gunn's method. The movements of right auricle and ventricle were recorded directly by attachment to writing levers. Records are thus obtained of the rate, rhythm, and size of the beats.

(a) Rubellin: In the guinea pig heart a concentration of 1 in 1 million produced increase in the size of auricular and ventricular contractions, dissociation, irregularities, and finally arrest of the heart beat; the ventricles ceased in systole. The coronary outflow revealed no change in the size of the vessels (figure 28). Similar results were obtained in the isolated rabbit heart.

(b) Prorubellidin: In the guinea pig heart a concentration of 1 in 1 million also produced improvement of the beat, auriculo-ventricular block, and finally systolic arrest of the heart.

(c) Transvaalin: The changes produced in the guinea pig heart by a solution of 1 in 1 million were essentially the same as described above (figure 29).

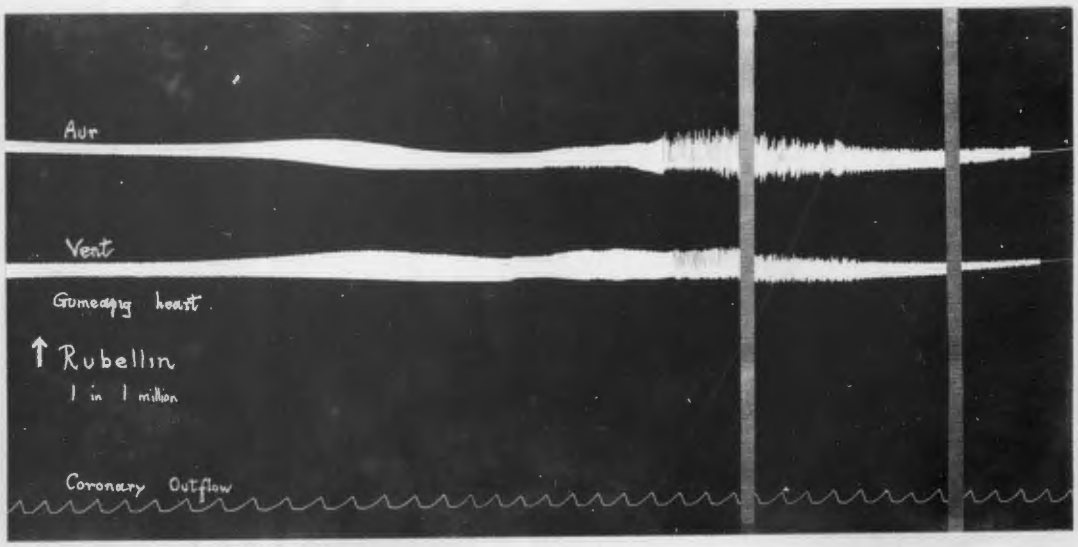


Fig. 28. Effect of rubellin 1 in 1 million on perfused isolated guinea pig heart.

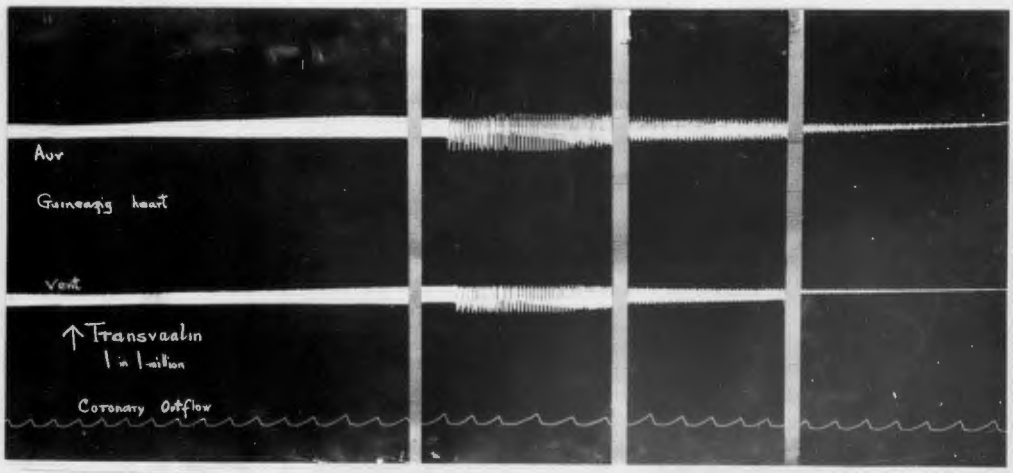


Fig. 29. Effect of transvaalin 1 in 1 million on perfused isolated guinea pig heart.

With hydro-alcoholic extracts of Urginea Burkei the most characteristic action on isolated mammalian hearts was found by Gunn (1921) to be slowing, increased amplitude, and systolic arrest; heart block sometimes appeared, or rapid irregularity, fibrillation, and cessation of the heart.

Premankur De (1927) noted that perfusion of isolated hearts of rabbits with scillaren 1 in 2,500,000 reduced the coronary outflow at first, but this was increased later when the heart beat more slowly with increased amplitude.

In the Langendorff method of perfusion of the mammalian heart primary effects upon the coronary circulation may be difficult to distinguish from effects secondary to changes in heart rate or the amplitude of the contractions.

Although the changes produced in the perfused isolated mammalian heart by the cardiac glycosides has long been accepted as evidence of a direct action of these drugs on the heart, some workers believe that this only occurs with "toxic" doses. Katz, Mendlowitz, and Kaplan (1938) showed that digitalis has no direct effect on the contractile power of the isolated mammalian heart; the only direct effects consistently observed were ectopic rhythms and effects on conduction.

In the whole animal (dog) digitalis has been shown to produce a fall of venous pressure; this action of the drug will be more fully discussed later.

In heart failure the action of digitalis may be explained by this peripheral effect of the drug. The clinical experiments of McMichael and Schafer (1944) tend to support this view.

ON BLOOD PRESSURE

The effect of the glycosides on the blood pressure was studied in anaesthetised cats and rabbits. The material was injected intravenously. Blood pressure was recorded from the carotid artery. The typical changes are illustrated in figures 21, 22, 23, 24, 27, 30, 31, 32.

With large doses both transvaalin and rubellin produce a temporary rise in blood pressure; this is followed by variations in the level, depending on the irregularities of the heart action as shown by the direct recordings of the heart movements by the Cushny myocardiograph. The fluctuations in blood pressure may take place below or above the normal level. Ultimately the blood pressure falls continuously, often falling suddenly to zero as the ventricle begins to fibrillate. During the stage of extreme cardiac irregularity the blood pressure might fall to a very low level only to rise rapidly to the original level. The results are fairly typical of the effect of digitalis glycosides on the blood pressure of the cat.

These changes in blood pressure appear to be mainly due to the action of the drugs on the heart. Transvaalin and rubellin produce no remarkable change in the size of the blood vessels. The progressive fall

and the irregularities in the blood pressure occurring later are due to failure of the heart.

While marked changes in the blood pressure of cat and rabbit occur with big doses of transvaalin and rubellin given intravenously, with sublethal doses little or no change occurs though heart action may be improved. In man rubellin apparently produced no remarkable elevation of the blood pressure (Isaacson, 1949).

Rothlin (1927) states that the blood pressure is always distinctly raised by the glycosides of squill, and that in animals this property of squill glycosides is more marked than with digitalis glycosides. Premankur De (1927) found that scillaren produced a slight rise in blood pressure in cats. In experiments on animals Stehle, Ross, and Dreyer (1931) found that scillaren B almost invariably produced a rise in blood pressure, usually moderate but occasionally marked in degree; they attributed the changes to cardiac and to vasoconstrictor effects. Clinically, urginin produces no specific effect on the systolic or diastolic levels (Chamberlain and Levy, 1937).

In the majority of cases in man "digitalis" glycosides produce no change in blood pressure; in a few cases the systolic level may be raised, or it may be lowered. The diastolic pressure on the other hand tends

to be lowered in the majority of patients, for in heart failure the anoxia may produce increased tone in the vasomotor centre which becomes reduced as the heart action and circulation are improved; the vessels then relax and the blood pressure may be lowered.

ON BLOOD VESSELS

(a) Perfusion of frog vessels: the pithed frog (Xenopus laevis) was opened and the heart exposed. A cannula was tied in the aorta. The perfusing fluid after having passed through the vessels of the whole body escaped from the incised auricles. The cannula was connected to Marriotte bottles giving 15 - 20 cm. (water) pressure.

Rubellin even in a concentration of 1 in 5,000 produced little or no diminution in the outflow. Even when 0.5 mg. was injected into the rubber tubing connected to the cannula little change was observed; adrenaline 0.1 mg. similarly administered produced rapid diminution and cessation of the outflow due to its marked vasoconstrictor action. Results from typical experiments are shown below. Drops per minute were counted over half a minute with stop watch every two minutes:

Ringer →	Rubellin →	Adrenaline
66,66,66,70,68,70	66,62,66,62,64,56,60,60	10,0

Transvaalin in a similar experiment produced the following result:

Ringer →	Transvaalin →	Adrenaline
24,24,24	26,20,20,14,22,20,18	12,6,0

Thus neither rubellin nor transvaalin were shown by this method to have any marked vasoconstrictor action even with the high concentrations used.

The following results have been reported in Rana with the glycoside scillaren.

Fahrenkamp and Nocke (1928) showed that in frogs scillaren produces vasoconstriction but with small doses vasodilatation was observed. Vasoconstriction was also found with scillaren B by Stehle et al. (1931). Premankur De (1927) noted with scillaren first a slight increase then a gradual and pronounced diminution in outflow with solutions containing 1 in 5,000 or 1 in 10,000 of the drug. Rothlin (1927) found that small doses of this glycoside produced constriction of the vessels in the extremity of the frog, though vasodilatation occurred in vessels of the kidneys and extremities, and vasoconstriction in the intestine of the rabbit. He concluded the therapeutic effect of scillaren is partly based on its action on the blood vessels.

(b) Volumetric curves from loops of small intestine (and blood pressure records) in experiments on the cat closely followed the cardiac changes produced by the glycosides, and so any action on the intestinal vessels was marked by the more powerful effect on the heart (figure 30, 31).

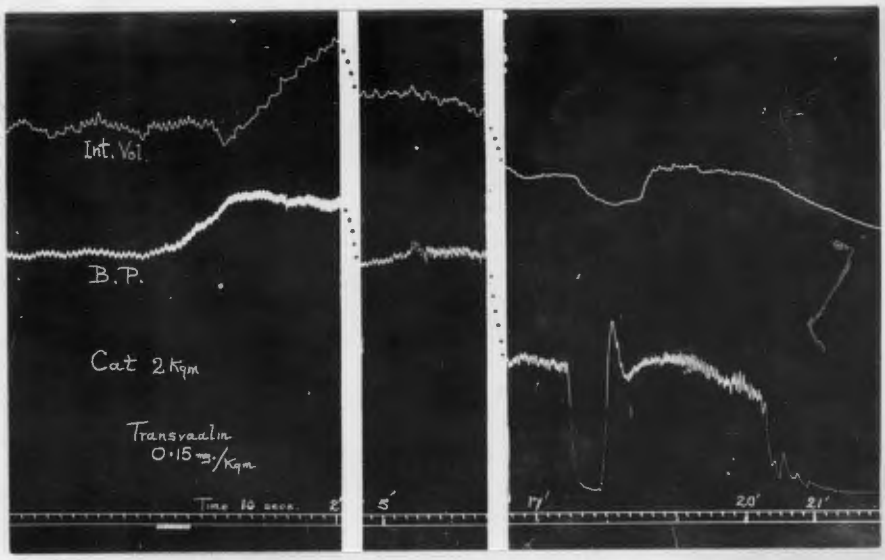


Fig. 30. Effect of transvaalin 0.15 mg. per kgm. on intestinal volume and blood pressure of the cat. Note changes occurring approximately in parallel.

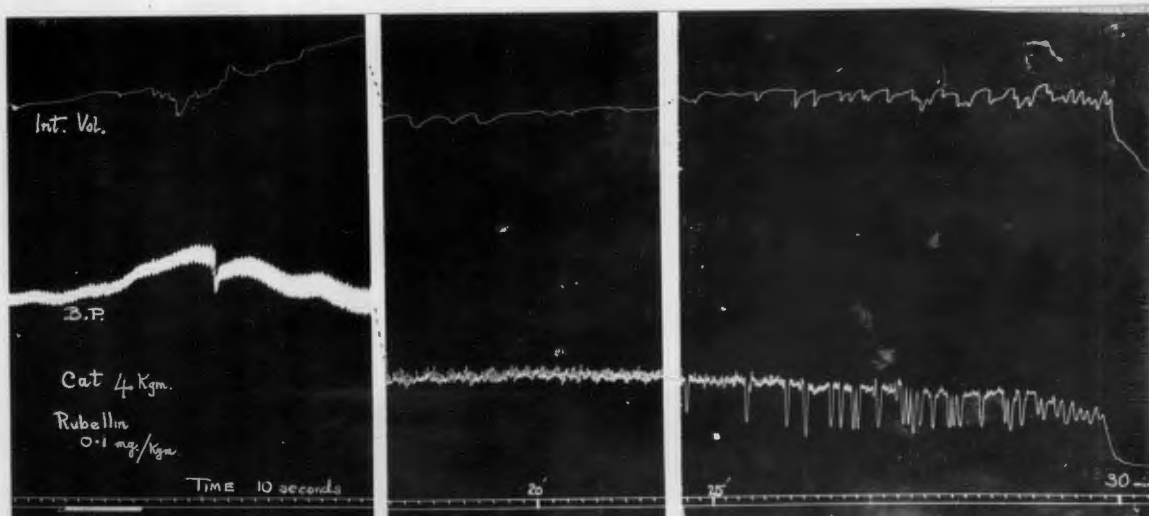


Fig. 31. Effect of rubellin 0.1 mg. per kgm. on intestinal volume and blood pressure of the cat. Note parallel change.

The fact that no increase in volume of the segment of small intestine occurred is perhaps important in showing that (in the cat) the cardiac glycosides under investigation produced no increase in venous pressure in the portal system such as occurs with therapeutic doses of digitalis in the dog (Dock and Tainter, 1930; Katz et al., 1938). It has been shown that in the latter animal the chief if not the sole site of action of such doses of digitalis is on the peripheral vessels, especially those of the liver including the hepatic vein sphincter. The significance of this mechanism will be referred to in more detail later.

(c) Coronary vessels: Perfusion of the isolated guinea pig or rabbit heart through the coronary arteries with transvaalin, rubellin, or prorubellidin, produced no significant change in the size of the vessels (figures 28, 29).

Premankur De (1927) found that the outflow through the coronary vessels is reduced during the first few minutes but is increased again later when the isolated rabbit heart is perfused with scillaren 1 in 2,500,000.

The "digitalis" glycosides vary considerably in the degree of vasoconstriction produced when vessels

are perfused directly, e.g. digitoxin is more powerful than strophanthin in this respect. Different vessels actually vary in their reaction, intestinal vessels contracting more readily than those of the kidneys, and limb and cerebral vessels to an even lesser degree. The coronary vessels are also contracted by digitoxin in perfusion experiments. Therapeutic doses may actually produce an increased blood flow in the kidneys, and slightly in the brain; toxic doses constrict the renal vessels. In his experiments with the thermostromuhr in anaesthetised dogs Hildebrandt (1941) found that after the administration of strophanthin the coronary flow follows cardiac output, decreasing for normal hearts and increasing for damaged hearts; in animal experiments the passage of blood through the coronary vessels is influenced in a secondary manner, and also differently under different conditions. The response of the coronary arteries to the cardiac glycosides may be variable and unpredictable when moderate doses are used. There is controversy regarding the effect, due to the numerous substances investigated in the past, and to the differences in the preparations and methods of measuring coronary outflow. Even with the same type of preparation and the same drug the results are variable. Sakai and Saneyoshi (1915) in the cat, Gilbert and Fenn (1932) in the isolated dog heart, Rühl and Wiehler (1934) on the dog

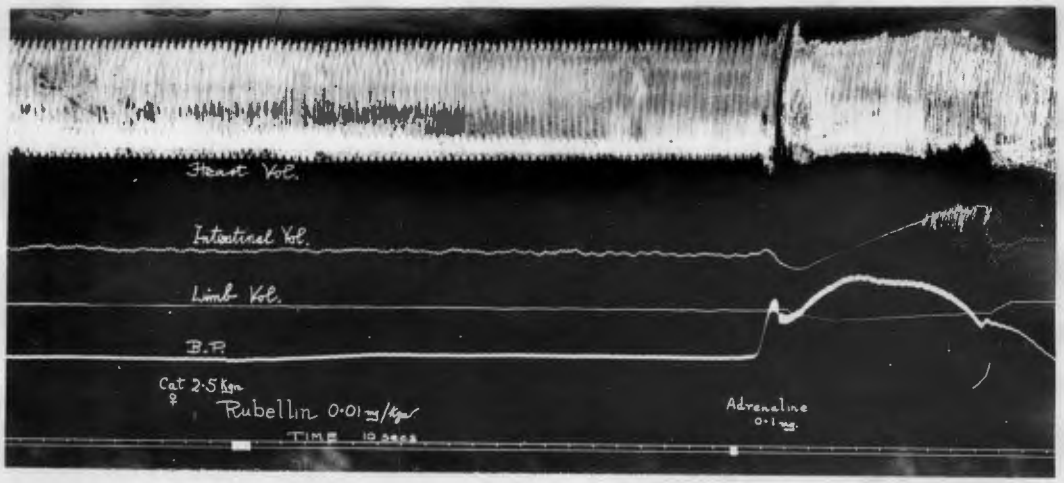
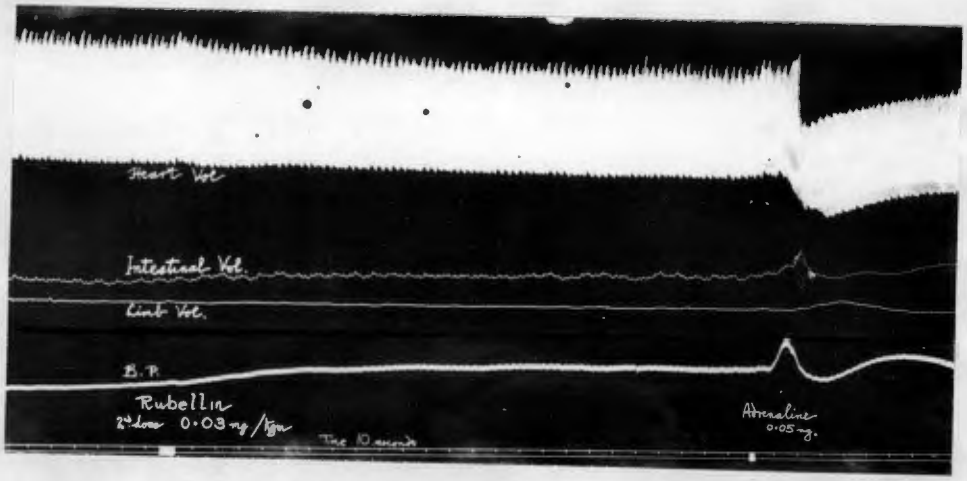


Fig. The action of rubellin on heart volume, intestinal volume, hind-limb volume, and arterial blood pressure ; tracing from cat, female, 2.5 kgm. under ether anaesthesia ; with artificial respiration.
Upper record: effect of rubellin 0.01 mg./kgm.
Lower record: effect of rubellin 0.03 mg./kgm.
Note the effect of adrenaline.



heart - lung preparation, Essex, Herrick and Visscher (1938) on the trained unanaesthetised dog, and Ginsberg, Stoland and Stiler (1938) on the dog, all obtained variable responses; Lindner and Katz (1941) found that certain glycosides may have a direct coronary constrictor action in dogs, even in therapeutic doses. Localised spasm of the coronary vessels may be the cause of the myocardial necrosis and electrocardiographic changes observed in dogs (La Due, 1942).

The glycosides studied in the present investigation produced no significant alteration in the vessels of the frog, cat intestine, or guinea pig and rabbit heart.

With all cardiac glycosides used in man the doses are unlikely to produce vasoconstriction such as is observed with big doses used in animals. In fact with certain glycosides, some observers have noted lowering of the venous pressure (reduction of venomotor tone) in certain types of cardiac failure which they regard as the primary action in relieving this condition (McMichael and Schafer, 1944; McMichael, 1948).

ON THE CIRCULATION

The cardiac glycosides studied in the present investigation have been shown to have the characteristic "digitalis" action, as shown for example in the perfusion experiments on isolated frog and mammalian hearts, and in the changes produced in the heart in the intact animal (systolic standstill of the frog ventricle, myocardiographic and electrocardiographic records of the mammalian heart). Such studies indicate that the glycosides have a direct action on the myocardium.

Some investigators state that these changes are produced in the heart mainly with toxic doses. The extensive literature on the pharmacodynamics actions of therapeutic doses of cardiac glycosides shows that they have both a cardiac and a peripheral (extracardiac; vascular) action in mammals. There is some controversy whether the effects on rhythmicity, conductivity, contractility and size of the heart, and on the energy cost and efficiency with which it does its work after administration of the glycosides are direct or indirect actions.

In previous experiments it was shown that in cats and rabbits certain doses of rubellin or transvaalin produced no change in the volume of loops of intestine i.e. in the calibre of the intestinal vessels; cardiometer studies on cardiac output and records of

the arterial pressure showed certain changes when relatively large doses of the drugs were given.

Further experiments were performed to elucidate a possible peripheral mode of action of the drugs; an attempt was made to study simultaneously as many of the variables as possible. Arterial pressure was measured with the mercury manometer, venous pressure with a water manometer, cardiac output with the Henderson glass cardiometer, and organ volume (intestine, limb or spleen) with the oncometer. Heparin was used as an anticoagulant. Since it is not desirable that any alcohol solvent be injected rubellin was used in these studies as it is water soluble. After a control period this glycoside was injected intravenously; the effect of small (therapeutic) doses was studied. Cats and rabbits were used in these studies.

Relatively small doses of rubellin (0.01 mg. per kgm. body weight) produced no obvious change in cardiac output, arterial pressure, or in intestinal, spleen or limb volume; the results of venous pressure records were not satisfactory. With larger doses, apart from such changes in the cardiometer and arterial pressure records as have already been described, no other alterations in the circulatory system were demonstrated. It is possible that the state of the circulation

in the deeply anaesthetised animal which has been operated on is not the same as in normal animals.

It would appear from these experiments that in the normal cat and rabbit at any rate the main action of rubellin is directly on the heart. While the results have shown that after large doses in the normal animal the heart decreases in size and the minute output decreases, the possibility of a decreased venous return following the administration of therapeutic doses would best be studied in man by special clinical methods such as those used by McMichael (1948). Rubellin, which has been shown in the present investigation to be more potent than ouabain, may perhaps resemble ouabain (g - strophanthin), which McMichael believes has a direct stimulating action on the myocardium; he found that in about half the cases of heart failure investigated that there is only a slight reduction in venous pressure although cardiac output becomes increased.

Comment:

The mode of action of the digitalis glycosides is still not yet settled though the subject has been investigated for many years by many workers. Some of the earlier contributions to this problem are cited by Katz et al. (1938).

In normal animals with an intact circulation

many workers have shown that the digitalis glycosides generally decrease cardiac output, raise the arterial pressure, lower venous pressure (diminished venous return) and decrease the size of the heart, and this also occurs in normal man (with the exception of the rise in arterial pressure). Digitalis increases the efficiency and the force of systole, but neither this action nor slowing of the heart nor the rise of blood pressure which usually occurs in animals, is adequate for explanation of the diminished cardiac output, according to Dock and Tainter (1930). These workers noted that in dogs a fall in venous pressure regularly occurred with consequent inadequate filling of the heart and diminished cardiac output; this indicated a peripheral (extracardiac) and not a cardiac action of digitalis. They demonstrated that (in dogs) digitalis in therapeutic doses causes constriction of the hepatic veins with pooling of blood in the liver and the portal system. Katz et al. (1938) also concluded from experiments on dogs that the chief site of action of toxic doses of digitalis is directly on the heart, but with therapeutic doses the action is mainly if not entirely on the peripheral vessels, especially on the hepatic vein sphincter; blood then accumulates in the liver and the splanchnic area, the circulatory blood volume and the venous return are decreased, causing a

reduction in the size of the heart and a decrease in the minute volume output.

The anatomical structures are not exactly the same in the dog and in man. In the human being the smooth muscle in the hepatic vein is scanty in amount and the effect of digitalis in this situation is insignificant; the reduced cardiac output produced in normal human subjects may be due to reduced muscle tone or to pooling of the blood in the skin, lungs, or other organs. Dock and Tainter have never meant their findings in the dog to be translated to the pharmacodynamic effects of digitalis in cardiac patients. They merely demonstrated that decrease in cardiac output in the absence of failure is due to a peripheral and not a direct cardiac action. Fishberg (1940) has explained the apparent difference of action of digitalis on cardiac output in health and disease as follows: (a) In health the venous return is the determining factor; the normal heart can expel any volume of blood delivered to it under physiological conditions. In these cases improvement of the functional capacity of the heart under the influence of digitalis does not come into play. (b) In congestive cardiac failure increase in cardiac efficiency due to digitalis may be expected to result in increased output. In some patients increased cardiac output may not occur if the

peripheral action of digitalis is prominent. Dock and Tainter thus made the important observation that digitalis lowers the venous pressure in dogs, but the explanation of the fall of venous pressure produced in man has been left uncertain since evidence has been obtained (by Wood, 1940) against an hepatic vein throttle mechanism in man. The observations on cardiac output and venous pressure made in man by McMichael and Schafer (1944) and McMichael (1948) by the catheter technique will be referred to in the discussion later in this work.

ON BLOOD CLOTTING

Several investigators have reported a reduction in coagulation time following the administration of certain "digitalis" preparations especially with relatively large doses in animals, but less obviously with therapeutic doses in man. In 1928 Tanaka noted that strophanthin when injected intravenously in rabbits hastened coagulation; the thrombin content of the serum and the fibrinogen of the plasma were increased. Macht (1943) found that not only tincture of digitalis but other digitaloid drugs including strophanthin, ouabain, digoxin, digitoxin, convallamarin, and scillaren, all definitely hastened coagulation in vitro (Howell's method). This finding was verified and amplified by some investigators. Werch (1943) found that digifoline given intravenously in rabbits produced a decrease in coagulation time. De Takats et al. (1944) observed that digitalis administered clinically and in dogs favoured a tendency to thrombosis, possibly by opposing heparin. Massie et al. (1944) stated that digitalis leaf in therapeutic doses shortens the coagulation time of the whole blood of man. Gilbert et al. (1944) suggested that this is responsible for the incidence of thrombosis in digitalised patients, while Massey and Steiner (1944) suggested that digitalis might be a possible cause of peripheral embolism.

May et al. (1945) found that digitalis decreases prothrombin time. On the other hand Sokoloff and Ferrer (1945) and Sokoloff, Ferrer and De Graff (1945) failed to find any significant alteration of coagulation times in whole blood (Lee-White method) during digitalis therapy. Moses (1945) could also not demonstrate any significant or consistent decrease in coagulation time after therapeutic digitalisation. In dogs Ramsey et al. (1945) and Richardson and Walton (1946) failed to demonstrate a significant change of coagulability after digitalis administration.

The variability in the findings of various workers is no doubt due to the different techniques used. It would appear that the more recent work is the more convincing.

The effect of rubellin and transvaalin on clotting time was studied in vitro on freshly drawn whole venous blood taken from medical students just before the test was performed. The Lee-White method was used. The effect of various concentrations of the drug was examined, the mixtures of drug solution and blood being kept throughout the experiment in a water bath at 37° C. The tubes were tilted at short intervals and the time of onset of coagulation estimated with a stop watch. No significant alteration in coagulation time was noted,

compared with the controls done simultaneously, as shown in some protocols below; numerals indicate minutes and seconds.

	Control	Control	Control	Rubellin 1 in 5000	1 in 50,000	1 in 500,000	1 in 5 million
(1)	3'40"	5'	5'	4'10"	4'50"	4'40"	3'55"
(2)	3'	3'15"	4'20"	4'25"	4'	4'15"	4'30"
(3)	3'30"	3'45"	5'	4'	3'20"	3'20"	3'15"
				Transvaalin			
(1)	3'15"	3'30"	3'40"	-	3'20"	4'5"	3'45"
(2)	3'20"	3'25"	3'28"	-	3'35"	4'10"	3'15"
(3)	4'10"	4'20"	4'45"	-	4'50"	5'	4'

Clot retraction in all cases was normal.

Those investigators who have reported a decrease in coagulation time suggest that the actions may be due to a thromboplastic property of the drugs, to mobilisation of prothrombin from the liver, or to an increase of fibrinogen or of thrombin. The possible danger from the therapeutic aspect is that thrombosis, and embolism, may be favoured by the use of cardiac glycosides. However, the findings in some of the investigations mentioned above and the long and widespread experience with "digitalis" preparations in clinical practice suggest that at least with therapeutic doses there is no great hazard of thrombosis.

EFFECT ON URINARY SECRETION

The effect of rubellin and transvaalin on the secretion of urine was studied in rabbits anaesthetised with urethane. Cannulae were tied in the ureters and the urine outflow recorded by means of a Condon drop counter. Blood pressure was recorded from the carotid artery, and the drug injected intravenously.

With non-lethal doses there was a small increase in the flow of urine (figure 32). This was observed after a latent period of several minutes, and a short while later the small rise in blood pressure produced by the drug. With fatal doses the output was diminished. Gunn (1921) also found that an extract of Urginea Burkei almost invariably caused an increase of urine, but large doses caused a decrease which he considered to be due to constriction of the renal vessels.

It has been stated by some workers that squill glycosides produce a diuretic effect in experimental animals, believed to be due to a direct effect on the renal epithelium (possibly on the convoluted tubules). Most workers believe the diuretic action of digitalis is an indirect one due to improvement of the circulation (filtration pressure) in the kidneys. The increase in the amount of urine secreted by normal animals is very small compared with that in patients with oedema, due

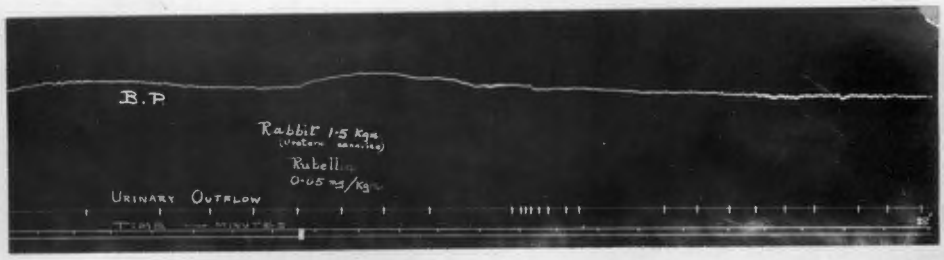


Fig. 32. Effect of rubellin 0.05 mg. per kgm. on urinary outflow in rabbit anaesthetised with urethane. Note slight increase in output, following small rise in blood pressure.

probably to constriction of the renal arterioles. In patients with congestive cardiac failure there is retention of sodium chloride; this may have some bearing on the better action of digitalis as a diuretic in cardiac oedema than in normal subjects. In cases of congestive cardiac failure squill preparations act as a diuretic and this is probably due to improvement in the state of the circulation.

As far as the animal experiments are concerned, attention is drawn to the following factors.

(1) An initial rise of blood pressure may cause the initial temporarily increased outflow of urine.

(2) In experiments where a cannula is tied in the bladder contraction of the part proximal to the cannula may expel some urine. Recording of outflow from the ureters is therefore much more desirable and accurate.

(3) The operative trauma in preparing the experimental animal may possibly produce reflex renal cortical ischaemia as recently demonstrated by Trueta et al. (1948)

This may account for the poor output of urine sometimes obtained especially in the initial stages in experiments on the rabbit.

While cardiac glycosides may produce some

increase in the quantity of urine secreted in the normal animal, in normal human subjects practically negative results are obtained as also in heart disease without oedema, and in purely nephritic oedema.

ON THE RESPIRATION

The intravenous injection of large doses of rubellin and transvaalin in cats produced an increase in the depth of respiration, followed by slowing of the rate, until respiratory paralysis occurred, usually a short while after the heart had ceased beating (figure 27).

In rats the injection of transvaalin produced slow and irregular respiration, probably due to an action on the central nervous system, as these animals also developed marked paresis of the hind limbs. With rubellin, however, which has a potent action on the heart in all animals studied, the respiratory difficulty would appear to be due to stagnant anoxia resulting from the impaired heart action, although there may also be a direct action on the respiratory centre.

ON THE CENTRAL NERVOUS SYSTEM

(a) Rubellin may have some action on the nervous system, but the action on the heart predominates. No convulsions were observed with this drug whether given orally or parenterally.

(b) Transvaalin, however, has a neurotoxic action in the rat which is demonstrable probably because of the resistance of the rat heart to this complex. When big doses are given subcutaneously (for example, 0.04 mg. per gramme body weight) the animal is first disinclined to move, and the respiration may be slowed. Within half an hour paralysis of the hind limbs develops; the forelimbs are not obviously affected as the animal is still able to drag itself forwards. No convulsions occurred, and within a few hours there is almost complete recovery. In some cases there may still be some disturbance of the gait twenty-four hours after the injection.

Rat (wt;G)	Dose mg. per G.	Results
105	0.01	No obvious effect
125	0.02	27' : dragging of hind limbs
65	0.03	25' : respiration slow irregular. Recovery.
		25' : respiration slow irregular. Recovery.
60	0.04	10' : sluggish; respiration slow
		55' : dragging hind limbs;
		80' : recovery.
		24 hours : weakness, peculiar stepping gait.
50	0.04	10' : sluggish; respiration slow;
		30' : marked paresis of hind limbs.
		24 hours : peculiar stepping gait (weakness) of hind limbs.

In addition to this neurotoxic action in the rat, in the frog and the cat transvaalin produces typical "digitalis" effects on the heart, so that this complex has both cardiotonic and neurotoxic actions.

The action of transvaalin in producing paralysis of the hind limbs of the albino rat without convulsions and with subsequent recovery suggests it has an action

on the spinal cord or on the muscles of the hind limbs.

With transvaalin the typical "digitalis" action in the frog and the cat must be due to its scillaren A content, and the paralysant action in the rat might be due to the small amount of "scilliroside" believed to be present. Amorphous scilliroside has an ultraviolet absorption spectrum very similar to and practically coincident with that of transvaalin (and rubellin). It is a highly purified material of red squill which produces weakness and ataxia in rats when small doses (0.00025 mg per gramme) are given intravenously, and convulsions when large doses (0.00125 mg. per gramme) are administered; doses of 0.0025 mg. per gramme intravenously produce death within a few minutes from cessation of the heart beat and without convulsions. This substance is one of the most potent constituents of red squill from the point of view of a convulsant action in rats, and has the typical actions of a cardiac glycoside in the rat, frog, cat, and man (Gold et al., 1947); it is not a single and pure principle, but the two actions are believed to be produced by one principle.

Strychnine-like convulsions are produced by digitoxin in the rat (Hatcher, 1912) and the cat (Weiss, 1932), and by strophanthin in the toad Bufo regularis (Epstein, 1931). Camphor-like convulsions have been

observed after intravenous injection of digitoxigenin in unanaesthetised rats. The differences in the activity of the various glycosides (in rats) have been attributed to differences in the rate of clearance, and to differences in the development of the convulsant factor in the molecular structure of the glycosides.

It is well known that in man the principles of the digitalis group exert an effect on the central nervous system producing such manifestations as vomiting, diarrhoea, headache, dizziness, drowsiness, depression, psychoses, and coloured vision. It is not established whether these are due to a direct toxic action on the brain cells. Some believe they are produced indirectly through circulatory changes, or result from reflex stimulation.

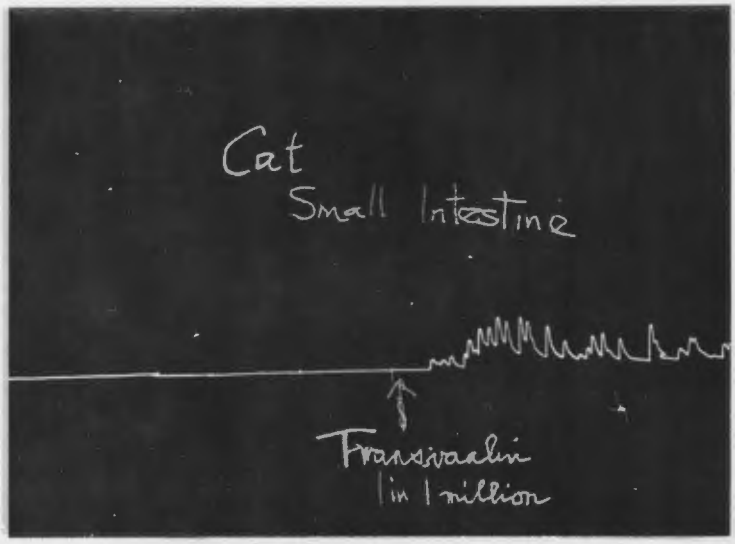


Fig. 33.

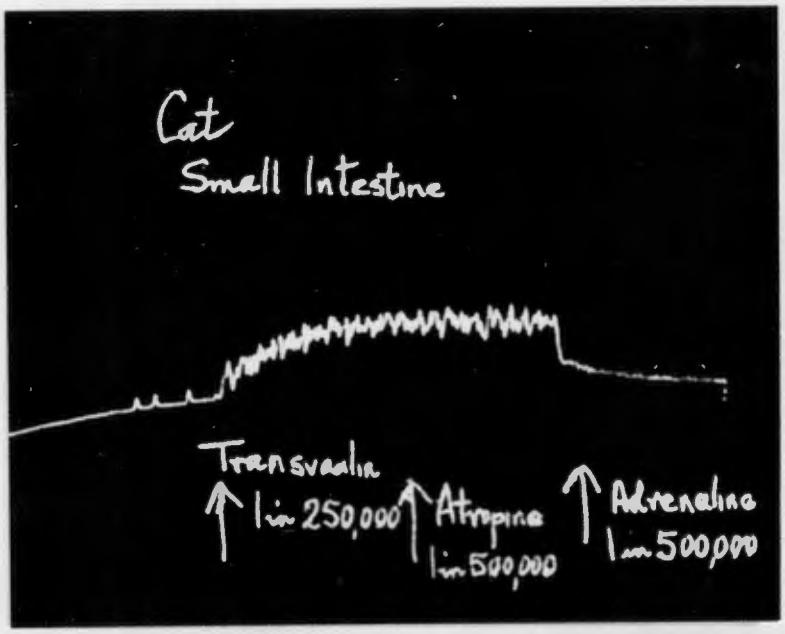


Fig. 34.

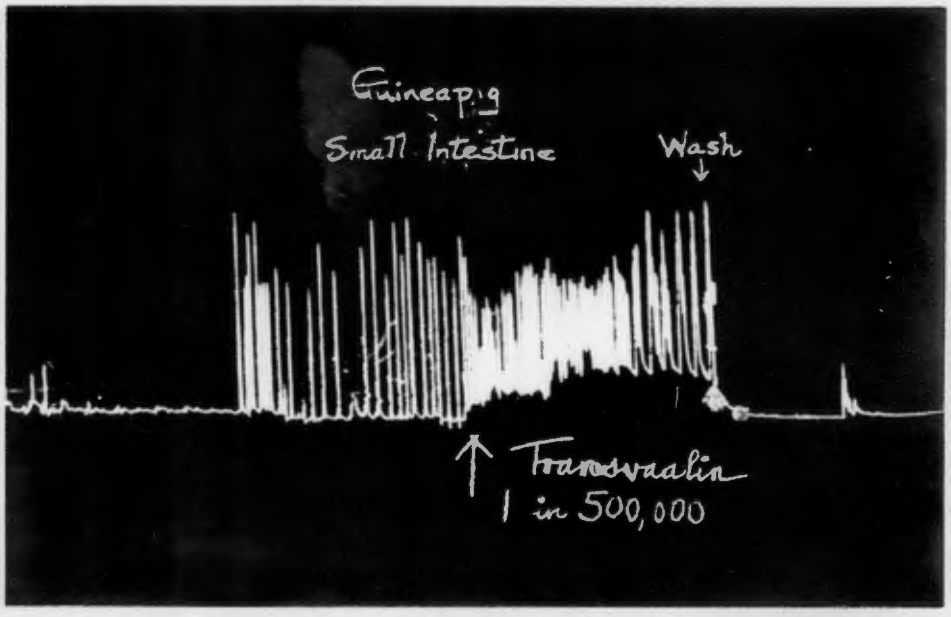


Fig. 35.

ACTION ON SMOOTH MUSCLE

All three compounds have a stimulant action direct on the smooth muscle of the intestine and uterus.

(a) Transvaalin: Isolated segments of the small intestine of the cat were stimulated by a concentration of 1 in 250,000 less strongly by 1 in 1,000,000 (figure 33). Increased contractions and an increase in tone were produced, with return to normal rhythm when the tissue was washed in Locke's solution. Atropine did not abolish the contraction of the intestine, but adrenaline 1 in 500,000 caused relaxation (figure 34). Guinea pig intestine is also stimulated (figure 35).

Animals poisoned with Urginea burkei develop marked diarrhoea with inflammatory changes in the intestine (Gunn, 1921; Steyn, 1934). On the other hand according to Premankur De (1927) the squill glycosides which have been demonstrated to be well tolerated and well absorbed show no gross or microscopic signs of irritation in the gastrointestinal tract in postmortem examination of animals to which toxic doses were administered.

Isolated segments of virgin and nonpregnant cat uterus and of nonpregnant guinea pig uterus showed marked increase in tone when a concentration of

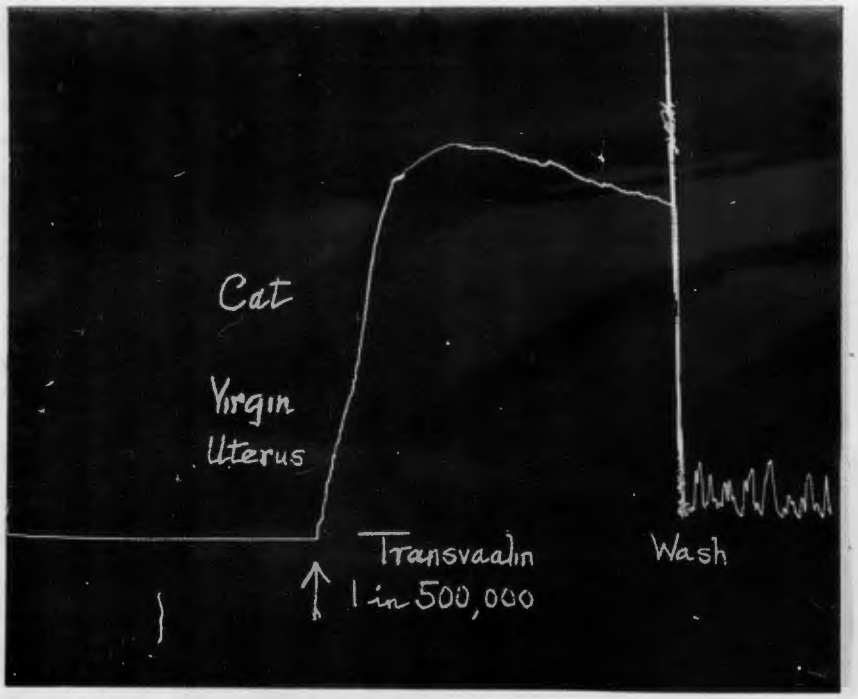


Fig. 36.

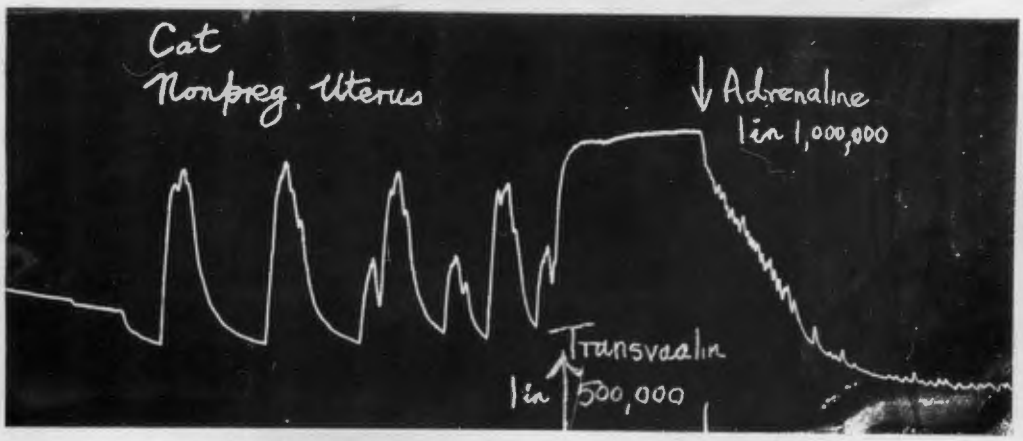


Fig. 37

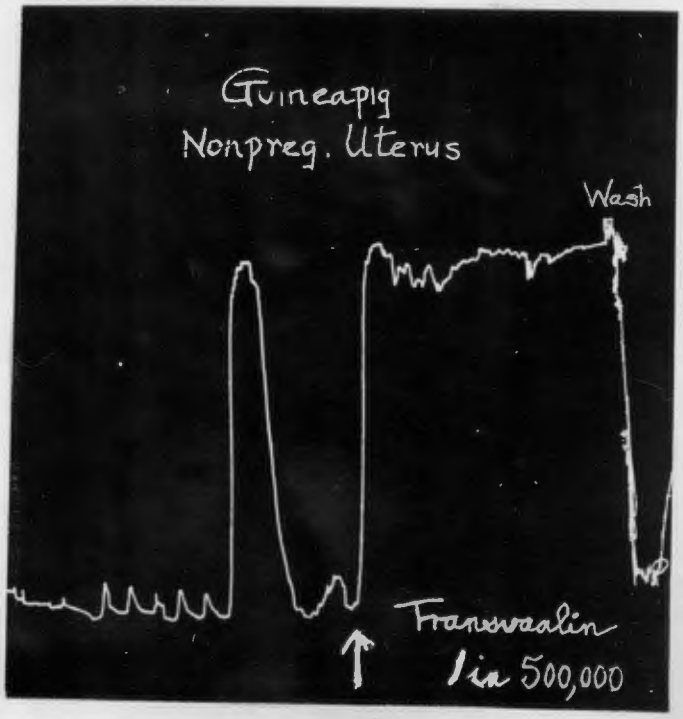


Fig. 38.

transvaalin 1 in 500,000 was used (figures 36, 37, 38), with return to normal tone when the tissues were washed with Locke's solution. Adrenaline 1 in 1,000,000 produced relaxation of the nonpregnant cat uterus stimulated by transvaalin.

It is of interest that the Sutos have used the bulb of Urginea burkei (Suto Sekanama) as an abortifacient as well as in the treatment of circulatory diseases.

(b) Rubellin: Segments of cat and guinea pig intestine and uterus were stimulated by a concentration of 1 in 500,000, prolonged contraction being produced; the small intestine of a cat was also stimulated by a concentration of 1 in 5,000,000 with increased tone and movement (figure 41). As with transvaalin atropine had no effect on the intestinal contraction produced by rubellin, but adrenaline 1 in 500,000 caused relaxation (figure 39). Adrenaline similarly relaxed the spasm of the uterus produced by rubellin (figure 40).

(c) Prorubellidin: This glycoside also stimulated smooth muscle of the small intestine and uterus of the guinea pig in a concentration of 1 in 1,000,000. A concentration of 1 in 5,000,000 also stimulated cat small intestine in one experiment (figure 41).

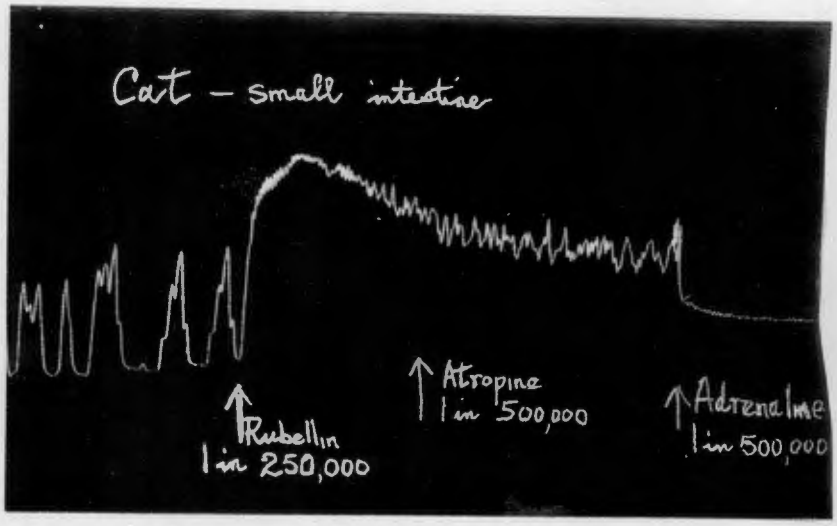


Fig. 39.

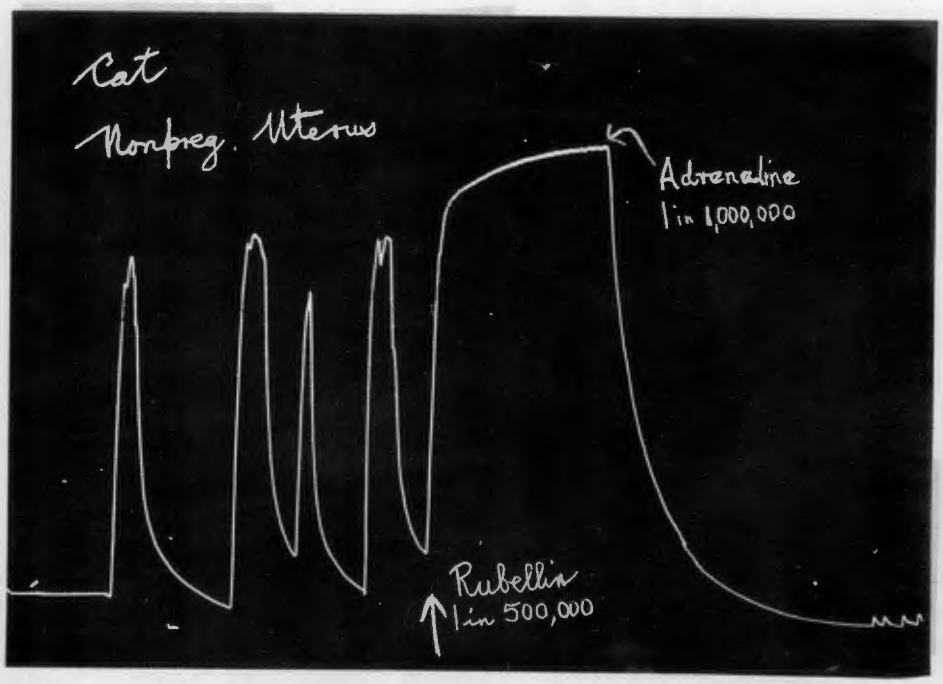


Fig. 40.

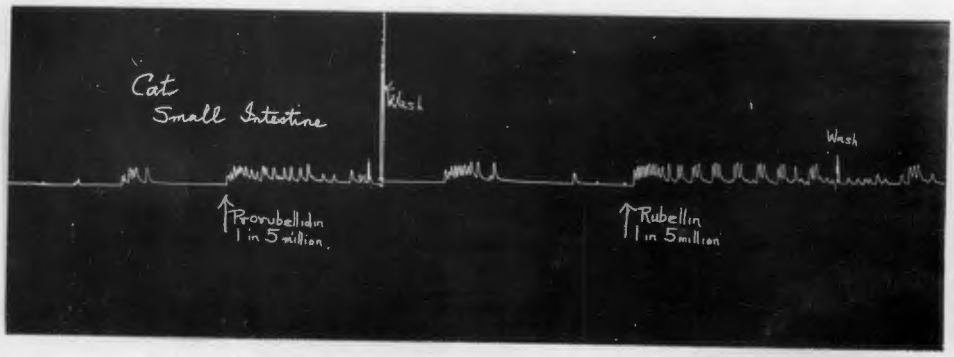


Fig. 41.

ACTION ON LYMPH HEARTS

The action of certain drugs including digitalis and strophanthus preparations on the lymph hearts of the frog has been investigated by a few workers. It was of interest to study the effect of rubellin and transvaalin on these structures in Xenopus laevis (previous workers have used Rana temporaria) with the Cossor-Robertson cathode ray tube as well as with the older optical lever method and naked-eye examination. In many experiments the electrocardiogram was recorded as well as the lymph-cardiogram.

Method: For each experiment a frog (Xenopus laevis) was anaesthetised with urethane, 1 ml. of 33% solution being injected into the ventral lymph sac; this is more satisfactory than to use decerebrate preparations. A small flap of skin overlying the posterior lymph heart was turned back. Pin electrodes connected to the Cossor-Robertson apparatus were then placed in position in direct contact with a lymph heart so as to get maximum lymph-cardiogram curves. The best position for the electrodes was with R A lead applied lightly to the upper part of the left (or right) lymph heart and LL lead in the midline at the posterior end of the lymph heart area; the LA lead was earthed to the apparatus, or was inserted into the right forelimb

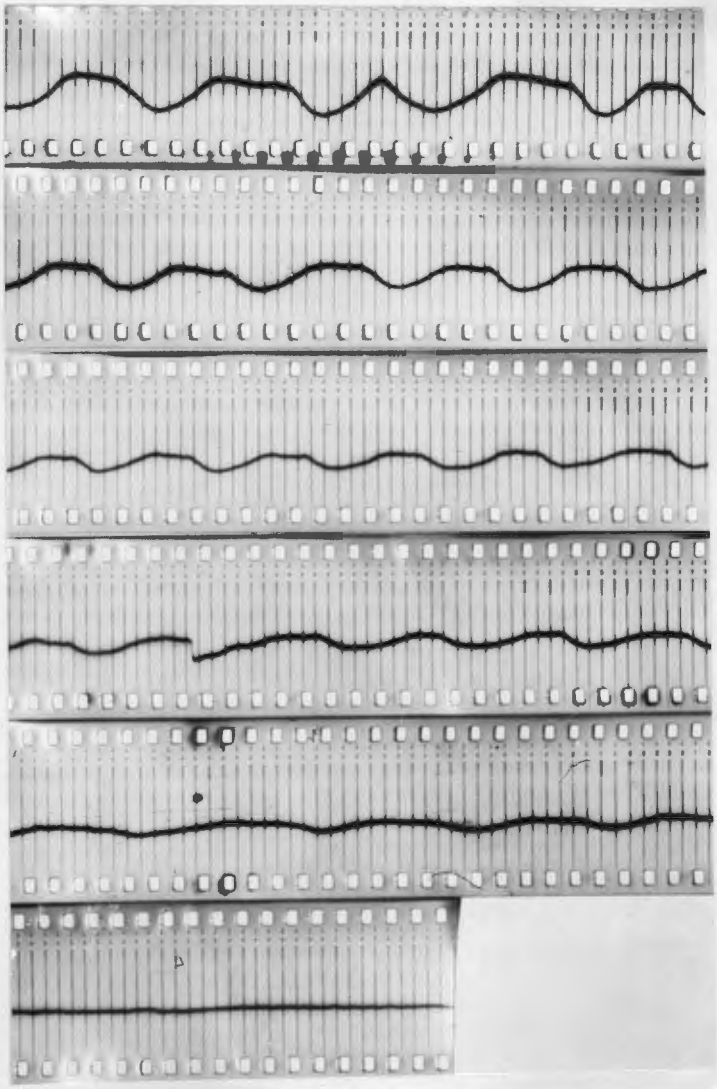


Fig. 42. Lymphcardiogram from frog (Xenopus laevis, 62 G) anaesthetised with urethane; rubellin 0.0005 mg. per gramme injected into the leg muscles. Note reduction of voltage. Normal tracing followed by tracings taken 4, 9, 15, 20, and 24 mins. after injection.

when it was desired to record the electrocardiogram as well as the lymph-cardiogram.

Results: By suitable adjustment of the electrodes uniform rhythmical curves were obtained in the lymph-cardiogram. With both rubellin and transvaalin, injected into the thigh muscle, the following changes were observed: a reduction in voltage, slowing of the lymph heart beat, occasional extrasystoles, and finally disappearance of the curves, as shown typically in figures 42 and 43.

Comment: Contrary to the observation of Vulpian (1856) who studied the effect of digitalis, it was noted that the lymph hearts (of Xenopus) may continue beating long after rubellin or transvaalin caused the systemic heart to pass into contracture. This was also observed by Fraser and MacKenzie (1909) with strophanthus sarmentosus.

Straub (1920) using light ether anaesthesia or decerebrate preparations observed the effect of strophanthin-K directly applied to the lymph hearts, and noted slowing and cessation of the beat, with recovery later. In the present study the glycosides were administered by intramuscular injection (thigh or leg mm) so as not to cause disturbance in the lymph hearts from possible local irritation, and also because with

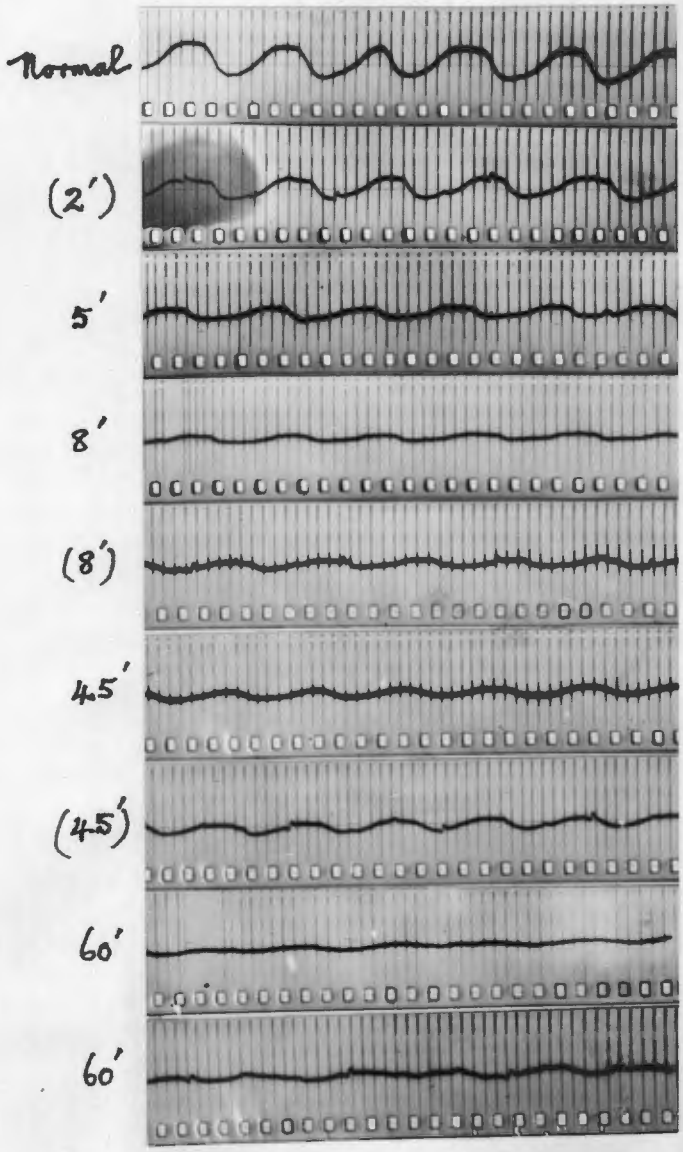


Fig. 43. Frog (Xenopus laevis, 60 G, anaesthetised with urethane); transvaalin0.0021 mg. per gramme injected into the leg muscles. Lymph-cardiograms show reduction in voltage. Normal tracing followed by tracings taken at 2,5,8, (8), 45, (45), and 60, minutes after injection. Note superimposed electrocardiograms at 2, (8), and (45) minutes. Last tracing is an electrocardiogram.

the pin electrodes the local application of fluid to these structures was found to interfere with the record.

Jolly (1934), like Straub (1920), used the optical lever method of v. Bruckes (1906), in studying the lymph hearts (of Xenopus laevis). In this method a small light mirror is placed on the skin overlying the lymph heart or on the lymph heart itself, and tracings are obtained with a beam of light recording on photographic paper. The normal record is that of a twitch. He referred to the tracing thus obtained as a lymph-cardiogram, but it should be noted that with this method these workers recorded movements or contractions of the lymph heart, whereas with the cathode ray tube used in the present study the action current or impulse is recorded. Small contractions may therefore not move the mirror placed on the overlying skin whereas current will still be recorded with the electrocardiographic apparatus.

In the present study electrocardiogram and lymph-cardiogram were sometimes recorded together as separate tracings or superimposed on each other (figure 43).

One feature demonstrable with the optical lever (mirror) technique but not with the electrical method is that the lymph heart unlike the systemic heart ceases in diastole.

GONADOTROPHIC ACTION

The cardiac glycosides are steroids and are therefore chemically related to the sex hormones, adrenocortical hormones, bile acids, vitamin D, and certain carcinogens. This group of substances illustrates the lack of relationship between chemical constitution and pharmacological action.

The sex hormones lack the lactone side chain essential to the "digitalis" action, but recently Giarman (1949) has shown that in isolated perfused frog hearts the cardiotonic action of certain lactones is potentiated by oestrone and to a lesser extent by androsterone. Cardiac glycosides have no carcinogenic action when tested on mice (Smith and Gardner, 1939).

It is of interest that neither transvaalin nor rubellin, nor certain other cardiac glycosides (unpublished observation) cause oviposition in Xenopus laevis even with doses that are sublethal or that cause death only many hours after injection. Certain other steroids have been shown to produce ovulation and even oviposition (Zwarenstein, 1937; with progesterone; Shapiro, 1939, with ten related steroids; Sapeika, 1943, with desoxycorticosterone). While there is no specific configuration of the steroid molecule which could be associated with its gonadotrophic action in Xenopus, it appears that long side-chains at

C_{17} destroy gonadotrophic activity, and that the most active compounds have a $\Delta 4.3$ keto constitution.

ABSORPTION, FIXATION, AND DURATION OF ACTION

The Urginea glycosides rubellin and transvaalin are less active when given by mouth than by injection. In this respect they resemble certain other glycosides such as strophanthus which is very poorly and irregularly absorbed when administered orally. This is of course a disadvantage for routine clinical use of these drugs since oral maintenance treatment is less inconvenient for a patient than repeated injections.

In cats and rats both glycosides were much more active when given by injection. Large doses were ineffective by mouth; in the cat the ratio of the oral to the intravenous dose of rubellin is more than 14 to 1. Thus rubellin given by mouth resembles ouabain and strophanthin which are not readily absorbed by this route of administration. From experiments on man it has been found by Gold that with digitoxin, which is completely absorbed so that oral and intravenous doses are the same, the ratio is 1 to 1, with digifoline 5 to 1, with lanatoside C about 10 to 1, while with digitalis only about one-fifth the dose is absorbed. These facts are important in the choice of a cardiac glycoside for oral use in patients requiring rapid and certain digitalisation. In one human subject rubellin 0.25 mg. given orally produced no gastrointestinal disturbance and no

change in the electrocardiogram. Transvaalin by mouth also produced no gastrointestinal irritation nor any obvious change in the electrocardiograms of "non-cardiac" patients who received a single dose of 0.25 mg., 0.25 mg., 0.5 mg., and 1.0 mg. respectively. The reduced activity when these drugs are given by mouth indicates that they undergo some change in, or are imperfectly absorbed from the intestine, although the factors of destruction and elimination after absorption are also involved.

The relatively slow development of the action and its duration after parenteral administration has stopped suggests some combination of the glycosides with the tissues or some alteration in the drug and its slow removal. Earlier in this work it was shown in experiments on the frog heart that the action of the glycosides is slow since the final arrest of the ventricle does not occur for many minutes, depending on the dose or concentration used. Similarly in experiments on the mammalian heart doses or concentrations of the glycosides such as would obtain with therapeutic use of the drugs produced effects which developed slowly and persisted for a considerable time.

A single dose of rubellin injected intravenously in man can produce full effects within two hours. This

may be compared with ouabain and strophanthin which produce full action within two hours, whereas with digitoxin it takes six hours, with digifoline and lanatoside C intermediate in speed of action. The dose of ouabain used in human beings is 0.25 mg. The single doses of rubellin given to man were mainly 0.21 mg. in 10 ml. sterile water, repeated according to the condition of the patient (Isaacson, 1949). This dose was based on experimental work done in the present investigation. Incidentally, no nausea, vomiting or diarrhoea occurred with 0.21 mg. or even 0.42 mg. rubellin, although marked electrocardiographic changes were recorded, and all urgent symptoms were alleviated in a short time in patients with normal and abnormal rhythms, with best results in auricular fibrillation and congestive cardiac failure. Larger doses may possibly produce nausea and vomiting which are important early signs of digitalis intoxication. With squill glycosides other workers have also noted that cardiac irregularities are more likely to be the first sign of overdosage, and nausea may be indicative of more advanced intoxication than is the case with digitalis.

The prolonged action noted in the animal experiments was well demonstrated when rubellin was injected intravenously in man. The size of the dose, the cardiac disorder and its cause are important factors determining

the duration of action which is variable, but may last for days. Judged by electrocardiographic changes the persistence of effect noted by Isaacson (1949) was as follows:

Case 1: 19 days following 0.84 mg. rubellin (divided doses)
" 2: 6½ " " 1.05 " " " "
" 8: 18 " " 0.63 " " " "
" 10: 5 " " 0.42 " " undivided dose

Judged by ventricular rate:

Case 3a: 3 days 19 hours following 0.42 mg. (divided doses)
" 3b: 11 " 9¼ " 0.21 " (single injectio
" 4 : 17 " plus " 0.42 " " "
" 6 : 13 " " 0.42 " " "
" 7 : 3 " ? " 0.63 " (divided doses)
" 9 : 2 " 19 hours " 0.21 " (single injectio

The action of the drug also persists after administration of the drug is stopped. There is great variation in the persistence of the "digitalis" effect, not only in different patients but even in the same patient at different times. As there is no known means of measuring the persistence of effect in the individual, a patient who has already received a large dose of rubellin, as of digitalis, should receive any further doses with great care in view of the dangers of poisoning.

In the case of ouabain only about one-third of the effect is stated to remain at the end of four days; with digitoxin less than one-third of the effect wears off in four days.

Squill glycosides have been shown to be well tolerated, well absorbed and rapidly active both in animals and in man. While they are not fixed in the heart so strongly as digitalis glycosides, cumulation, for example with scillaren, appears to be sufficient to allow useful application in clinical practice. Carr and Mayer (1936) commented on the long duration of action of urginin.

An interesting modern development is the study of radioactive glycosides. Kimura and Geiling (1949) have shown that higher levels of radioactivity are present in the ventricles than in the atria of hearts perfused with radioactive digitoxin.

DISCUSSION

Among the large number of naturally-occurring steroids (including sex hormones, adrenocortical hormones, vitamin D preparations) the digitalis-like glycosides form an important group from a therapeutic point of view. Many of these glycosides have been obtained in crystalline form and their structure elucidated. Although synthetic compounds have been prepared as for instance by Chen and Elderfeld (1942, Chen et al. (1943) no synthetic products have yet taken the place of the natural cardiotrophic drugs.

Crystalline preparations of individual glycosides have the advantage of constant composition, freedom from impurities, stability, accurate dosage, and suitability for oral or intravenous administration. Whether they have any advantage over digitalis can only be proved by thorough and comparative therapeutic tests in man. There appears to be little doubt that they have various and nonproportional degrees of activity in their several actions, so that for clinical use it may be possible to select the glycoside producing the maximum desired effect with a minimum of concomitant unfavourable actions. The introduction of new purified cardiac glycosides has therefore enlarged the therapeutic possibilities with these drugs. Some have not been widely used;

others such as lanatoside C have become established as useful drugs.

The increasing use of pure cardiac glycosides has made it desirable to determine any important differences in individual glycosides. There is little agreement in the studies on potency of cardiac glycosides, owing to the differences in the methods used and the criteria for comparison. The biological assays reported in this work indicate the relative potencies of the glycosides transvaalin, rubellin, and prorubellidin in laboratory animals.

The drugs can be measured gravimetrically and the dosage for man (in milligrammes) will have to be determined by adequate therapeutic investigations. It is well known that cardiac glycosides have varying potency or toxicity in different animal species. Rubellin, which has been shown to be very potent in animals, has also proved very active in man when given by intravenous injection. It is to be anticipated that acute or chronic poisoning will occur in man if wrong doses are used. Isaacson (1949) has noted some toxic effect following intravenous doses of rubellin. The stability of rubellin is shown by the fact that assays performed with the same sample five years after the original communication on its potency revealed no loss of activity.

With the isolated heart of the frog (Xenopus laevis), and the heart of the cat and rabbit in situ all three glycosides investigated in this work were shown to produce the familiar effects of cardioactive drugs; a period of latency, increased force of contraction, and finally cardiac standstill. Rubellin is more active, as assayed on the frog, than transvaalin and prorubellidin, and several other well known glycosides, namely, ouabain, standard strophanthin, lanatoside C, digoxin, digitoxin.

Visscher and la Due (1941) found that when the basis for comparison was the single intravenous dose required for "full digitalisation" in auricular fibrillation there was a remarkably small difference in the molar quantity of ouabain, digitoxin, digoxin, and lanatoside C required to produce comparable effects. Cattell and Gold (1941) studied the effect of various glycosides on the isolated papillary muscle of the cat using as a criterion of therapeutic effect increased systolic tension, and of toxicity subsequent decline which occurred with stronger concentrations; ouabain and digitoxin were found to be of equal potency, while the activity of lanatoside compounds was about one-tenth as great. Recently White and Salter (1946) using a similar technique found ouabain to be approximately twice as potent as digitoxin. De Graaff and his collaborators (1941) used the appearance of

atrioventricular block in the embryonic chick heart as a test for activity, and found the concentration ratios for digitoxin, digoxin and lanatoside C to be 1 : 3 : 4.5. More recently Wedd and Blair (1947) found ouabain, digitoxin, digoxin and lanatoside C to be qualitatively and quantitatively similar in their ability to shorten the QT interval of the electrogram.

The greater potency of rubellin as compared with its derivative prorubellidin is not easily explained. The difference probably lies in the aglycone. The sugar portion most likely has an influence on the solubility of the compounds and their fixation within the heart muscle. Rothlin (1939) stated that as the sugar content of a cardiac glycoside increases so does sometimes the pharmacological activity of the molecule, but Chen et al. (1941) found that a decrease in the number of molecules of sugar may or may not be accompanied by an increase of activity.

The conclusions drawn from animal experiments, although accurate and suggesting the potency of the glycosides, are not necessarily transferable to the problem of therapeutics in human beings. Data obtained by the use of large doses in experimental work may be misapplied if used as a basis for the effects of the therapeutic doses given to man. Also important is the fact that

observations of the effects on the normal heart are not applicable to the diseased organ.

The choice of a cardiac glycoside for clinical use is determined by the potency, the speed of action, and the rate of elimination of the drug. Potency and speed of action of orally administered drugs is related to the speed and completeness of absorption. Rubellin and transvaalin do not appear to be satisfactory for oral use. Where great speed of action is required, rubellin certainly is of great value by intravenous injection, as a full effect on the failing heart is produced within two hours. In this respect the drug resembles strophanthin administered intravenously. Intravenous digitoxin requires about six hours. With regard to speed of elimination it has already been shown that the action of rubellin after a single intravenous dose may persist for many days. There has been much controversy regarding the advisability of giving cardiac glycosides by intravenous injection. Some workers fear the dangers; others claim as advantages for the method that good results can be obtained rapidly and the dosage is accurate and controllable; still others advocate an initial injection to be followed by oral administration. Rubellin should probably be reserved for emergency cases of cardiac failure, the initial intravenous injection of this drug to be

followed by oral administration of a digitalis preparation.

Little is known of the mode of action of the cardiac glycosides. They appear to penetrate the heart muscle and there the active aglycone is split off the glycoside to exert its action on the metabolism and activity of the cell. The various glycosides differ from one another in the speed with which this penetration and intracellular liberation occurs, and on the length of time they remain fixed in the cell. Kimura and Geiling (1949) have shown that in the perfused heart of the rat and guinea pig radioactive digitoxin is present to a greater extent in the ventricles than in the atria. Du Bois et al. (1949) have shown that bufagin, the glycoside present in the parotid secretion of the tropical toad Bufo agua, decreases the rate of oxidation of glucose in heart slices to a much greater extent than in liver and kidney slices; the drug had no effect on anaerobic glycolysis nor on the cytochrome oxidase and succinic dehydrogenase systems. Slices of left ventricular muscle taken from dogs which had received ouabain respired for the first 30 to 60 minutes at a somewhat higher rate than cardiac slices from control dogs but subsequently the respiratory rate fell below the control level,

especially in the case of slices from fibrillating hearts (Wollenberger, 1949). The effect of the cardiac glycosides (scilliroside, ouabain, digitoxin) on the respiration of cardiac muscle has also been reported by Finklestein and Bodansky (1948), who found that Ca ions were essential to the acceleration of respiration by the cardiac glycosides. Modern studies such as those quoted above will help towards a better understanding of the mechanism of action of the cardiac glycosides.

It has been repeatedly demonstrated that a lactone substituent on C17 of the cyclopentanoperhydrophenanthrene nucleus of the cardiac glycosides is essential for the cardiac activity, although it is recognised that a favourable configuration of the steroid nucleus can enhance the action. Stoll (1937) has suggested that fixation of glycosides in the heart may occur through the cholesterol present in the myocardium, while Giarman (1949) suggests on the basis of certain experimental work that a steroid-enzyme complex is formed which is capable of metabolising a lactone moiety in a manner which yields energy to the contractile mechanism of the heart.

The mode of action of cardiac glycosides on the failing heart is not settled. Observations on the heart in situ do not establish a direct action on the cardiac muscle because of other influences which may

alter the force of contraction, such as heart rate, diastolic size, peripheral resistance, return flow and coronary flow; most of the objections also hold in the studies on the isolated heart. Cattell and Gold (1938), and White and Salter (1946) have investigated the action of various glycosides on the isolated hypodynamic papillary muscles from the right ventricle of the cat, which tissue avoids many complexities involved in a more elaborate biological preparation. The method permits the testing of extremely small amounts of the drugs both quantitatively and qualitatively. However, from a technical standpoint the method is not easy, and experiments performed with the glycosides used in the present study were not satisfactory. An increase in systolic tension was noted when rubellin was added to the oxygenated solution in which the muscle was bathed, but only small contractions could be recorded. Large numbers of cats may have to be sacrificed in order to obtain suitably reactive specimens of papillary muscle. Recently White, Bedford, and Salter (1948) compared the inotropic effects of twelve pure cardiac glycosides on the isolated hypodynamic papillary muscle and found the comparison to agree closely with results reported for the mean lethal dose in intact cats. They found the papillary muscle preparation open to the same criticism

as all surviving tissues. Care is required in translating results from one type of experiment to another. For example digilanid C is highly potent in the intact animal whereas on the papillary muscle it exerts an indifferent effect (Sciarinć, Ackerman, and Salter, 1948); this drug must therefore by hydrolysed somewhere in the intact animal before it can affect the myocardium.

The action of transvaalin and rubellin is mainly on the heart muscle. They have little or no action on the blood vessels of animals (intestinal volume, isolated mammalian heart, and frog perfusion experiments). Conclusions regarding their effect on the blood vessels of man cannot of course be drawn from experiments on portions of the vascular tree in animals. The mechanism by which a cardio-active drug such as rubellin produces beneficial results in heart failure may be the direct action on the myocardium. The fact that a good response can occur without slowing of the heart being produced shows that this factor cannot play a decisive role; any slowing following the action of the drug could be due to improved function of the heart. On the other hand it is possible that the action of the glycoside in congestive cardiac failure in man may be predominantly on the venomotor tone, although the drug definitely

acts directly on the heart as shown by the changes observed in the ventricular rate in auricular fibrillation, in the electrocardiographic studies, and in the heart experiments in animals.

From clinical experiments conducted in man by McMichael and Schafer (1944), and from pharmacological experiments performed by Dock and Tainter (1930) and Katz et al. (1938), evidence has been produced that the primary action of digitalis in relieving congestive cardiac failure is not on the heart muscle but on the venous side of the circulation, venous pressure being lowered, decreasing the load on the heart so that its efficiency is improved. Comment was made earlier on the views that cardiac glycosides have peripheral (vascular) action when therapeutic doses are used, and a direct cardiac action only when toxic doses are administered.

Gold and Cattell (1940) oppose the views of Dock and Tainter; they believe that in heart failure the high venous pressure is the result and not usually the cause of it, and that digitalis improves the function of the heart in heart failure by direct action on the heart muscle which, without primary changes in the diastolic length of the muscle, increases the tension which the muscle can develop during systolic contraction. Another observation against the extra-cardiac action of digitalis

is that in isolated left ventricular failure the drug causes restoration of compensation without significant changes in venous pressure.

McMichael (1948) has shown by accurate serial measurements of venous pressure and cardiac output that the action of digitalis in low output cardiac failure (e.g. in myocardial hypertensive or valvular disease) is beneficial; by lowering the venous pressure the cardiac output is increased. On the other hand in high output failure (e.g. in anaemia or emphysema) digitalis by lowering the venous return reduces the cardiac output and is harmful to the circulation. He suggests that ouabain differs from other cardiac glycosides in acting on the cardiac muscle rather than on the veins, and may be the drug of choice in cardiac failure secondary to pulmonary disease. Clinical research studies such as those by McMichael would be of great value in the search for a cardiotonic drug with minimal action on the veins for use in cardiac failure associated with a high cardiac output. Rubellin, which has an especially potent action on the heart, is one such drug which appears well worthy of special clinical investigation.

Dock (1947) points out that the observation of McMichael may be true of cases in advanced low-output failure in whom rise in venous pressure causes a further

decrease in cardiac output, but not in ambulatory patients whose cardiac output rises with exercise or recumbency. In the latter group the disappearance of gallop rhythm, or a rise in vital capacity and definite clinical improvement may be caused by digitalis, and in such cases the only site of action must be in the heart. The beneficial effect on the function of the myocardium in failure is thoroughly proved by work on isolated hearts and on isolated strips of heart muscle. The catheter technique permits observations only of the immediate effect of intravenous digitalis preparations. Dock suggests that the shortening of systole and the lengthening of diastole which "digitalis" produces in many failing hearts probably permits a gradual recovery of the muscle, which will only become apparent many hours after full digitalisation.

Bloomfield et al. (1948) found that in human beings with congestive cardiac failure the physiological effects of ouabain were similar to those described in experimental animals as well as those reported in patients by less exact clinical measurements than they used. They find that ouabain acts directly on the failing heart; that cardiac slowing or any decrease in peripheral resistance or right ventricular filling pressure occur only later, and that improvement in cardiac output precedes any fall in venous pressure. The results of these workers are at

variance with those reported by McMichael and Sharpey-Schafer (1944) with digoxin; the latter workers ascribed the improvement in cardiac output to a fall in right auricular pressure. (It should also be pointed out that McMichael and Sharpey-Schafer implied alterations in peripheral venous pressure, although they measured only central (auricular) pressures; from the haemodynamic standpoint the two may not be regarded as synonymous. The data of these authors also indicate a significant rise in cardiac output in patients with congestive cardiac failure even in the lower ranges of venous pressure. This evidence points to some other primary factor such as increased myocardial efficiency, improving the cardiac output).

McMichael (1948) states that ouabain (g-strophanthin) has a definite stimulating action on the myocardium in man; in about 50 per cent of cases of heart failure venous pressure was found to be only slightly reduced in cardiac output. Rubellin appears from animal experiments to be closer to ouabain than to digoxin in its site of action.

In experiments on the frog heart, and the mammalian heart, both isolated and in situ, disturbances

of conduction between auricles and ventricles were observed, especially after the administration of rubellin. In man, Isaacson (1949) found that rubellin by intravenous injection prolonged the PR interval in three cases:

Case 2 (a) : from 0.16 to 0.2 secs. after 0.42 mg. rubellin (in divided doses);

Case 2 (b) : from 0.16 to 0.32 secs. after 0.63 mg. rubellin (in divided doses);

Case 6 : from 0.12 to 0.16 secs. after 0.42 mg. rubellin (single injection);

Case 10 : from 0.12 to 0.14 secs. after 0.42 mg. rubellin (single injection).

Prolonged auriculoventricular conduction was observed by Chamberlain and Levy (1937) in the electrocardiograms of patients after the administration of "Urginin"m a commercial preparation of squill, and similar findings have been reported by other investigators.

The toxic effects produced by squill preparations are similar to those of digitalis. With the urginea glycoside rubellin and transvaalin no gastrointestinal disturbances were produced in normal human subjects who took the drug

by mouth. Rubellin did not produce nausea, vomiting, or diarrhoea when given intravenously in the treatment of cardiac disorders in man (Isaacson, 1949), yet marked prolongation of the PR interval and other electrocardiographic changes such as the Wenckebach phenomenon, auricular fibrillation, depression of the ST segments and T waves, premature contractions and pulsus bigeminus were also observed, all transient in nature as they disappeared after treatment was stopped. It is of interest in this connection that Carr and Mayer (1936) found in their experience that cardiac irregularities are likely to be the first signs of dangerous intoxication. While nausea may precede the appearance of arrythmias they believe that nausea due to over-dosage with the squill derivative urginin indicates more advanced intoxication than in the case of digitalis. Urginin may produce premature contractions and auricular fibrillation (Maher and Sittler, 1936), nodal rhythm (Van der Veer et al., 1939), vomiting and diarrhoea (Chamberlain and Levy, 1937; Van der Veer et al., 1939).

As patients may show symptoms of intoxication with cardiac glycosides in the absence of changes in the electrocardiogram, it is unsafe to estimate the amount of a drug such as digitalis or rubellin from the electrocardiographic record. The factors responsible for the

presence or absence of electrocardiographic changes are still unknown.

Numerous plants in South Africa are known to have caused severe poisoning and death in animals, but much less is known about the dangers to man from the accidental or therapeutic administration of plant preparations. Information about toxic plants is of value to practitioners who may have to treat cases of poisoning; treatment would also be more rational e.g. digitalis preparations are contra-indicated in poisoning with plants such as tulip or Urginea Burkei which contain cardio-active glycosides. From a medicolegal standpoint the information is also of great importance. A study of the plant principles as in the present work may help to introduce new cardiac glycosides with special advantage in the therapy of cardiac disorders.

Although there is definite or presumptive evidence that very many South African plants are toxic, only about forty species have been reported to have caused death in man (Sapeika, 1944). Numerous unrecorded cases of poisoning no doubt occur among native tribes. There appear to be no definite published reports about deaths in man from the ingestion of squill

or urginea preparations, although a few other plants with "digitalis action" have caused serious poisoning e.g. acokanthera venenata (Watt and Breyer-Brandwyk (1932); Homeria and moraea species (Watt and Breyer-Brandwyk, 1932, Steyn, 1934); cotyledon species (Sapeika, 1936); adenium oleifolium (Sapeika, 1941). Approximately thirty plants in South Africa have been shown to have a digitalis-like action; from only a half of these have active principles been isolated.

SUMMARY

The genus Urginea, a group of bulbous rooted plants, is closely related to the genus Scilla. More than 25 species of Urginea occur in South Africa. Their importance lies in the fact that some have proved toxic to stock, and that they contain glycosides which may prove to be of value in the treatment of heart disease in man.

Few active principles from Urginea plants have been isolated. Rubellin (from Urginea rubella), a recently isolated glycoside, has been investigated in this work. Some of the actions of its derivative prorubellidin were also studied. Transvaalin, a crystalline product from Urginea Burkei Baker, consisting mainly of a glycoside identical with scillaren A with possibly small amounts of scilliroside, or a related glycoside, was also examined. Frogs, cats, rabbits, and guinea pigs were found to be sensitive to the compounds, rubellin being very potent.

In the frog (Xenopus laevis) all three compounds cause death by a selective action on the heart which ceases in characteristic systolic standstill. By the 16-hour frog assay method the relative potency of the glycosides is, in descending order: rubellin (121), ouabain (international anhydrous, used as standard)(100), prorubellidin (50.5); other well known glycosides compared with these by the same method have the following potency - strophanthin (44), lanatoside C (31), digoxin (19), digitoxin (3.6).

On isolated frog hearts the typical "digitalis" action was observed with concentration as low as 1 in 6 million. Transvaalin did not produce A-V block as frequently as rubellin or prorubellidin. Electrocardiographic studies revealed interesting changes; widening of the R wave, depression of the ST segment which became progressively shortened until the T wave was lost in the downward limb of R or became inverted. Electrocardiographic records taken while kymographic tracings were being made showed changes in the heart's action not revealed by the latter method. The effect of saponins, barium chloride and methyl violet, which produce changes in the frog heart resembling digitalis, was also studied electrocardiographically for comparison with the cardiac glycosides. Dibenamine did not prevent the action of the glycosides under investigation.

With the Du Bois-Reymond induction apparatus rubellin was found to increase but later to decrease the excitability of the frog heart; the ventricle did not respond eventually when in systolic arrest. Studies on the contracture of the isolated frog ventricle showed that rubellin increases the coefficient of elasticity.

On the mammalian heart (cat) in situ the glycosides administered in suitably large doses produced the typical "digitalis effect" e.g. with transvaalin 0.18 mg. per kgm.

body weight, and rubellin 0.13 mg. per kgm. ; increased excitability, periodic grouped beats in auricles and ventricles, extrasystoles, auriculoventricular dissociation, auricular flutter and fibrillation, and finally ventricular fibrillation were observed. Cardiometer experiments showed that the glycosides produce a rapid diminution in output when large doses of the glycosides were injected. Electrocardiographic records in cats gave convincing evidence of the digitalis-like action of the glycosides, showing for example, slowing of the rate, prolongation of PR interval, widening of QRS complex, ectopic rhythm, tachycardia; in man, rubellin has been observed to produce prolonged PR interval, the Wenckebach phenomenon, auricular fibrillation, depression of ST and digitalis-like T waves, extrasystoles, and pulsus bigeminus, after intravenous injection of the drug.

The isolated mammalian heart (guinea pig) when perfused with the glycosides in a concentration of 1 in 1 million showed increase in the auricular and ventricular contractions, dissociation, irregularities, and finally arrest of the heart with the ventricle in systole. Isolated papillary muscle from the cat's heart showed increase in systolic tension when the glycosides were added.

The blood pressure in anaesthetised cats and

rabbits showed at first a temporary rise, followed by fluctuations at about the normal level, ultimately falling to zero. Such changes are due to the action of toxic doses of the glycosides on the heart; the blood vessels do not appear to be significantly affected by these drugs. In man rubellin did not affect the blood pressure to any significant degree.

Studies on the blood vessels in perfused frogs, in loops of small intestine of the cat, and in the isolated mammalian heart (coronary vessels) showed no significant change in the size of the vessels. The question of the site of action of digitalis, directly on the heart, or primarily on the vessels, is discussed.

Urinary secretion was only slightly increased after intravenous administration of rubellin and transvaalin, and with fatal doses the output was diminished.

The respiration became slow and increased in depth in cats which received large doses of the drug by injection. In rats transvaalin produced slow, irregular respiration.

The central nervous system may be stimulated by rubellin, but no convulsions were produced by this drug. With transvaalin however when big doses (0.04 mg. per gramme body weight) are given torats by subcutaneous injection a neurotoxic action was observed, the hind limbs

becoming paralysed, with slow recovery; in the frog and the cat transvaalin produces typical digitalis effects on the heart, but the rat is more resistant. Rodents have long been known to be resistant to digitalis bodies. Rubellin, however, produced death in rats with relatively small doses (0.001 mg. per gramme body weight) given subcutaneously, although oral administration of 0.006 mg. per gramme failed to cause death. Prorubellidin did not kill rats with these doses.

The smooth muscle of the intestine and the uterus of the cat and the guinea pig is stimulated by the glycosides in a concentration of 1 in 500,000 or less. Atropine failed to abolish the contraction of the intestine, but adrenaline produced relaxation of the stimulated intestine and uterus.

The action of rubellin and transvaalin on the posterior lymph hearts of the frog (Xenopus laevis) was studied with the aid of the Cossor-Robertson cathode ray tube. In the animal anaesthetised with urethane the drug was injected into the thigh or leg muscles and produced a reduction in voltage, slowing, extrasystoles, and finally disappearance of the lymph-cardiogram curves. Simultaneous records of the electrocardiogram and lymph-cardiogram showed that the lymph heart may continue to beat long after the systemic heart has been arrested by

the glycosides. Unlike the systemic heart the lymph heart stops in diastole.

Neither transvaalin nor rubellin, like certain other cardiac glycosides, produces ovulation in Xenopus laevis, though certain chemically related steroids such as progesterone and deoxycortone have a gonadotrophic action in the frog.

When administered orally the glycosides are less effective both in animals and in man than when given by injection. This may be due to alteration of the drugs in the intestine or to imperfect absorption. Absorption, fixation, and the duration of action of the glycosides is considered.

Certain questions about chemically pure glycosides and potency are discussed; also the problem of their mode of action and the mechanism by which the heart is affected, whether by a direct action or secondary to an effect (extra-cardiac or peripheral) on the vessels.

Rubellin which is a very potent cardiac glycoside would appear to resemble ouabain in having a direct stimulating action on the myocardium.

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