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STUDIES ON SOUTH AFRICAN CARDIAC GLYCOSIDES. II. OBSERVATIONS ON THE CLINICAL AND HAEMODYNAMIC EFFECTS OF COTYLEDOSIDE

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ABSTRACT

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Cotyledoside, a bufadienolide isolated from *Tylecodon wallichii* (Harv.) Toelken, subs. *wallichii (=Cotyledon wallichii* Harv.), was dosed to guinea-pigs and sheep.

In guinea-pigs, the oral and subcutaneous LD_{50} values were very similar (*cf.* 0,173 mg/kg over 48 h with 0,116 mg/kg over 24 and 48 h). When dosed subcutaneously, a cumulative effect was observed. Intravenous administration of cotyledoside to anaesthetized guinea-pigs resulted in: dyspnoea, increased heart rates and blood pressures, and electrocardiagraphic changes typical of cardiac glycoside poisoning. A positive cardiac inotropic effect was succeeded by a positive chronotropic one.

In sheep, acute and subacute intoxication resulted in ruminal, respiratory and cardiac changes. The signs included ruminal stasis, cyanosis, cardiac arrhythmia, ectopic foci and AV dissociation, followed by hypotension and progressive respiratory and cardiac failure. The skeletal muscles were affected in only 1 sheep *vide infra*.

In chronically intoxicated sheep typical clinical signs of "krimpsiekte" developed, e.g. weakness, reluctance to stand, unsteadiness on feet, tremor and paresis of hindquarter muscles, paresis of the neck, arching of the back and standing with the feet close together. Respiratory function was affected in all 3 cases; ruminal stasis, with concomitant loss of appetite occurring in one, and a transient change in heart function in another.

The syndrome induced by acute cotyledoside poisoning is similar to that of other cardiac glycosides, but the paretic signs of chronic intoxication resemble "krimpsiekte", a disease associated only with intoxication with the plants of the family Crassulaceae.

INTRODUCTION

The "plakkie" group of poisonous plants comprise *Cotyledon, Tylecodon* and *Kalanchoe* spp. of the family Crassulaceae. They cause cardiac glycoside poisoning and are of considerable economic importance in South Africa (Naudé, 1977).

The main toxic principle of *Tylecodon wallichii* (Harv.) Toelken, subsp. *wallichii (=Cotyledon wallichii)* Toelken (1978) (Fig. 1a and 1b), is a bufadienolide glycoside, which was isolated and characterized by Van Rooyen & Pieterse (1968). Its structure (Fig. 2) was determined by Van Wyk (1975).

Henning (1926), Steyn (1932, 1934 & 1949), Sapeika (1936) and Terblanche & Adelaar (1965) described the clinical signs of the disease caused by *Cotyledon* and *Tylecodon* spp. and alcoholic extracts of the plants.

It was felt, however, that more information was required on the effect on the heart during acute and chronic intoxication to facilitate comparison with other forms of cardiac glycoside poisoning. Experiments were also conducted to compare the effects of pure cotyledoside with those of the plants causing the "krimpsiekte" in the field.

Experiment I. LD_{50} and cumulative effect of cotyledoside in guinea-pigs

Materials and Methods

This experiment was conducted in a manner similar to that of Naudé & Potgieter (1971).

Young male albino guinea-pigs (Wistar strain), with a mass of 300–500 g, were deprived of food and water for *c.* 24 h prior to the experiment.

A fresh solution of chromatographically pure cotyledoside (Fig. 2) was prepared by first dissolving a specific mass of cotyledoside in a small quantity of warm ethanol and then diluting this to a final concentration of *c.* 10% ethanol (v/v) with isotonic saline. In all cases the dosage of ethanol was far below the limit of 2,5 mℓ/kg given by Naudé & Potgieter (1971) for the production of transient symptoms of ethanol intoxication. The guinea-pigs were either dosed by stomach tube or injected subcutaneously.

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Pilot trials were done with 1 or 2 animals per dose in order to determine the approximate range that had to be covered.

Five groups of 5 guinea-pigs each were used for the LD_{50} determinations. After administration of cotyledoside the clinical signs and time of death were noted. The LD_{50} was determined at 24 h, 48 h and 7 days.

To estimate the cumulative effect of cotyledoside, 2 groups of 3 ginea-pigs were injected subcutaneously with 25% and 50% respectively of the subcutaneous LD_{50}/day, until they died.

Post-mortem examinations were performed in order to verify that death had not been due to other causes.

Results

Subcutaneous LD_{50}

The LD_{50}, as determined by the method of Lictchfield & Wilcoxon (1949), was 0,116 mg/kg (95% confidence limits of 0,107–0,126 mg/kg) over 24 h as well as over 48 h.

The 7 day LD_{50} could not be statistically determined because too few animals survived the experiment. It was evident, however, that the 7 day LD_{50} would not differ greatly from that at 24 h.

At the highest dosage used (0,15 mg/kg), paresis of the neck became evident 30 min after administration, whereas it was only noticeable after 1–2 h at the LD_{50}. Depending on the dosage this progressed to ataxia and general paresis and paralysis. In severely affected cases bradycardia and arrhythmia was followed by cardiac arrest. Dyspnoea was also in evidence. These nervous signs sometimes lasted for several days and in those cases which recovered disappeared in the reverse order.

Post-mortem examinations were performed routinely on guinea-pigs but, except for pulmonary haemorrhages and emphysema in a few cases, nothing unusual was observed.

Oral LD_{50}

The 48 h LD_{50} for guinea-pigs dosed orally was 0,173 mg/kg (95% confidence limits of 0,148–0,201 mg/kg), and the 7 day LD_{50} was 0,160 mg/kg (95% confidence limits of 0,128–0,2 mg/kg).

Here, in contrast to the above, the first clinical sign, namely, neck paresis, was noticed only on the morning after the animals had been dosed and this was followed by the same symptoms and dyspnoea. In addition, diarrhoea was occasionally encountered. Some severely affected animals recovered and in these cases neck paresis was the last sign to disappear.

At necropsy, pulmonary haemorrhages, emphysema and cyanosis were present and the contents of the caecum and colon were often soft or liquid.

Cumulative effect

Though no clinical signs appeared before the LD_{50} was reached, conspicuous signs occurred when 5×25% or 3 × 50 % the LD_{50} were administered. All the animals eventually died.

Discussion

Irrespective of whether cotyledoside was administered subcutaneously or orally, the LD_{50} was virtually identical. When the toxin was injected subcutaneously, death usually occurred within 24 h; when dosed *per os*, it usually occurred within 48 h, but further mortality was recorded up to 7 days.

The clinical signs included cardiac and respiratory dysfunction and nervous signs, such as neck paresis, ataxia and paralysis. Sapeika (1936) observed the same clinical signs in guinea-pigs injected subcutaneously and intraperitoneally with *C. wallichii (= T. wallichii)* extract. Clonic spasms/convulsive movements were also seen in guinea-pigs fed with dried *C. reticulata (=T. reticulatus* subsp. *reticulatus)* and after intraperitoneal and subcutaneous injection with extracts of the plant. Steyn (1932) observed attacks of convulsions and complete paralysis in guinea-pigs injected subcutaneously with *C. decussata* extracts.

Although the same type of symptoms were observed by Naudé & Potgieter (1971) in guinea-pigs intoxicated with 1 α, 2 α-epoxyscillirosidin, all nervous signs had disappeared 48 h after intoxication.

The cumulative effect of cotyledoside under these experimental conditions was clearly demonstrated. By contrast, Naudé & Potgieter (1971) could not elicit symptoms with 20 × 25% the guinea-pig LD_{50} of 1 α, 2 α-epoxyscillirosidin under similar conditions.

Experiment II. Acute heamodynamic and respiratory effects of cotyledoside on anaesthetized guinea-pigs

Materials and Methods

Nine male guinea-pigs, with a mean live mass of *c.* 340 g, were deprived of food and water for *c.* 24 h and anaesthetized with sodium pentobarbital* injected intra peritoneally at the rate of *c.* 17,5 mg/kg. They were then heparinized** with 1,5 iu/g introduced through catheters into the *vena jugularis* and *arteria carotis*.

Electrocardiographic (ECG) recordings, using standard limb leads, were made with the animals in a supine position. Respiratory movements (Siemens nasal thermistor), heart sounds (Elema microphone) and carotid blood pressure (electromanometer and transducer) were recorded simultaneously on an Elema Mingograph 81.

Recordings were made both before and after administration of the cotyledoside solutions, prepared as previously described, and injected via a catheter into the *vena jugularis*, at 4 dosage levels, namely 0,66×, 1×, 1,25× and 2,25× the subcutaneous LD_{50}.

* Sagatal (Maybaker)
** Medical and Hospital Supplies

Results

The different time intervals and wave amplitudes of the ECG, as well as heart frequency, duration of mechanical systole, arterial blood pressure and respiration frequency registered before the administration of cotyledoside, are given in Table 1.

TABLE 1 Parameter values before administration of cotyledoside to 9 anaesthetized guinea-pigs

Parameter	Mean ± SD	Range
ECG time intervals (S)†		
PQ	0,090 ± 0,021	0,065–0,140
QRS	0,023 ± 0,004	0,020–0,030
QT	0,129 ± 0,017	0,110–0,160
ECG wave amplitudes (mV)‡		
P	0,17 ±0,03	0,10–0,20
QRS	1,05 ± 0,34	0,90–1,60
T	0,10 ± 0,04	0,05–0,20
Heart frequency (beats/min)	235 ± 21	190–260*
Mechanical systole (s)†	0,155 ± 0,012	0,140–0,180
Arterial blood pressure (mm Hg)		
Systolic	68,8 ± 37,1	46–100
Diastolic	36,2 ± 15,3	20–58
Respiratory frequency (rate/min)	37,1 ± 11,9	18–52

* Low HF probably due to anaesthetic
† s=seconds
‡ mV=milli volt

Two distinct groups of time-dependent changes occurred in all the guinea-pigs after the administration of cotyledoside. Initially, within 12 min of administration, the first changes (Table 2, Fig. 3), namely, a rise in the blood pressure, shortening of the mechanical systolic time and a tendency for the heart frequency to rise, occurred. The electrical activity of the heart showed small changes (PQ time), contradictory changes (QRS time, QT time) and/or none (P wave amplitude, QRS amplitude) (Table 2). The T wave amplitude, however, showed a marked increase in most cases. In addition, the respiratory frequency tended to increase in guinea-pigs that received higher doses, e.g. 3a, 3b and 3c (Table 2).

Guinea-pigs 1, 2a and 2b showed no further changes after that. The other guinea-pigs showed the following reactions (Fig. 3):

The amplitude of the T wave increased and confluence with the P wave took place until eventually they became superimposed and could not be distinguished (12 min after administration). Ectopic foci (marked 1 and 2, in Fig. 1) followed 15 min after administration and increased progressively in frequency and dissociation, with runs of ventricular tachycardia occurring 27 min after administration. A bizarre ECG pattern was recorded 90 min after administration.

Increased fluctuations in the blood pressure, which correlated with respiratory movements, were encountered, but towards the end the blood pressure values decreased markedly (Fig. 3).

Respiration was clearly influenced, while double expiratory movements and low respiratory frequencies occurred in animals that received the higher doses.

Fig. 1a & 1b *Tylecodon wallichii* subsp. *wallichii (=Cotyledon wallichii)*

TABLE 2 Observations on guinea-pigs acutely intoxicated with a single dose cotyledoside

Guinea-pig	Dosage i.v.		Time of recording (min after administration)	HF	Time intervals			Amplitudes			Mechanical systole	Blood pressure	Respiratory frequency	Further ECG changes (min after administration)	
	mg/kg	s.c. LD_{50}			PQ	QRS	QT	P	QRS	T				ST depression	ectopic foci
1	0,077	0,66×	7	+15,5	+7,7	0	−13,3	+33,3	+27,3	0	−12,5	+100 / +160	−16,6	—	—
2a	0,116	1×	6	0	+6,6	+33,3	−4	0	−11	−50	−7,1	+ 19 / 0	—	—	—
2b	0,116	1×	7	+10,5	0	−16,6	−21,9	0	+5,2	−33,3	−5,8	+ 93 / + 93	−16,6	—	—
2c	0,116	1×	5	+16,3	−28,6	−33,3	0	0	−23	0	+25	+ 35 / + 46	−14,2	7	10,5
2d	0,116	1×	5,5	+32	0	−33,3	—	+25	0	+100	−21,7	− 5 / +110	−11.1	11	16,5
3a	0,174	1,5×	6	+4,5	0	0	+27,2	0	+20	+100	−22	+132 / +183	+33,3	12	13
3b	0,174	1,5×	12	+4	—	+25	+7	—	0	+266	+14	+ 16 / + 13	+200	5	14
3c	0,174	1,5×	9	+8	0	+33	+21	−75	−38	+100	−13	+ 20 / + 21	+21	4,5	12
4	0,262	2,25×	4	−12,5	+6,6	0	+25	−20	−20	+100	+10,3	+ 16 / + 34	—	4	6

+ = % increase
− = % decrease
i.v. = intravenous
s.c. = subcutaneous

Discussion

The initial changes suggest a positive inotropic effect of cotyledoside on the heart followed by a positive chronotropic effect.

The haemodynamic and respiratory changes encountered after administration of cotyledoside are comparable with those obtained in anaesthetized guinea-pigs with cardiac glycoside intoxications like digoxin and 1α, 2α-epoxiscillirosidin (Kruger, 1971). Sapeika (1936) observed the same changes in respiratory movements and blood pressure, and in the excitability and conduction of the heart muscle in anaesthetized cats after administration of an alcoholic extract of *C. wallichii(=T. wallichii)*.

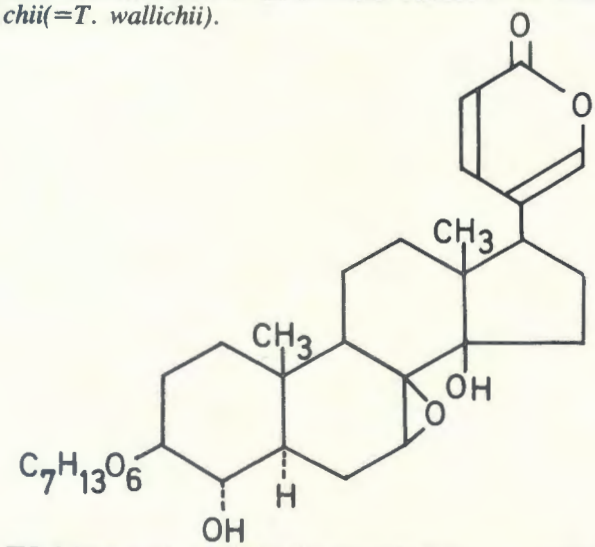

FIG. 2 Cotyledoside, a bufadienolide glycoside, in *Tylecodon wallichii* subsp. *wallichii*

Experiment III. The toxicity of cotyledoside for sheep

Materials and Methods

In order to facilitate the direct recording of arterial blood pressure of unanaesthetized sheep simultaneously with other cardiovascular parameters, the carotid arteries of 3 sheep were exteriorized as described by Jha, Lumb & Johnston (1961) and O'Brien, Chapman, Rudd & McRoberts (1971). The sheep could be used for experimental purposes within 3–4 weeks of the operation.

Seven Merino wethers (2 tooth-full mouth) were deprived of food and water for *c.* 24 h before the experiment. At this stage their live masses varied between 25 and 43 kg (corrected for fleece mass).

The ECG recording were made in accordance with those described by Schultz, Pretorius & Terblanche, 1972. A Siemens nasal thermister was used to register respiratory movements, and the carotid blood pressure was directly recorded by means of a transducer and external manometer (Elema Schönander, 0-300 mm Hg). An Elema differentiator recorded the first derivative (dp/dt) of the blood pressure. The animals were heparinized with 200 iu/kg body mass injected through the catheter immediately after it was inserted into the exteriorized artery. Recordings were made and the animals were clinically examined before and after administration of cotyledoside. The following routine chemical pathological determinations were done periodically: erythrocyte sedimentation rate, haematocrit, haemoglobin and glucose on the blood; and glutamic oxaloacetic transaminase (SGOT), pyruvic transaminase (SGPT), bilirubin, urea nitrogen (SUN), calcium, sodium, potassium, magnesium and inorganic phosphorus on the serum. Sometimes the acid/base balance of the blood was studied with the use of standard techniques (PO_2 and PCO_2).

The cotyledoside solutions were prepared as before and injected intravenously at doses varying between 0,005-0,1 mg/kg (Tables 3 & 4), the object being to produce symptoms of varying severity. Three of the sheep (Sheep 6, 7 and 8) were injected repeatedly with small doses of cotyledoside at varying intervals in order to produce a paralytic syndrome. One of these, Sheep 7 had been used 8 days previously in an acute experiment (Table 4).

All the aminals that died were necropsied.

Results

The nature of the disease produced varied in severity largely in accordance with the magnitude of the dose of cotyledoside they received. Acute, subacute and chronic manifestations of intoxication were observed.

The haemodynamic, respiratory and clinical changes as well as survival time at various dosages are given in Tables 3 and 4.

Acute and subacute intoxication

Ruminal stasis (Sheep 2, 4 and 5) and respiratory distress [Sheep 1, 2, 3, 4 (Fig. 4) and 5], were followed by slight ECG changes as seen in Table 3. These changes were progressive, terminating in excitability and conduction changes such as firing of ectopic foci and AV dissociation [Sheep 1, 2 and 4 (Fig. 4)]. Eventually, Sheep 1 developed apnoea. These cardiac and respiratory crises were either transient or terminated in death.

Consistent, increased T wave amplitudes were probably related to respiratory insufficienty—the respiratory distress and cyanosis lasting until death (Fig. 4). An initial rise in blood pressure (Sheep 2) was followed by a progressive decline (Sheep 2 and 4, Fig. 4).

At 0,1 mg/kg clinical signs (including dyspnoea), haemodynamic changes typical of bufadienolides (Naudé & Pretorius, 1969, unpublished data) and other cardiac glycoside poisoning were observed.

At 0,05 mg/kg, 1 of 2 sheep died after developing typical cardiac glycoside intoxication. The other showed no marked haemodynamic changes, but the effect on respiration was pronounced.

At 0,025 mg/kg, no ill effects except ruminal stasis and polypnoea were noted in the 2 sheep. However, a second dose of 0,025 mg/kg given 3,5 h later resulted in the death of Sheep 4, with typical signs of cardiac glycoside poisoning. In Sheep 5, a second dose after 24 h resulted in death, with only mild haemodynamic changes but with severe respiratory involvement and clear-cut paresis.

Thus, although the acutely intoxicated sheep (Sheep 2 and 3) occasionally collapsed, definite though transient paresis was evident only in the subacutely intoxicated one (Sheep 5).

The necropsy findings (Table 3) resembled those expected in cardiac and respiratory failure.

Chronic intoxication

Of the 3 sheep, Sheep 6 was found to be comparatively resistant to intoxication (Table 4). Five consecutive daily doses of 0,01 mg/kg were needed to produce weakness and collapse on exercise. A sixth dose the next day resulted in the typical paretic syndrome described below. However, it recovered from this within 2 days.

After a rest period of 5 days doses on Days 11 and 13 resulted in mild paretic symptoms which were still detectable on Day 19, when a further injection was given,

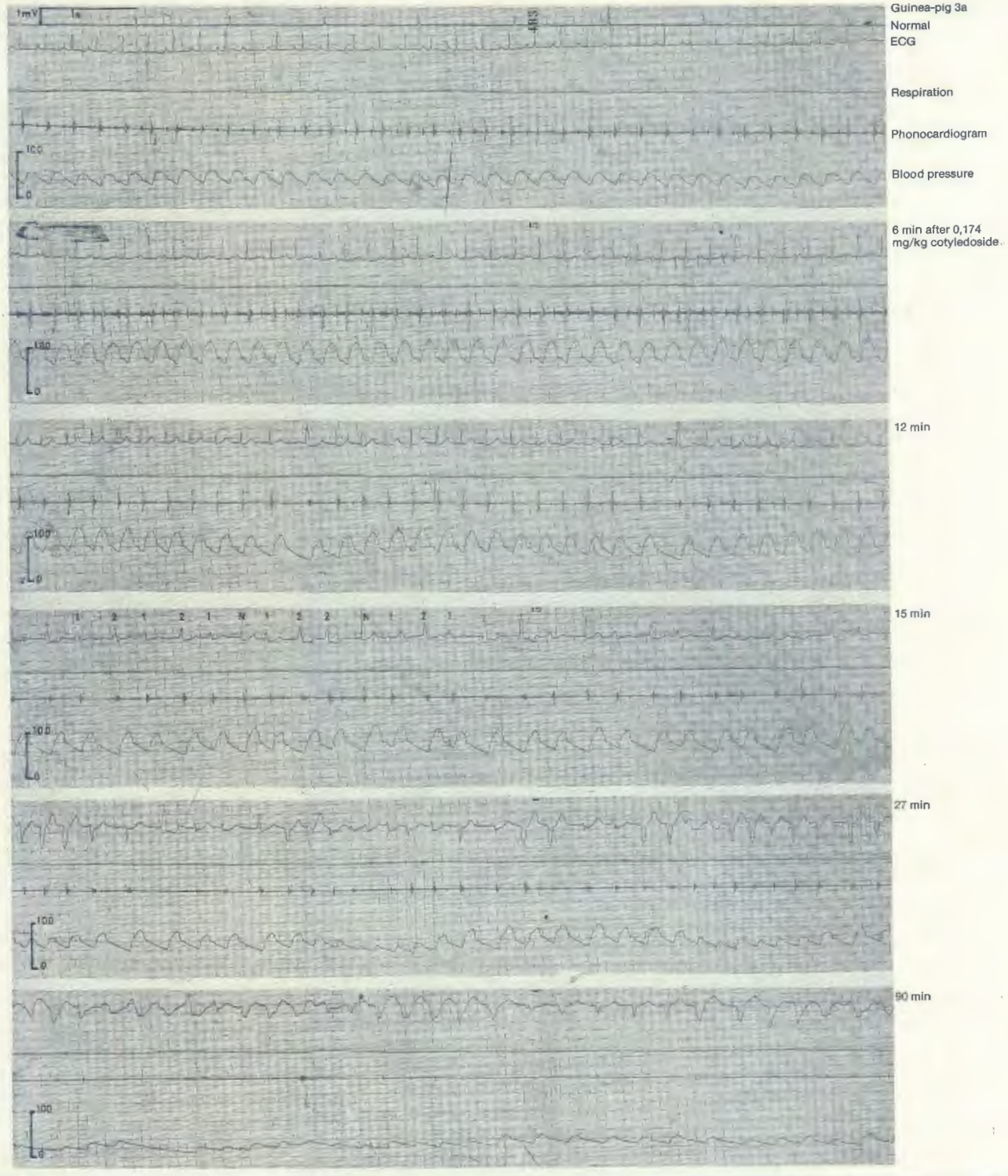

FIG. 3 A typical example of the haemodynamic changes in a guinea-pig encountered after intravenous administration of cotyledoside at 0,174 mg/kg (1,5 × subcutaneous LD_{50}).
6 min: Confluence of P and T waves. BP increased.
12 min: Ventricular tachycardia: coincidental AV synchronization with marked influence on phonocardiogram and BP.
15 min: Normal beats (N) and ectopic foci (1 and 2) are indicated.
27 min: Different ectopic foci firing and ventricular tachycardia; decreased BP.
90 min: Bizarre ECG, very low BP.

followed by another on Day 21. Paresis was more marked but not excessive, and on Day 26 a final dose inexpectedly resulted in death overnight.

In Sheep 7 it was found that a single dose of 0,01 mg/kg/d produced no untoward effect but that a second dose the next day resulted in the paretic syndrome. These symptoms gradually abated until after *c.* 5 days the sheep appeared to be quite normal again. By judicious intermittent dosings at varying intervals, depending on the symptoms, the paretic syndrome could be reproduced and its severity as well as duration controlled at will.

In Sheep 8, 3 consecutive daily injections of 0,01 mg/kg resulted in an acute syndrome, with typical cardiac and respiratory signs from which the animal soon recovered. Two consecutive injections on Day 6 and 7 resulted in mild paresis which became very pronounced after a further dose on Day 13 and lasted for 11 days, i.e. up to Day 24.

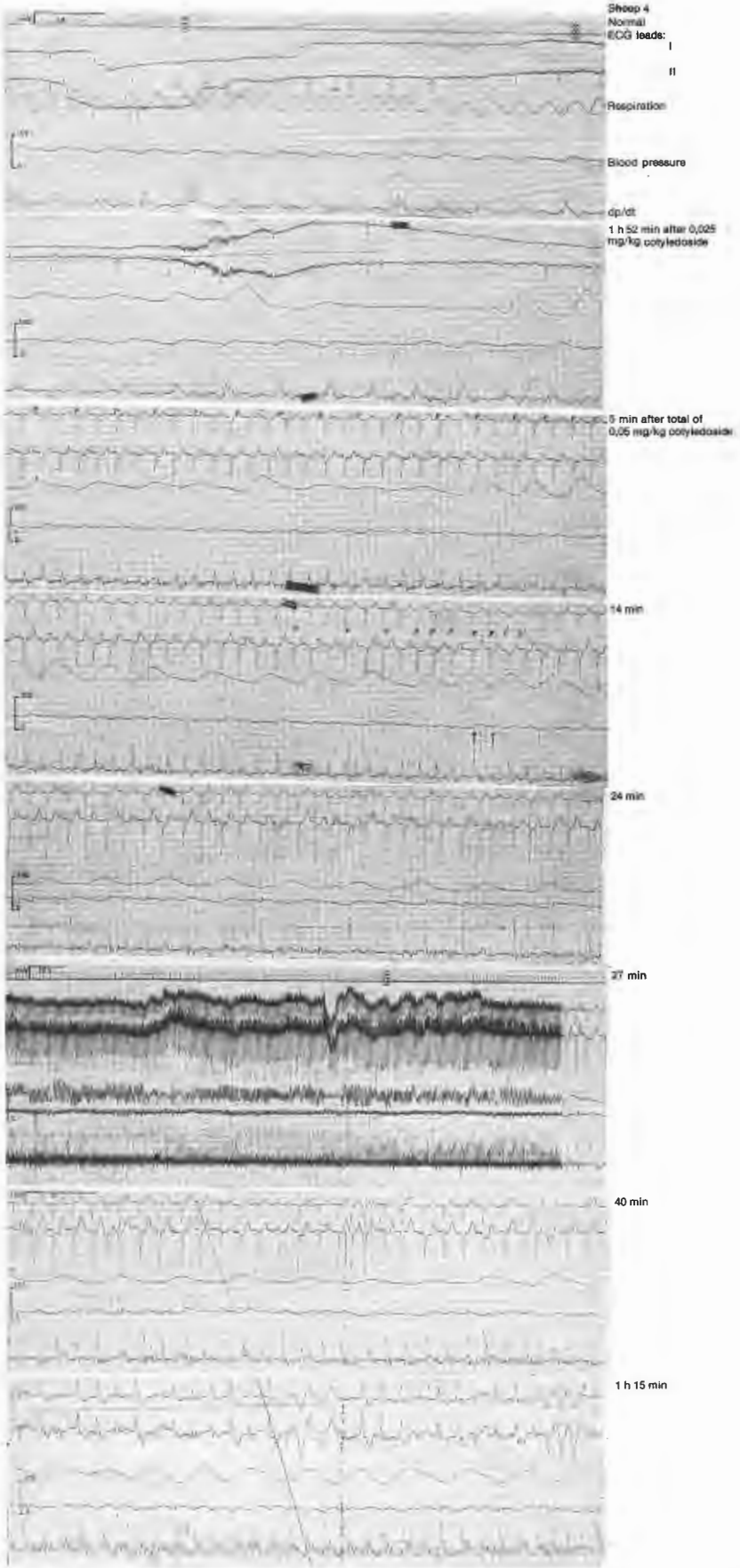

FIG. 4 Parameters (Sheep 4) before and after administration of 0,025 mg cotyledoside/kg intravenously followed by 0,025 mg/kg 3,5 h later.
1 h 52 min after 0,025 mg/kg: Struggling and arhythmic respiratory movement (respiratory crisis); decreased BP.
5 min after 0,05 mg/kg: AV dissociation with ventricular tachycardia; P waves are indicated. ST segment suppression. BP decreased with changed configuration of the anacrotic notch.
14 min: AV dissociation: P waves are indicated. Electrical alternans. Longer ventricular filling time corresponds with higher BP and dp/dt but not with the QRS amplitude (indicated by 1st and 2nd arrow respectively).
24 min: AV dissociation. Note the effect of ventricular filling time on BP and dp/dt. Double respiratory movements.
27 min: Note variation in dept of respiration and respiratory crisis with little effect on the BP.
40 min: Different ectopic foci were firing. Note effect of diastolic time (filling time) on the BP and dp/dt.
1 h 15 min: Bizarre ECG, but respiration at this moment rhythmic although double. Pulse wave configuration differed again.

Fig. 5 Sheep 7 in the typical "krimpsiekte" attitude, after being repeatedly dosed with 0,01 mg/kg/day cotyledoside. Note the position of the feet.

TABLE 3 Observations on sheep acutely and subacutely intoxicated with relatively high doses of cotyledoside

Sheep No.	Dosage mg/kg	Survival time	Haemodynamic changes	Clinical signs	Necropsy
1	0,1	45 min	Tachycardia, transient sinus arhythmia, followed by ectopic foci and AV dissociation (12 min). Later T wave aplitude increased; wave configuration reversed and biphasic; QT segment depressed; QRS time prolonged. Terminally, tachycardia, ectopic foci and bizarre ECG patterns	Polypnoea; irregular, jerky abdominal breathing; transient apnoea (17 min). Later irregular deep. slow respiration with consequent cyanosis. Terminally, erratic breathing and severe struggling	Cyanosis, marked lung emphysema and slight subepicardial petechiae
2*	0,05	1 h 35 min	Rise in BP (20% after 20 min) followed by a drop (40% after 45 min); enlarged QRS and T wave amplitudes, slightly prolonged PR time ending in ectopic foci, AV dissociation, arhythmia	Ruminal stasis, dyspnoea, cyanosis, increased blood CO_2, coma and death	Cyanosis, general venous congestion, marked lung emphysema and slight hydropericardium
3	0,05	—	Transiently decreased PR time interval; T wave amplitude slightly increased. Returned to normal after 4th day	Respiration abdominal and jerky; cyanosis; inability to stand for long. Complete recovery by Day 4	—
4*	0,025 After 3,5 h 0,025	4 h 50 min	After 2nd dose, increased QRS and T wave amplitudes; elevation of BP (16%) and dp/dt (31%) followed by AV dissociation; electrical alternance and decrease in BP	1st dose produced only ruminal stasis and polypnoea. After 2nd dose, dyspnoea; cyanosis; cardiac arhythmia; death	Cyanosis, congestion of lungs, ascites, slight hydropericardium and subendocardial petechiae of left ventricle
5	0,025 After 24 h 0,025	31 h	After 1st dose, only increased T wave amplitudes; tachycardia after mild exercise. After 2nd dose also transient episodes of tachycardia	After 1st dose, ruminal stasis and polypnoea. After 2nd dose, deep abdominal respiratory movements; cyanosis; shivering hindquarters and difficulty in standing upright	General congestion and cyanosis, lung oedema, hydropericardium, subepi- and endocardial petechiae, slight intestinal atony, suspected nephrosis and congestion of liver

* Blood pressure (BP) recorded

TABLE 4 Observations on sheep chronically intoxicated with repeated low doses of cotyledoside

Sheep No.	Dosage schedule	Survival time	Haemodynamic changes	Clinical signs	Necropsy
6	11 × 0,01 mg/kg over 27 d on Day 0, 1, 2, 3, 4, 5, 11, 13, 19, 21 and 26	27	T wave amplitudes increased. Sinus tachycardia occurred once after exercise. Myograms, caused by unsteadiness and shivering occurred frequently on the ECG recordings	Dyspnoea and inappetence followed by locomotory/postural changes: unsteadiness; weakness; muscular tremors; ataxia; neck paresis; standing with back arched and feet close together. Frequent urination and diarrhoea (faeces dark, putty-like). Locomotary changes exacerbated by exercise, but disappeared between doses	Cyanosis of liver, kidney and lungs. Pulmonary emphysema, subepicardial petechiae
7†	10 × 0,01 mg/kg over 36 d on Day 0, 1, 6, 13, 14, 16, 24, 28, 31 and 35 1 × 0,005 mg/kg Day 37	46 (slaughtered)	Transient QRS and T wave configurational changes	A systolic murmer occurred throughout the trial. Locomotory changes as observed in Sheep 6. Terminally respiratory movements were jerky and the animal became very weak	Pulmonary emphysema Light hydropericardium
8‡	6 × 0,01 mg/kg over 14 days on Day 0, 1, 2, 6, 7 and 13 5 × 0,005 mg/kg on Day 27, 35, 42, 43 and 44	45 (slaughtered)	On Day 3, HF increased (140/min); BP (44%) elevated and dp/dt (53%) depressed; QRS and T wave amplitudes slightly increased, followed by AV dissociation, ventricular tachycardia, QT segment depression. The T wave remained slightly enlarged throughout the experiment. Terminally the BP was lowered	Polypnoea, inappetence and ruminal atony (3/5 min) followed by ruminal stasis. Locomotory changes as observed in Sheep 6. Blood analysis showed only transient, slight rises in PCO_2 values corresponding to clinical signs	Weak condition; atrophy of rumen

† Sheep 3 in previous experiment
‡ Blood pressure recorded

A single injection of 0,005 mg/kg on Day 27 caused a return of mild signs of paresis. The same dosage on Day 35 produced even milder reactions. However, on Day 42–44, 3 consecutive daily doses were needed to obtain a similar response.

The most conspicuous clinical signs in these experiments involved the locomotor system of the sheep. This syndrome, which became pronounced during exercise, included paresis, weakness, postural changes and ataxia (Table 4). All the chronically affected sheep assumed a typical "krimpsiekte" posture, the sheep standing on tiptoe, with the feet close together and the back arched (Fig. 5). These abnormalities were evident for a few days after the sheep were dosed, but disappeared when no further doses were given.

In 2 sheep respiration was influenced throughout the trial in a manner comparable with that of the acute disease. These signs were exacerbated by light exercise.

Only Sheep 8, amongst the chronically intoxicated ones, developed marked ECG changes. Blood pressure, recorded only in this sheep, was mildly elevated for most of the experiment. Unlike the other sheep, Sheep 8 had recurrent bouts of ruminal stasis accompanied by anorexia and a loss in mass of 7,5 kg over 35 days.

Discussion

During acute intoxication ruminal movements, respiratory and cardiac functions were noticeably affected.

Blood pressure and ECG changes followed the same pattern during this investigation as those described by Pretorius, Van der Walt, Kruger & Naudé (1969) after administration of dried *Homeria glauca* via rumen fistula and 1α, 2 α-epoxyscillirosidin (isolated from *H. glauca*) intravenously. The clinical signs during the acute experiments are comparable with those obtained by Terblanche & Adelaar (1965) in sheep poisoned with *C. orbiculata* L.

Chronic intoxication gave rise to typical signs of "krimpsiekte" with characteristic locomotor system changes (Henning, 1926). The heart was markedly affected in only 1 case and this occurred after a relatively high dose had been given in a short period. The respiratory system was less affected than in the acute cases, but after light exercise the respiratory involvement became more pronounced. The PCO_2 was only slightly affected. Diarrhoea occurred only in 1 case where the faeces were putty-like. Progressive ruminal atony, as found in sheep poisoned by *C. orbiculata* (Terblanche & Adelaar, 1965), was absent. Bulbar paralysis as described by Henning (1926) was not seen.

In the case of intoxication of sheep by digoxin and the bufadienolide 1α, 2 α-epoxyscillirosidin, Naudé & Pretorius (unpublished data, 1969) recorded the important contribution of extra cardiac symptoms to the poisoning syndrome, particularly respiratory involvement and skeletal muscle weakness, and paralysis. 1α, 2 α-epoxyscillirosidin was however not found to be a cumulative poison in guinea-pigs when 0,25 × LD_{50}/d was administered for 20 days (Naudé & Potgieter, 1971). Furthermore, in Sheep 3 repeated daily doses of 1 g of dried *H. glauca*/kg (LD_{100}=1,5 g/kg) followed by 1,25 g/kg on the fourth day, did not result in cumulative toxicity (Naudé & Pretorius, unpublished data, 1969).

However, with cotyledoside it was possible to produce typical cardiac glycoside intoxication with a single lethal dose of *c.* 0,05 mg/kg, predominantly respiratory involvement at *c.* 0,025 mg/kg, and with repeated doses of 0,01 mg/kg at intervals of 24 h or more, the paralytic syndrome wich is identical with the condition known as "krimpsiekte" (Henning, 1926).

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