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Studies on the Action of Potassium Monofluoroacetate (CH_2FCOOK) [*Dichapetalum cymosum* (Hook) Engl.] Toxin on Animals.

By J. I. QUIN and R. CLARK, Section of Physiology, Onderstepoort.

Introduction.

Marais (1944) identified the toxic principle of the plant "Gifblaar" [*Dichapetalum cymosum* (Hook) Engl.] as being monofluoroacetic acid. In the same publication he described the synthesis of this compound. Subsequently the action of this synthetic product on various animals and organ systems was studied in the Section of Physiology at Onderstepoort. All the experiments described in this report were carried out with synthetically prepared potassium monofluoroacetate supplied by Dr. Marais.

Species Susceptibility.

It was found that there was a marked species variation in susceptibility to the compound as is indicated by the results shown in Table 1.

Table 1.

Species.	Dose per Kilogram Body Weight.	Number Treated.	Deaths.	Route of Administration.
	Mgm.			
Rabbit	0·5	70	5	Various.
	1·0	2	2	Subcut.
White mice	4·0	3	0	Subcut.
	8·0	12	4	Subcut.
	16·0	16	8	Subcut.
Guinea pig	0·25	20	20	Various.
Sheep	2·0	3	3	Intravenous.
Sheep	2·0	3	3	Intravenous.
Fowl	50·0	1	0	*Per os.*
	50·0	1	1	Subcut.
	20·0	1	0	*Per os.*
	20·0	1	1	Subcut.
	10·0	2	0	Subcut.
Xenopis laevis (clawed toad)	500·0	2	0	Subcut.
	500·0	2	0	Intraperitoneal.

Owing to the limited supplies of the compound available, no attempt was made to ascertain the M.L.D. for each species tested. It will, however, be seen that, in the case of the rabbit and guinea-pig, less than one mgm. per Kilogram body weight was lethal. These figures for the rabbit are in accordance with the earlier findings of Marais (1943), who found the M.L.D. of the purified toxic principle extracted from the plant to be between 0·5 and 0·75 mgm. per kilogram body weight in the case of this species. Two mgm. per Kilogram body weight killed all three sheep in less than six hours, thus indicating that much smaller doses than this would be fatal. The white mouse was relatively resistant. The fowl was extremely resistant when dosed, but of equal susceptibility to the mouse when injected subcutaneously. The clawed toad (*Xenopis laevis*) was not affected by doses of up to 500 mgm. per Kilogram administered parentally.

Route of Administration.

The effect of the route of administration on the toxicity of the compound was tested on rabbits and guinea-pigs. Groups of these animals were given equal doses, calculated on body weight, by the following routes, viz. *per os*, subcutaneous, intramuscular, intraperitoneal, and intravenous. In no case was there any significant difference between any of these groups as regards either the mortality or the rapidity with which death took place. The poison produced no local reaction at the site of introduction and post-mortem lesions were constant, regardless of the mode of administration.

Symptoms and Microscopic Lesions.

In all species tested there was a considerable latent period between the administration of potassium monofluoroacetate and the advent of gross clinical symptoms. This period was seldom less than two hours, even after 10 to 20 times the lethal dose was given. Auscultation of the heart in sheep, however, revealed that marked disturbances occurred 15 to 20 minutes after the intravenous injection of 2 mgm. per Kilogram body weight. The heart sounds became accelerated, sometimes up to 300 per minute, while the pulse itself was feeble and irregular. Later, progressive respiratory distress appeared. The rabbits and guinea-pigs usually showed no signs for three or four hours after administration of the poison, when sudden spasms of respiratory distress occurred. These were often violent in character and accompanied by the emination of froth from the nose and mouth. The white mice became extremely sluggish in their movements about an hour after the injection, often appearing moribund but frequently recovering. Death was usually immediately preceded by tetanic spasms almost identical with those seen in acute strychnine poisoning in this species. No definite symptoms were noted in the fowls prior to death. The clawed toads showed no reaction whatever to the poison.

The lesions found at post-mortem were all indicative of failure of the heart. There was a generalised cyanosis and venous congestion, especially severe in the heart and lungs. In addition the lungs showed marked oedema and, in some cases, intra-pulmonary haemorrhage. There was no sign of any inflammatory reaction, either at the site of introduction, or in any other part of the body.

Effect of Repeated Sub-lethal Doses.

A group of ten white mice received 4 mgm. of the poison subcutaneously on seven consecutive days. All the animals were visibly affected by each

injection but only two deaths occurred, one after the second injection and the other after the sixth. There was, therefore, no evidence of a cumulative effect and the poison was, in all probability, being rapidly eliminated after each injection.

Development of Tolerance.

A week after the completion of the above experiment the surviving eight mice, together with eight fresh controls were injected subcutaneously with 14 mgm. of the poison per Kilogram body weight. The results are shown in Table 2.

Table 2.

Group.	Number.	Deaths and Period after Injection at which these occurred.				Total.
		4 Hrs.	5 Hrs.	6–22 Hrs.	48 Hrs.	
Previously treated.....	8	0	0	0	2	2
Controls.....	8	1	1	2	0	4

As seen in the above table one of the control mice died after four hours, another after five hours and two more were found dead the following morning. Among the previously treated mice two died 48 hours after injection. The lower mortality in this group, together with the very much longer survival period of those which did succumb, point to the development of a tolerance to this poison in mice.

The Effect of Fluoroacetate on the Fermentation of Ruminal Flora.

Duplicate samples of ruminal contents were withdrawn into Erlenmeyer flasks from healthy sheep with permanent ruminal fistulae. To one set of samples the poison was added so as to yield final concentration of 1:10,000 —1:5,000—1:2,000—1:1,000 and 1:700 respectively. Glucose, in a final concentration of 0·1 per cent. was added to all the samples which were then incubated at 39° C. in a water bath while being constantly shaken mechanically. The gas evolved was led off to individual manometers and its volume recorded every five minutes. From this it was found that, in the concentrations given above, the poison had no effect on the gas-producing activity of the ruminal flora. After incubation, samples of the ingesta were stained with iodine solution for starch and glycogen and examined microscopically. This showed that the presence of the poison had had no effect on either the number of bacteria and pseudo-yeasts present or on the amount of starch and glycogen normally accumulated in these organisms. These results indicate that the presence of fluoroacetate in the fore-stomachs would exert no harmful effect on the normal fermentation processes in these organs.

The Effect of Ruminal Fermentation on the Toxicity of Fluoroacetate.

After incubation for four hours the above ruminal ingesta, containing fluoroacetate, were dosed to four guinea-pigs and one rabbit. The amount dosed to each animal was calculated to have contained 0·5 mgm. of the

poison per Kilogram live weight prior to incubation. As controls two guinea-pigs were also dosed with comparable amounts of the poison freshly dissolved in water. Two of the guinea-pigs dosed with ruminal ingesta and the rabbit died. Of the two controls one died. The ruminal ingesta which had been incubated without the addition of fluoroacetate proved innocuous to other guinea-pigs. There was, therefore, no loss in the activity of the toxin during fermentation by normal ruminal flora, thereby indicating that under natural conditions of poisoning with the plant *Dichapetalum cymosum* its toxicity is not reduced during its stay in the fore-stomachs.

Influence on Specific Organs.

A. *Duodenal Strip of the Rabbit.*

As will be seen from Figure 1, the addition of 1:22,000 fluoroacetate to the Ringer-Locke fluid in the Dale bath caused a marked decrease in the size of the contractions and a slight loss of tone after an interval of one to two minutes. The rhythm, however, was not affected. An increase of the concentration to 1:1,750 had no further effect, neither did the tissue return to normal after replacement of the toxic solution by fresh physiological saline.

B. *Rabbit Spleen and Uterus (pregnant and non-pregnant).*

No effect could be demonstrated on these organs by concentrations up to 1:2,000.

C. *Rabbit Heart.*

The isolated rabbit's heart was perfused in the usual way with oxygenated Ringer-Locke solution. Owing to limited supply the poison was not added to the perfusion in the reservoir but, after a normal tracing had been obtained, 0·25 mgm. fluoroacetate dissolved in Ringer-Locke was injected into the inlet tube immediately above the heart. The results can be seen in Figure 2.

Although the material passed rapidly through the heart in relatively high concentration, there was no effect for 30 seconds. Ventricular arrhythmia due to partial heart-block then set in, every second auricular beat only being transmitted to the ventricles. This continued for about 100 seconds when the arrhythmia disappeared and was followed by a marked increase in the force of the heart beat and, to a lesser extent, acceleration, giving a typical adrenalin-like effect. After a further 60 seconds this reaction in turn passed off and, after a period of normality, the ventricular arrhythmia again appeared. All the changes described above occurred as a result of a single injection of the poison. A subsequent injection of 0·5 mgm. caused complete heart-block, during which the auricles continued to beat but the ventricles were entirely quiescent.

Repetition of this experiment gave a similar result except that in the second case the adrenalin-like effect preceded the ventricular arrhythmia.

2. *The Rabbit's Heart* in Situ.—A rabbit was narcotised with nembutal and the ventral portion of the thoracic wall removed under artificial respiration. The right ventricle was connected to a recording apparatus and a tracing of its movements made. When the normal heart beat had been recorded, 5 mgm. fluoroacetate was injected into the animal intravenously, representing a dose of 3 mgm. per Kilogram body weight.

As will be seen from the tracing (Figure 3) there was slight augmentation in the excursion of the ventricle one minute after injection. This effect was progressively increased for a further period of five minutes after which marked ventricular arrhythmia appeared. Twenty minutes after the injection the auricles were beating at 120 to the minute while the ventricles were quiescent, the condition being one of complete heart-block and auricular flutter. The excursions shown on the tracing during this stage are due solely to the movement of the lungs.

D. *The Effect on the Carotid Blood Pressure of the Sheep.*

A sheep was narcotised with chloral hydrate intravenously and a canula inserted into the carotid artery. This canula was connected with a mercury manometer and the blood pressure recorded. The tracing so obtained is reproduced in Figure 4.

After the normal carotid pressure had been recorded, 500 mgm. fluoroacetate was injected intravenously (9·6 mgm. per Kilogram body weight).

Six minutes after the injection there was an increase in the frequency of both the pulse and the respiratory movements, together with a slight drop in arterial pressure. For the following 30 minutes the only change noted was a gradual but constant decrease in the carotid blood pressure. A progressively increasing arrhythmia of the pulse then appeared, accompanied by respiratory distress. Finally a violent dyspnoeic spasm dislodged the canula and terminated the recording.

E. *Direct Observation on the Exposed Heart of the Sheep.*

In order to gain more information on the action of this poison on the heart, a series of three sheep were narcotised with chloral hydrate intravenously and the ventral portion of the thoracic wall removed under artificial respiration, in order to expose the heart. An intravenous injection of 500 mgm. was then given and the effect on the heart noted by direct observation. In place of the usual graphic record of heart action, a cinematograph film was made.

In these experiments it was found that even after the intravenous injection of excessive doses (ca. 15 mgm. per Kilogram live weight) there was no visible effect on the heart for a period of 15 to 20 minutes. After this initial latent period the heart beat showed a sudden and dramatic change. In all cases the first change noted was an acceleration accompanied by an increase in the force of the contraction. The ventricular rhythm often increased suddenly from the normal 70 or 80 beats per minute to 160 or more. These periods of tachycardia were usually of one to two minutes duration and were frequently followed by similar periods of extreme bradycardia. Interspersed between these variations of rhythm the heart frequently beat normally for five to ten minutes at a stretch.

In all three cases this state of affairs continued till about an hour after the injection, when definite signs of heart-block supervened. The auricular beat became greatly accelerated, often exhibiting typical auricular flutter, while both ventricles, although contracting in unison, showed a very much slower and irregular rhythm. In one instance the auricles were contracting at a rate of 280 to the minute while a simultaneous count of the ventricular contractions gave a value of 92. Often the ventricles would fail to contract for two or three seconds at a time, during which

period they were subjected to excessive dilatation especially affecting the right ventricle. Even at this stage the heart showed remarkable powers of recovery and for periods of two or three minutes the beat would be perfectly normal. The interference with heart action gradually became more and more frequent and severe while signs of circulatory failure were revealed as a progressive cyanosis especially noticeable over the heart itself. With the failure of the pulmonary circulation and the consequent rise in the carbon dioxide level of the blood, forced diaphragmatic and abdominal respiratory movements made their appearance despite the fact that pulmonary ventilation by means of the respiratory pump was being fully maintained. Finally the heart-block became complete, the auricles continuing to beat while the ventricular musculature went into fibrillation. The heart went into a final and irreversible distention and violent inspiratory efforts marked the onset of death.

Discussion.

It has been suspected in the past that the plant *Dichapetalum cymosum* (gifblaar) acted as a heart poison. It was not till the active principle had been isolated and synthesised, however, that critical experiments on the action of the toxin could be undertaken. The findings reported above leave no doubt that potassium mono-fluoroacetate acts essentially as a cardiac poison, probably with primary effect on the conducting mechanism of the heart. The initial variations in the rate of the heart beat might be explained as due to either a direct effect on the pace-maker or to an interference with the nervous control of the heart. The fact that the isolated perfused heart also showed this reaction would indicate that the sino-auricular node itself is involved.

The heart-block which subsequently developed was, in all respects, typical of an interference in the conducting mechanism. From the symptoms noted in these experiments, fluoroacetate lends itself very well to further investigation concerning the nature of heart-block and cardiac failure.

Summary.

1. Considerable variation in susceptibility to potassium mono-fluoroacetate has been found to exist among various species of laboratory animals.

2. The compound is not affected by actively fermenting ruminal flora nor does its presence affect such fermentation.

3. Direct observation on the hearts of rabbits and sheep injected with this compound have proved it to be a heart poison probably affecting the cardiac conducting mechanism thus leading to partial or complete heart-block.

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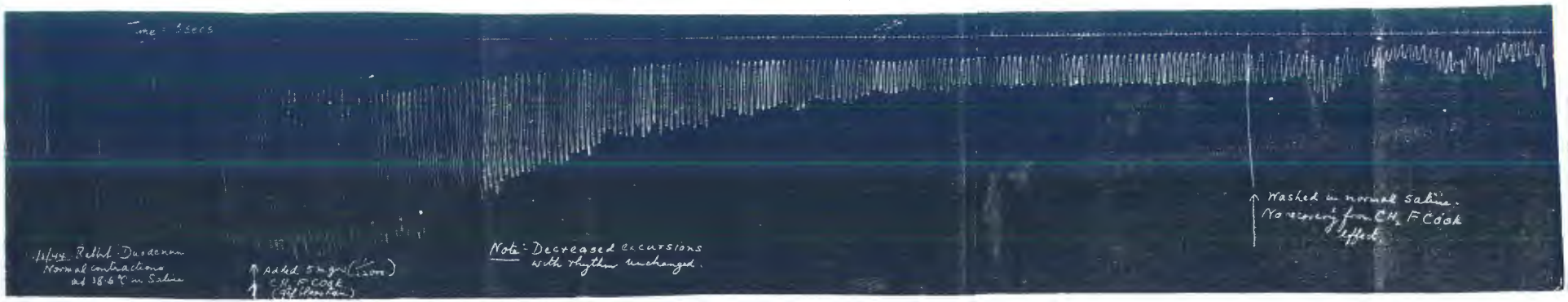
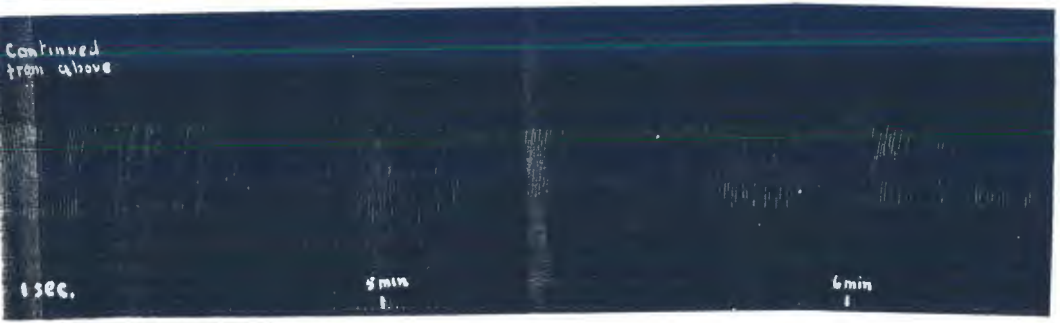
MARAIS, J. S. C. (1944). Monofluoroacetic acid, the toxic principle of " gifblaar " *Dichapetalum cymosum* (Hook). Engl. *Onderstepoort J.*, Vol. 20, No. 1, pp. 67-74.

FIGURE I.

The Effect of Fluoroacetate on the Isolated Duodenal Strip of the Rabbit.





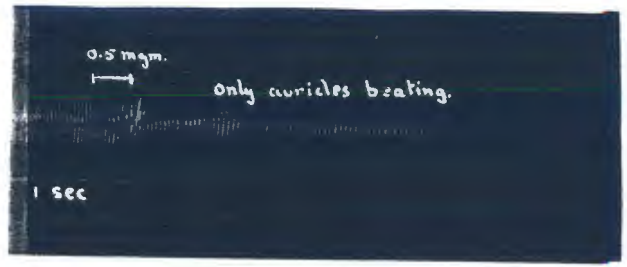


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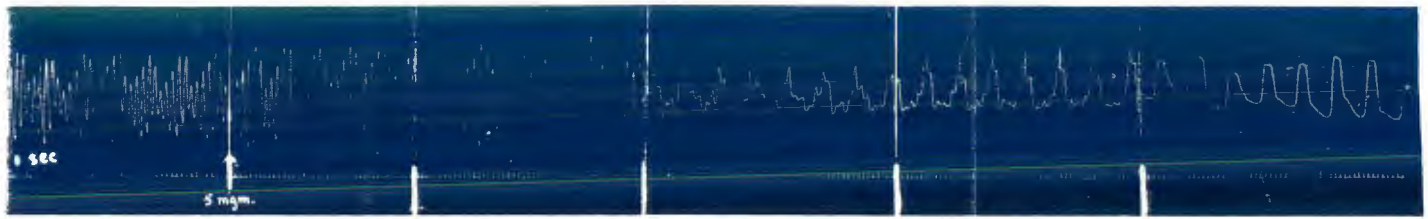
FIGURE 3.

Effect of 5 mgm. CH_2FCOOK on the Right Ventricle of the Rabbit's Heart in Situ (Injection Intravenous).

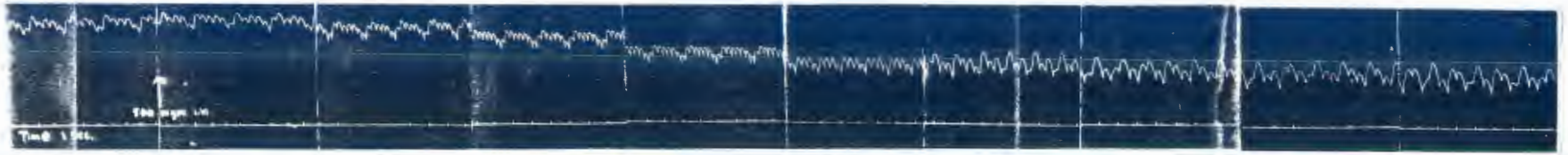


Time after Injection:—	Before.	1 min.	3 min.	6 min.	12 min.	20 min.
Remarks:—	Normal.	Increased rate and excursion.		Marked arrhythmia and partial heart block.		Complete heart block. Movement of lungs only. Auricles beating 120/min.

Notes:—Nembutal anaesthesia. Exposed thoracic cavity. Down stroke = systole. Time tracing 1 sec.

FIGURE 4.

Effect of 500 mgm. NH_2FCOOK on the Carotid Pressure of Sheep 66466 (Wt. 115 lb.). Injection Intravenous.



Time after Injection	Before.	1 min.	7 min.	8 min.	10 min.	30 min.	38 min.	44 min.	55 min.	58 min.
Remarks:—	Normal.	No change.		Increased pulse and respiration rates, fall in pressure.				Arrhythmia. Variations in pressure mainly respiratory origin.		

Notes:—Chloral hydrate anaesthesia. Time tracing 1 sec. Original pressure = 200 mm. Hg. Heart sounds at 55 min. = 300/min.