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by

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In Northern Queensland there are found two species of very large elapine snakes. One of these is *Oxyuranus maclennani,* described by J. R. Kinghorn (1923) from two specimens obtained by Mr. W. McLennan, who, after killing the snakes, procured their venom by expressing it from the poison glands. These two samples of venom, now some years old, after being powdered and dried *in vacuo* over calcium chloride, weighed 0·475 and 0·356 grammes respectively. They were recently sent to us from the Australian Museum by Mr. Kinghorn. A little earlier, through the kindness of Mr. Donald Thomson, we had become possessed of a third sample of venom, also collected in the field by Mr. McLennan from a large brown snake which he believed to be identical with those of which specimens had been sent to the Australian Museum, and which had been described as *Oxyuranus.* Mr. Kinghorn, in sending the two authentic specimens of *Oxyuranus* venom, warned us that this third sample might prove to be that of another large snake, *Pseudechis scutellatus* Peters, indistinguishable from *Oxyuranus* in its external characters, and differentiated by certain osteological and dental characters. The investigation of this third sample of venom has led us to the belief that this is the case, and we are provisionally describing it as the venom of *Pseudechis scutellatus.*

These two snakes are the largest venomous snakes in Australia. They are from 8 to 10 feet long. *Oxyuranus* has a formidable biting apparatus, and the large venom yield and the high potency of its venom makes it beyond question the most dangerous snake in this country. *Pseudechis scutellatus* must also be regarded as highly dangerous, though its venom is much less potent. We have not attempted to determine finally the toxicity of these venoms, using large numbers of animals, since the samples of *Oxyuranus* have undergone some deterioration, neither being completely soluble in physiological saline, and since the sample obtained from Mr. Thomson is not certainly identified as the venom of *Pseudechis scutellatus.*

[1] This research was carried out under a grant from the Department of Health, Commonwealth Government.

1. *The Venom of Oxyuranus maclennani.*

For the estimation of toxicity the larger sample of venom was used. This had been extracted from the venom glands of a snake 8 feet 6 inches long and 7 inches in girth, from which a skull, now in the Australian Museum, was obtained. This sample before being powdered was a hard, translucent mass, which had shrunk from the sides of its containing vessel.

The finely powdered venom, in weighed amounts, was placed in physiological saline, and after an hour at room temperature the insoluble residue was removed by centrifugation before the solution was used for testing. The doses recorded are those of the original dried venom, though only the soluble portion of this has been injected. Since the venom was obtained immediately after death by expression from the venom glands, it may possibly have been diluted to some extent with saliva. For these reasons the venom of this snake must be regarded as even more toxic than our results indicate.

The Results of the Injection of Oxyuranus maclennani in Monkeys.

Three monkeys *(Macacus rhesus)* were injected subcutaneously with varying doses of this venom, in a concentration of 2·0 mgm. per c.cm. The injections were made just below the knee.

Monkey 1. 3·16 kilos, received 1·2 mgm. per kilo. He went off his food, and became weak and ataxic in his movements. Twenty hours after the injection he fell over and lay on his side, without dyspnoea or any distress. The respirations were quiet and entirely diaphragmatic, about 16 per minute. The limbs were not completely paralyzed, occasional movements being observed. There was no circulatory collapse, and the animal remained warm till shortly before death, which took place quietly, without convulsions, 24 hours after the injection. At no stage was any ptosis observed, as with other venoms. The facial muscles contracted rhythmically until shortly before the end, the angles of the mouth being drawn back with each breath. The pupils were neither dilated nor constricted.

Post-mortem. There was no local lesion at the site of injection. The blood was fluid, and the clotting time 14 minutes. There were no gross lesions in any of the organs.

Monkey 2. 2·6 kilos, 1·0 mgm. per kilo injected subcutaneously. After 29 hours the animal was not eating, and there was some weakness and ataxia in the movements. Forty-six hours after the injection the animal fell over, and the only movements observed were those of the facial muscles contracting with each respiration and the passive movements of the abdominal wall. The animal was warm and the heart beating strongly. Under full ether anaesthesia the carotid was cannulized. The mean blood pressure was 90 mm. Hg., the rise and fall at each beat being from 106 mm. to 74 mm. The rate of the heart-beat was 74 per minute, and of the respirations 15 per minute. Stimulation of

the sciatic nerves evoked a good response in the innervated muscles, but there was no appreciable rise in the blood pressure. Stimulation of the phrenic nerves produced a brisk contraction of the diaphragm. The animal died of asphyxia when its thorax was open, and there was no rise in blood pressure. Apart from a few haemorrhages in the lungs there were no post-mortem changes due to the venom.

Monkey 3. 2·71 kilos; 0·5 mgm. per kilo of venom was administered subcutaneously. This animal showed no symptoms, and remained perfectly well.

The lethal dose of this sample of venom for monkeys appears to be about 1·0 mgm. per kilo. The action of the venom is predominantly neurotoxic, and appears to be on the central nervous system, and not on the nerves or nerve-endings, though, as will be seen later, in one or two rabbits the response of the muscles innervated by the sciatic and phrenic nerves was feeble when tested immediately after death while the heart was still beating.

Though the evidence so far as it goes suggests a central rather than a peripheral action of the venom, it should be remembered that Houssay and his colleagues (1922) have produced good evidence that nearly all the venoms they have investigated, including that of *Notechis scutatus,* the Australian tiger snake, have a curarizing action on nerve-endings in skeletal muscle. Whether stimulation of the phrenic will or will not cause brisk contraction of the diaphragm just before death, while the heart is still beating, appears to depend on the time during which the venom has been acting, and upon whether the dose is so large as to produce rapid death from other causes. It seems probable, in the light of Houssay's experiments, that the venom of *Oxyuranus* also has this peripheral action, and experiments to elucidate this point and to decide whether there is a central action as well, are in progress.

The Results of the Injection of the Venom of Oxyuranus maclennani in Rabbits.

Most of the rabbits used were domestic long-eared strains, but a few were young wild rabbits, as indicated by (wild) in brackets after the weight. The results of subcutaneous injection of the venom in a concentration of 2·0 mgm. per c.cm. into the subcutaneous tissues of the abdominal wall are set out in Table 1.

TABLE 1.

The Results of the Subcutaneous Injection of the Venom of Oxyuranus maclennani in Rabbits.

Weight in kilos.	Dose in mgm. per kilogram.	Result.
1.73	2.0	Died in between 6 and 15 hours.
1.84	1.5	Died in between 6 and 15 hours.
2.16	1.0	Died in less than 24 hours.
1.59	0.5	Died in 23½ hours.
1.43	0.3	Paralyzed in 18 hours and remained so till the end of the second day. Survived.

The lethal dose is in the neighbourhood of 0·5 mgm. per kilo. Unfortunately, only one of these animals (that which received 0·5 mgm. per kilo) was observed throughout its illness. After 21 hours it was completely paralyzed, save for the muscles of the face and the muscles of respiration. The respirations were shallow, rapid, and regular. The circulation appeared good, and the animal remained warm till shortly before death. Death took place by failure of respiration. The heart was beating strongly when the thorax was opened immediately before death, and stimulation of the sciatic and phrenic nerves at this time caused only a feeble response in the innervated muscles, though these reacted briskly to direct stimulation. The clotting time was 4½ minutes. Apart from a few petechial haemorrhages at the site of injection, and variable haemorrhages in the lungs and thymus, no gross post-mortem changes were observed in any of these animals.

The results of intravenous injection are set out in Table 2. The venom was injected into a marginal vein of the ear in a concentration of 2·0 mgm. per c.cm., except in the case of the last three animals in the table, in which it was used in a solution containing 0·1 mgm. per c.cm.

TABLE 2.

The Results of the Intravenous Injection of the Venom of Oxyuranus maclennani in Rabbits.

Number of animals.	Average weight in kilos.	Dose in mgm. per kilogram.	Result.
1	1.71	0.9	Died in 5 minutes.
3	2.15	0.8	Died in 3 minutes, 5 minutes, and in 1 hour 39 minutes.
2 (wild)	1.45	0.6	Died in 8½ minutes and in 4 hours 41 minutes.
2	1.83	0.6	Died in 1 hour 20 minutes and in 2 hours 3 minutes.
1	1.89	0.4	Died in less than 24 hours.
1	2.14	0.2	Died in 3 hours.
1	2.0	0.1	Died in 5 hours.
1	2.07	0.05	Severe paralysis on the second day. Survived.
1	1.46	0.03	Paralysis of the hind limbs during the third day. Survived.

The animals which received the smallest doses exhibited striking neurotoxic symptoms, but survived, and those which received 0·8 and 0·9 mgm. per kilo died following intravascular coagulation with typical symptoms (sudden collapse and convulsions). In these there was fibrin whipped out in the right side of the heart, and patchy thrombosis in the portal and mesenteric veins. In no case was massive coagulation observed. Since the "thrombase" factor in venoms is usually more readily destructible, being associated with the coagulable fraction of the venom, it seems likely that the fresh venom, or venom collected and stored under better conditions, would be found to be much more potent in its coagulant action. This suggestion is supported by the examination of the second specimen, which was "denatured" to a greater extent, and was found to have

no demonstrable coagulable action *in vitro*. Injected intravenously into rabbits, in a dose of 2·0 mgm. per kilo, it caused death in 2½ hours, with neurotoxic symptoms, but without any evidence of intravascular clotting. For this reason no attempt has been made to determine accurately the subcutaneous-intravenous index (Fairley, 1929), which is probably greater than 5·0, the certainly lethal dose by the intravenous route being about 0·1 mgm. per kilo.

Animals which, owing to insufficient dosage, escaped the coagulant action of the venom, died quietly without convulsions, with paralysis and failure of respiration. These presented no symptoms immediately following the injection, and, indeed, remained apparently well until a short time before death. The heart was still beating when the thorax was opened after unconsciousness had supervened, the blood was everywhere fluid, and coagulation was somewhat delayed, partial clotting occuring in 7½ to 15 minutes. The phrenic and sciatic nerves were relatively insensitive to faradic stimuli, though the muscles reacted briskly. There were no gross lesions in the organs.

The Effects of the Venom of Oxyuranus maclennani in Guinea-pigs.

The results of subcutaneous injection in the tissues of the abdominal wall of guinea-pigs are shown in Table 3. The venom was used in a concentration of 0·02 mgm. per c.cm.

TABLE 3.

Results of Subcutaneous Injection of the Venom of Oxyuranus maclennani in Guinea-pigs.

Number of animals	Average weight in grams.	Dose in mgm. per 100 grams.	Result.
4	255	0·01	All died in less than 18 hours.
6	215	0·008	Two died in less than 18 hours, one in 24 hours, one on the second day, and two on the fifth day.
2	280	0·005	No symptoms. Survived.

The certainly lethal dose of this sample of venom appears to be about 0·008 mgm. per 100 grammes.

The symptoms following injection are very similar to those observed in rabbits. The animals appear normal till the onset of paralysis, which progresses rapidly, till death occurs from respiratory failure. There is no wetness of the mouth, no obvious dyspnoea, and the circulation appears to be well maintained till shortly before death. Apart from petechial haemorrhages at the site of injection, and haemorrhages in the lungs, there were no obvious pathological changes found at autopsy. The clotting time was normal, 3 to 4 minutes.

The Effect of the Venom of Oxyuranus maclennani in Rats.

A few rats were injected subcutaneously with the venom in a concentration of 0·2 mgm. per c.cm. The results are set out in Table 4.

TABLE 4.

The Results of the Subcutaneous Injection of the Venom of Oxyuranus maclennani in Rats.

Number of animals	Average weight in grams.	Dose in mgm. per 100 grams.	Result.
2	135	0.08	Both died in less than 18 hours.
6	140	0.06	One died in 18 hours, one in 22 hours, one in 28 hours, two on the second day, and one on the fifth day.
2	145	0.04	One died on the fifth day and one survived.
2	150	0.02	Both survived without symptoms.

The lethal dose is about 0·06 mgm. per 100 grammes. The symptoms were closely similar to those observed in guinea-pigs and rabbits, and were predominantly neurotoxic, death occurring from respiratory failure. Apart from haemorrhages in the lungs there were no obvious lesions at autopsy.

The Coagulant Action of the Venom of Oxyuranus maclennani.

This was further investigated, using Lamb's (1903) technique on the citrated plasmas of the rabbit, guinea-pig, and sheep, and on two samples of human plasma. The tests were performed in glass tubes 7·5 cm. long and 12 mm. in internal diameter. These were specially cleaned by boiling in *aqua regia*, and afterwards thoroughly washed in distilled water and dried in the hot-air oven. The venom, in a volume of 0·1 c.cm. of saline, was placed in the bottom of these tubes, and citrated plasma (prepared by shedding blood into an equal volume of 2% sodium citrate in physiological saline and centrifuging out the corpuscles) was added in a volume of 0·2 c.cm., using a dropping pipette. The clotting times in the tubes were observed minute by minute at 37°C. The results are set out in Table 5, where for comparison the clotting times of one of the human plasmas with black snake venom are shown.

TABLE 5.

The Coagulation of Plasma with Oxyuranus Venom in vitro.

Amount of venom in mgms.	Clotting times in minutes. Guinea-pig. (1)	Guinea-pig. (2)	Rabbit.	Sheep.	Human. (1)	Human. (2)	Black Snake. Human. (2)
0.1	15	15	1½	30	5	9	8
0.05	25	20	2	60	7	10	8
0.25	40	40	3		13	12	10
0.012	90 (soft)	90	5		22	20	18
0.0062	not clotted in 20 hour		10	not clotted in 20 hours	30	60 (soft)	23
0.0031			20				
0.0016			30				
0.00078			20 hours				

The differences between plasmas from different species may be dependent on genuine differences in the reaction of the species which with other venoms

have been demonstrated by intravenous injection, or they may be in part related to the methods of bleeding. The human blood was obtained from the median basilic vein, rabbit blood from the marginal vein of the warmed ear, and guinea-pig and sheep blood from the divided vessels of the neck, the citrated sheep blood being procured at the abattoirs.

Experiments with the second smaller sample of venom, to which it was suspected spirit had at some time been added, were of some interest because, though we failed to demonstrate any trace of coagulant activity *in vitro* with any of these plasmas, even using 2·0 mgm. of venom to 0·2 c.cm. of plasma, the venom exhibited a feeble anticoagulant action in doses of 0·1, 0·05, 0·025, 0·012, and 0·0062 mgm. For example, after incubation with guinea-pig plasma for 1 hour, the addition of calcium chloride 0·5% solution in an amount sufficient to cause clotting in control tubes within 4 minutes, only resulted in clotting after the lapse of 50 minutes with 0·1, 0·05, and 0·025, of 40 minutes with 0·012, and of 15 minutes with 0·0062 mgm. Plasma incubated with smaller amounts of the venom exhibited normal clotting on the addition of calcium chloride.

The Haemolytic Effects of the Venom of Oxyuranus maclennani.

The haemolytic action of the venom was tested on the red blood cells of man and of the sheep, rabbit, and guinea-pig. The cells were used in a 3% suspension. The total volume of the haemolytic system was 0·3 c.cm. (0·1 c.cm. of saline or added guinea-pig serum, 0·1 c.cm. of saline containing venom, and 0·1 c.cm. of 3% suspension of red blood corpuscles). "Washed" red blood cells were three times washed in normal saline by centrifugation, and "unwashed" cells were made up at once after spinning them down from the citrated blood. A few experiments were also made using defibrinated blood diluted with saline so as to contain the same proportion of red blood corpuscles as the 3% suspensions in saline.

The tubes used for these haemolysis tests were boiled in *aqua regia*, thoroughly washed in distilled water, and subsequently dried in the hot-air oven. The venom was used in doses of 0·1 mgm., 0·1 x 10^{-1} mgm. . . . . 0·1 x 10^{-9} mgm., or in a closer spaced series, 0·1, 0·05, 0·025, 0·012 . . . . 0·000195 mgm.

With *human* red blood cells, both "washed" and "unwashed," and venom in doses of 0·1, 0·1 x 10^{-1} . . . . 0·1 x 10^{-9} mgm., there was no haemolysis after 4½ hours at 37°C. The addition of 0·1 c.cm. of guinea-pig serum to either "washed" or "unwashed" cells after incubation with venom for 3 hours caused traces of haemolysis in the first three tubes in the series after a further 45 minutes' incubation. Red blood cells from another individual were tested with 0·1, 0·05 . . . . 0·000195 mgm. of venom. Incomplete haemolysis of "washed" cells was observed with 0·1 mgm. of venom after 2½ hours, and complete haemolysis with 0·1 and 0·05 mgm. of venom after 3 hours, but no haemolysis was observed in the parallel experiment with defibrinated blood. In the case

of "washed" cells, incubation in the presence of complement caused some haemolysis in all the tubes in 15 minutes and complete haemolysis in ¾-hour, but with defibrinated blood no haemolysis was observed until ¾-hour, and then only in the first four tubes, containing 0·1, 0·05, 0·025, and 0·0125 mgm. of venom. After 1¼ hours the first seven of the ten tubes in the series were completely haemolyzed. This experiment affords an excellent example of the "protective" action of homologous serum, like that described by Duhig and Jones (1928) in their experiments on the venom of the stone fish, *Synanceja horrida*. A similar phenomenon was noted by Welsh and Chapman (1910) in their study of haemolysis by Australian venoms.

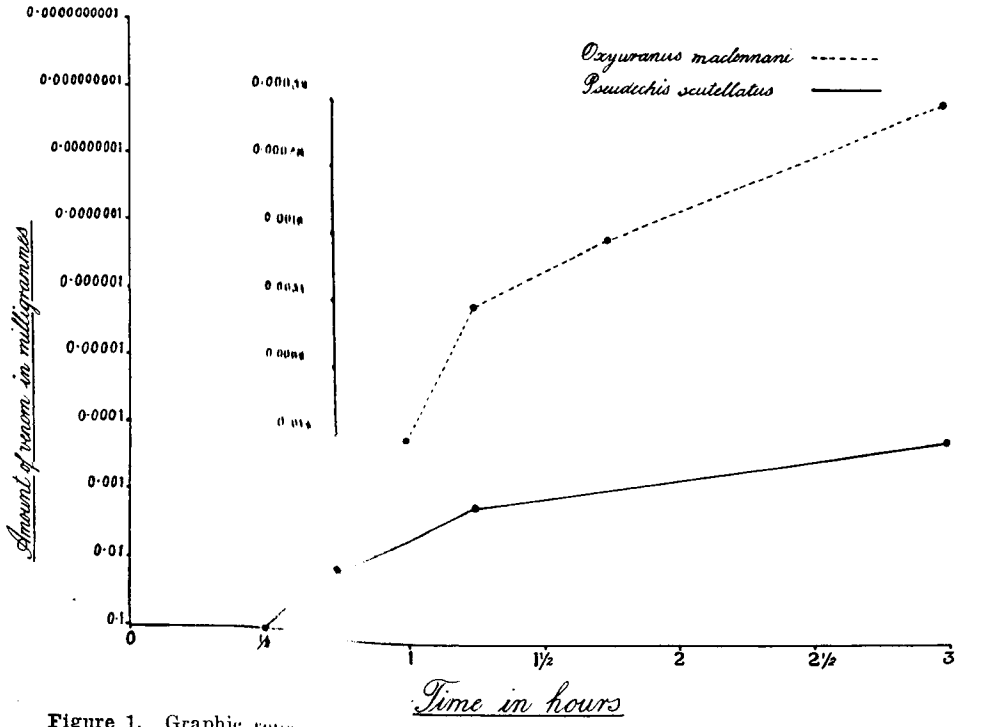

Figure 1. Graphic representation of haemolysis of washed cells of the guinea-pig by the venom of *Oxyuranus macdonnani* in doses of 0·1, 0·05, 0·025, 0·012 . . . . 0.00039 mgm., and of the venom of *Pseudechis scutellatus* in doses of 0·1, 0·01, 0·001, and 0·0001 mgm.

With *sheep* red blood cells no haemolysis was observed in 20 hours, and the addition of complement failed to cause any haemolysis. With *rabbit* red blood cells both "washed" and "unwashed," there was also no haemolysis, but the effect of the addition of guinea-pig serum was not tested.

Guinea-pig red blood cells (figure 1) were much more sensitive to this venom. Complete haemolysis of "unwashed" cells took place with 0·1, 0·05,

0·025, and 0·012 mgm. in 1 hour, with 0·031 mgm. of venom in 1 hour 20 minutes, with 0·0016 in 1¾ hours, and with 0·00078 and 0·00039 mgm. in 3 hours.

The experiments with human, rabbit, and guinea-pig red cells demonstrate the feeble nature of the haemolytic action of this venom, a result which was already suggested by the post-mortem findings in animals killed with venom.

Experiments with guinea-pig red cells showed only a very slight difference in haemolytic activity between the two samples of this venom. The haemolytic, unlike the coagulant, activity of the venom, has not been greatly impaired by the excessive denaturation which has occurred in the smaller sample.

The Effects of the Venom of Oxyuranus on the Circulation.

A number of experiments of a preliminary nature have been made on cats and rabbits under ether anaesthesia without artificial respiration, in which the blood pressure has been recorded by cannulization of the carotid artery. In some the vagi have been intact, and in others divided. The venom used in most of these experiments has been the smaller ("denatured") sample, which does not cause intravascular coagulation when injected intravenously

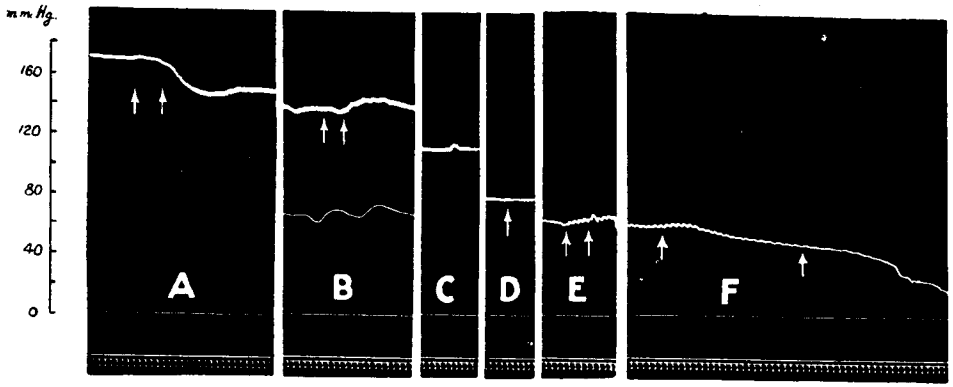

Figure 2. Blood pressure tracing from a cat (Protocol 1) which was injected at A with 10 mgm. of *Oxyuranus* venom intravenously. At B a second dose of 8·0 mgm. was administered. A portion of a plethysmograph record of the small bowel is shown here. As this appeared to be recording only passively the bowel was restored to the abdomen. C, tracing at 1.46 p.m. (Protocol 1). D, record at 1.53 p.m.; vagi cut, no rise of pressure resulting. E, record at 2.20. Right sciatic nerve stimulated, only a very small rise of pressure. F, 5·0 mgm. of *Oxyuranus* (undenatured); rapid fall of blood pressure with coagulation in the right heart.

It has been administered intravenously in a concentration of 2·0 mgm. per c.cm., the injections being made slowly from a syringe. Rabbits were found to be more suitable than cats, as they are much less resistant to the action of the venom, and a dose of about 10 mgm. is sufficiently large to produce death within an hour or two, obviating the necessity for prolonged anaesthesia.

It was hoped that by first injecting a dose of denatured venom it might be possible to induce a negative phase, as Martin (1894) did by injection of minute doses of coagulant venoms, and so permit of the study of the less denatured sample of venom used in most of the other experiments, by injection at a sufficiently rapid rate to ascertain its immediate effects on the blood pressure. This was not possible, and the injection of this sample of venom invariably caused a rapid fall of blood pressure associated with intravascular clotting. Protocols 1 and 2 illustrate the general effects of the venom in the cat and rabbit.

The immediate effect of the injection of a large dose of this venom is to cause a small fall in blood pressure. This is illustrated in figure 2 at A, where a dose of 10 mgm. of *Oxyuranus* was injected. The pressure fell from 169 mm. to 145 mm., shortly recovering to 148 mm. A condensed protocol of this experiment is given here.

Protocol 1. Cat, 2·24 kilos.

12.10 p.m. Ether anaesthesia. Trachea and carotid cannulized. Vagi intact. Time marker, 5 second intervals.
12.34 p.m. Blood pressure recording, 180 mm. Hg.
12.54 p.m. 10 mgm. Oxyuranus ("denatured" sample) injected into femoral vein. Blood pressure 169, falls to 145 mm. and rises again to 148 mm.
1.12 p.m. Blood pressure 142 mm.
1.23 p.m. Blood pressure 135 mm. 8·0 mgm. Oxyuranus "denatured" venom injected.
1.46 p.m. Blood pressure 110 mm.
1.53 p.m. Blood pressure 76 mm. Vagi cut, no rise in blood pressure.
2.20 p.m. Blood pressure 62 mm. Right sciatic nerve stimulated, very slight rise in blood pressure to 66 mm. Good contraction of innervated muscles.
2.25 p.m. Blood pressure 60 mm. 5·0 mgm. of undenatured Oxyuranus venom. Rapid fall in blood pressure and death in four minutes with fibrin whipped out in the right heart but no other sign of intravascular clotting.

Protocol 2. Rabbit, 2·6 kilos. Ether anaesthesia; trachea and carotid cannulized, Vagi cut.

3.50 p.m. Blood pressure recording. 135 mm. Hg.
3.52 p.m. Blood pressure 130 mm. 8·0 mgm. denatured Oxyuranus venom. Blood pressure falls to 117 mm.
3.57 p.m. Respirations 88 per minute. Blood pressure 123 mm.
4.10 p.m. Blood pressure 120 mm. 1·0 mgm. Oxyuranus venom (undenatured). No change in blood pressure.
4.14 p.m. Respirations 80 per minute.
4.16 p.m. Blood pressure 122 mm. 1·2 mgm. Oxyuranus venom (undenatured).
4.26 p.m. Blood pressure 125 mm. Traube waves. Blood not so well oxygenated.
4.30 p.m. Blood pressure 126. Traube waves about seven per minute. 1·8 mgm. Oxyuranus venom (undenatured).
Death in four minutes from the commencement of this injection. Traube waves beautifully marked as the pressure falls. Fibrin whipped out in the heart. Stimulation of the phrenic nerves causes brisk contraction of the diaphragm.

Smaller doses of venom (2·0 mgm.) also cause a small immediate fall in pressure, sometimes preceded by a still smaller rise, which is possibly a volume effect. The later effects of a single dose of the venom on the circulation, when the effects of changes in lung ventilation are eliminated by section of the vagi, are illustrated in Protocol 3.

Protocol 3. Rabbit, 2·67 kilos. Ether anaesthesia; trachea and carotid cannulized, vagi divided, and 9 mgm. of *Oxyuranus* administered intravenously.

Time after injection.	Blood pressure.	Respiration Rate.
1 minute	Falls from 64 to 56 mm.	84
6 minutes	56 mm.	87
13 „	74 mm.	
18 „	66 mm.	94
23 „	66 mm.	84
34 „	72 mm.	74
41 „	84 mm. (Feeble output per beat, oxygenation not so good)	48
48 „	78 mm. (Oxygenation improved)	54
55 „	84 mm. (Traube curves)	66
64 „	80 mm.	44
70 „	70 mm.	34
74 „	Blood pressure falls rapidly and respiration ceases. Artificial respiration ineffective.	

The blood pressure for some time is moderately well maintained, though after about an hour the output of the heart per beat becomes very small. At this stage the respiratory rate, which has been steadily declining, becomes slower, and the blood in the arterial cannula is obviously less well aerated. The blood pressure often rises somewhat, possibly owing to asphyxia, and at this stage Traube curves may appear in the tracing. Finally, the respiratory rate becoming progressively slower, there is a sudden fall of blood pressure, respiration failing at about the same time. There are never convulsions, though stimulation of the sciatic and phrenic nerves always causes brisk responses in the innervated muscles. The failure to demonstrate any peripheral action of the venom in these experiments is probably related to the size of the dose employed and the short period of time during which it has been acting.

Further experiments are in progress for the analysis of the action of this and other venoms on the circulation and respiration, using decerebrate preparations to eliminate the complicating effect of anaesthesia, and decapitate and spinal preparations for the study of the effects on the cardio-vascular system under artificial ventilation. The results of these experiments will form the subject of a further communication.

2. *The Venom of Pseudechis scutellatus.*

This sample of venom was in excellent condition, and was completely soluble in water and saline. Its toxic action will be seen to be strikingly different from that of *Oxyuranus maclennani*. Instead of being coagulant, powerfully neurotoxic and feebly haemolytic in its action, it is anticoagulant, not very strongly neurotoxic, but powerfully haemolytic, and resembles rather closely the venom of *Pseudechis guttatus* De Vis, recently examined by one of us (Kellaway, 1929).

The Effect of the Venom on Rabbits.

Four rabbits were injected with this venom, in a concentration of 2·0 mgm. per c.cm., into the subcutaneous tissues of the abdominal wall. One weighing 1·38 kilos received 3·0 mgm. per kilo, and died in 5¾ hours. Another, also a wild rabbit, weighing 1·3 kilos, received 2·0 mgm. per kilo, and died in less than 18 hours. A domestic rabbit, weighing 1·73 kilos, which received the same dose per kilo, and another weighing nearly 2 kilos, which received half this dose, survived without symptoms. The symptoms which were exhibited by the two rabbits which died were similar to those (shortly to be described) exhibited by rabbits injected intravenously, but were longer delayed in onset. The animals appeared to be sick, but no neurotoxic symptoms were observed, haemolytic changes dominating the picture.

Post-mortem.

The abdominal subcutaneous tissues were the seat of an extensive haemorrhagic oedema spreading from the place of injection. The suprarenals were congested, and there was haemorrhagic congestion of the bowel. There was intense haemoglobinuria. The lungs and thymus contained haemorrhages. The blood was fluid in the heart, and great vessels, and its clotting time was delayed. The serum which exuded from the clot was deeply stained with haemoglobin.

The results of intravenous injection were more dramatic. Even with very large doses there were no immediate symptoms suggestive of intravascular coagulation, though death took place very rapidly in one animal. The results are set out in Table 6. The venom was injected in a concentration of 2 mgm. per c.cm., except in the first animal, where it was used in a concentration of 5 mgm. per c.cm.

TABLE 6.

The Results of Intravenous Injection in Rabbits.

Number of animals.	Average weight in kilos.	Dose in mgm. per kilo.	Result.
1	2·02	5·0	Died in 20 minutes.
1	1·54	2·0	Died in 3½ minutes.
1	1·4	1·0	Died in between 1 hour 20 minutes and 2 hours 50 minutes.
2 (wild)	1·3	0·9	Both died in 1 hour 15 minutes.
2 (wild)	1·25	0·7	Died in 41 minutes and in 1 hour 39 minutes.
2	1·75	0·6	No symptoms. Survived.

The certainly lethal dose is more than 2·0 mgm. per kilo by subcutaneous injection, and about 0·7 mgm. per kilo intravenously. The animal which received the largest dose had no symptoms for 10 minutes. It then exhibited slight weakness, and inco-ordination of its movements. After 19 minutes it had a very brief convulsion, and respiration ceased in 20 minutes. The blood

was everywhere fluid, and the clotting time 10 minutes. The serum which exuded from the clot was deeply blood-stained. There was a little staining of the peritoneum. There were no other lesions. The phrenic and sciatic nerves were stimulated immediately after death, and brisk contraction of the innervated muscles resulted. The symptoms in the remaining animals were very similar. There was a latent interval of varying length, in which there were no obvious symptoms, and then quite suddenly the animal went over on its side and died in a brief convulsive seizure. One animal had a terminal pulmonary oedema, with blood-stained frothy fluid oozing from the mouth and nose. All exhibited intense intravascular haemolysis. Clotting was much delayed, frequently over an hour, and the serum was deeply coloured with blood pigment. The peritoneum and subperitoneal tissues were stained with blood pigment. There was much blood-stained fluid free in the peritoneal cavity, and some animals had similar effusions in the pleural cavities. The bladder in rabbits surviving sufficiently long contained much blood pigment in the urine, and the cut surface of the kidneys was black with altered blood. There was intense haemorrhagic congestion of the small bowel, and there was no obvious clotting anywhere in the vascular system.

Effects in the Guinea-pig.

The effect of subcutaneous injection was also observed in guinea-pigs, and the results of injection in a concentration of 2·0 mgm. per c.cm. are set out in Table 7.

TABLE 7.

Results of Subcutaneous Injection in Guinea-pigs.

Number of animals	Average weight in grams.	Dose in mgm. per 100 grams.	Result.
6	262	0·2	Three died in between 9 and 12 hours, one in less than 18 hours, one in 27 hours, and one survived.
6	261	0·15	Two died on the first day, three on the second day, and one on the third day.
6	268	0·1	One died in less than 18 hours, two on the second day, and three survived.
5	256	0·08	One died on the third day and four survived.

The certainly lethal dose is somewhat more than 0·2 mgm. per 100 grammes. The symptoms were very similar to those observed in rabbits. Apart from a slight roughness of the coat and anorexia, there were no symptoms till shortly before death. The animals did not appear to be paralyzed, though there were sometimes muscular weakness and ataxia. Haemoglobinuria was frequent, and death evidently resulted from the haemolytic action of the venom. The post-mortem findings were closely similar to those found following the injection of diphtheria toxin or of the venom of *Pseudechis guttatus*. All the animals had intense haemorrhagic oedema of the abdominal wall, almost black haemorrhagic

adrenals, and haemorrhagic small bowel. There was much free blood-stained fluid in the peritoneum, and sometimes in the pleural cavities. Haemorrhages in the lungs were constantly present.

The Effects of the Venom of Pseudechis scutellatus in Rats.

The effects of subcutaneous injection of this venom in a few rats are set out in Table 8. The venom was given in the flank in a concentration of 2·0 mgm. per c.cm.

TABLE 8.

The Effects of Subcutaneous Injection in Rats.

Number of animals	Average weight in grams.	Dose in mgm. per 100 grams.	Result.
2	120	0·8	Died in 3½ and 3¾ hours.
6	156	0·4	One died in 23 hours, one in 24 hours, one in 26 hours, two later on in the second day, and one survived.
4	134	0·3	One died in 26 hours, one later on in the second day, and two survived.
2	150	0·2	No definite symptoms. Survived.
2	135	0·15	One died on the third day and the other survived.

The certainly lethal dose is more than 0·4 mgm. per 100 grammes. The symptoms exhibited after injection were very similar to those observed in rabbits and guinea-pigs. The coat was ruffled, and there was often haemoglobinuria. The animals did not become paralyzed, but some appeared to be weak and ataxic before they fell over very shortly before death. The post-mortem findings were similar to those observed in guinea-pigs. There were haemorrhagic oedema spreading from the region of the injection, haemorrhages in the lungs, and haemorrhage and congestion of the bowel. Haemorrhagic adrenals were seldom observed.

The Anticoagulant Action of the Venom.

In vitro experiments were made with this venom side by side with those earlier described on the venom of *Oxyuranus maclennani*. With none of the plasmas was any coagulant action observed with doses of venom up to 2·0 mgm., but, on the contrary, after incubation for 1 hour, and the addition of sufficient calcium chloride to cause rapid coagulation in control tubes, clotting failed to occur in the tubes containing larger amounts of venom in the series 0·1, $0{\cdot}1 \times 10^{-1}$ . . . . $0{\cdot}1 \times 10^{-7}$ mgm. The clotting times after the addition of varying amounts of calcium chloride to the different plasmas which had been incubated with venom, and the clotting times in control tubes, are given in Table 9.

TABLE 9.

Anticoagulant Action of the Venom of Pseudechis scutellatus.

Dose of venom in mgm.	Clotting times in minutes after addition of $CaCl_2$. Guineapig.	Rabbit.	Sheep.	Human.
Nil	4	9½	4	18
.1	No clotting	No clotting	No clotting	No clotting
.01	"	"	"	"
.001	"	"	25 (soft)	"
.0001	12 hours	"	7	"
.00001	4½ mins.	30 mins. (soft)	4½	30 mins.
.000001	4 "	15 " "	4	25 "
.0000001	4 "	9½ " "	4	18 "

The anticoagulant action of this venom is much more striking than that of the venom of the copper-head and death adder (Kellaway, 1929), and approximates closely to that of cobra venom.

The Haemolytic Action of the Venom of Pseudechis scutellatus.

The results of the injection of this venom into animals, and particularly those of intravenous injection in rabbits, demonstrate its striking haemolytic activity. Tests *in vitro* with washed human and rabbit red blood corpuscles, using the methods earlier described, in which comparison was made with the

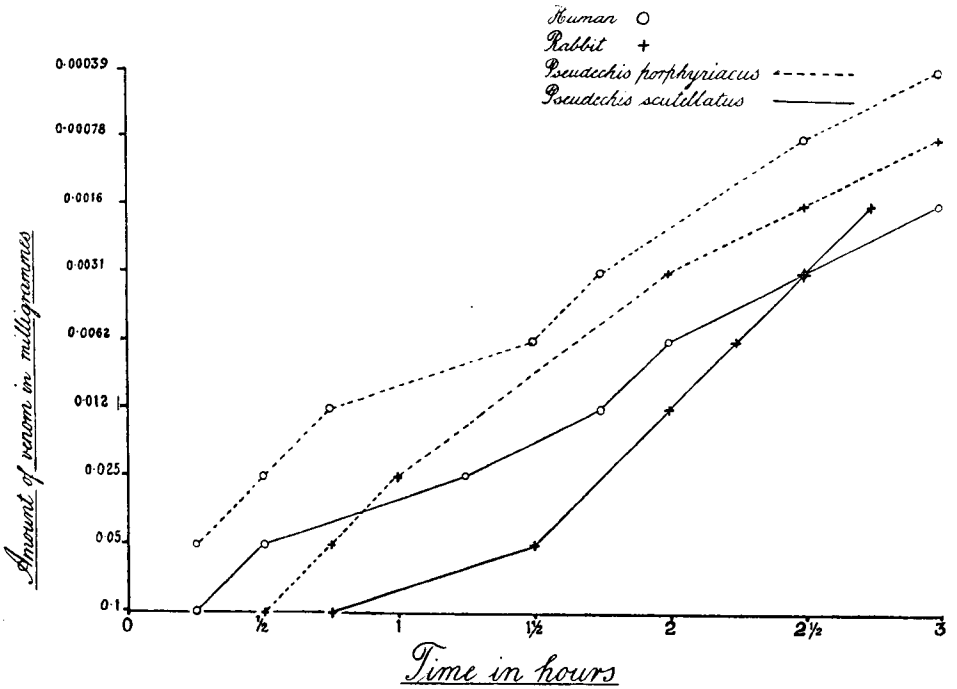

Figure 3. Graphic representation of haemolysis of washed human and rabbit red blood cells by the venom of *Pseudechis porphyriacus* and *Pseudechis scutellatus.*

venom of the black snake *(Pseudechis porphyriacus)*, are graphically represented in figure 3. This is a convenient method of displaying the results, and has no strict quantitative significance. The times at which the various quantities of venom in the series, 0·1, 0·05 . . . . 0·00039 mgm., cause complete haemolysis, furnish the points on the curve. It will be seen that for red blood corpuscles of these two species the venom is but little inferior in activity to that of *Pseudechis porphyriacus*. With defibrinated human blood no haemolysis took place in 3 hours with either venom, but in defibrinated rabbit blood the red cells were hardly protected at all by the presence of homologous serum. The addition of guinea-pig serum to washed human cells and venom caused complete haemolysis in all the tubes in ¾-hour at 37°C., and even with defibrinated blood, though the process was slower, haemolysis was complete in 1¼ hours. With "washed" guinea-pig red blood cells (figure 1) 0·1 mgm. caused complete haemolysis in ½-hour, 0·01 mgm. in ¾-hour, 0·001 in 1 hour 20 minutes. 0·0001 in 3 hours. The sensitiveness of these cells to haemolysis by this venom is therefore about the same as those of man and of the rabbit. With sheep red cells no haemolysis was observed.

The Effect of the Venom of Pseudechis scutellatus on the Circulation.

A number of experiments were made on rabbits under ether anaesthesia without artificial ventilation, in which the trachea and carotid were cannulized and the blood pressure recorded. In some the vagi were intact, and in others they were divided.

The effects of this venom on the circulation were much more striking than those produced by the injection of the denatured specimen of the venom of *Oxyuranus*. The immediate rise and fall of blood pressure produced by doses of 5–8 mgm. intravenously is strikingly like the effect produced by a very small dose of adrenaline, and it is possible that the immediate effect of the venom is actually produced by the liberation of adrenaline from the suprarenals. No proof of this suggestion is, however, offered in the present communication.

Figures 4 and 5 illustrate the circulatory changes which follow the injection of this venom, and the experiments from which they were taken are set out briefly in Protocols 5 and 6.

Protocol 5 (figure 4). Rabbit, 1·44 kilos. Ether anaesthesia; trachea and carotid cannulized, vagi intact. Time in 5-second intervals.

2.50 p.m. Blood pressure 100 mm. Hg.
2.51 p.m. 8·0 mgm. *Pseudechis scutellatus* venom injected intravenously. Blood pressure rises from 91 mm. to 98 mm. and then falls to 55 mm. within 15 seconds.
2.53 p.m. Blood pressure 34 mm.
3.1 p.m. Blood pressure 48 mm. Blood well aerated.
3.5 p.m. Respirations 120 per minute, regular. Blood pressure 60 mm.
3.12 p.m. Respirations 106. Blood pressure 90 mm. Marked Traube curves, 11 per minute.
3.15 p.m. Respirations 96 per minute. Blood not so well oxygenated. Blood pressure 95 mm.
3.16 p.m. to 3.20 p.m. Rapid fall of blood pressure from 80 mm. to 18 mm. Heart fails before respirations which at the beginning of this period are 58 per minute.

Protocol 6 (figure 5). Rabbit, 2·5 kilos. Ether anaesthesia; trachea and carrotid cannulized; loop of bowel enclosed in plethysmograph, and vagi divided. Time in 5-second intervals.

11.50 a.m. 8·0 mgm. *Pseudechis scutellatus* venom injected intravenously. Blood pressure rises from 76 to 85 mm. and then falls in the next 20 seconds to 56 mm. Plethysmograph shows a small increase in volume coincident with this fall. (Plethysmograph record a little behind the blood pressure.)
11.52 a.m. Blood pressure 70 mm.
11.57 a.m. Blood pressure 73 mm. Blood not so well oxygenated.
11.58 a.m. Blood pressure 80 mm. Bowel volume decreases as blood pressure rises.
11.59 a.m. Mean blood pressure 84 mm.
12.6 p.m. Fall in blood pressure.
12.7 p.m. Respiration fails.
12.8½ p.m. Animal dead. The bowel volume falls passively with the blood pressure.

A number of other experiments, both with and without a bowel plethysmograph, was made, and gave closely similar results. In all cases the volume of the bowel increased with the initial fall of blood pressure, and diminished as the pressure rose later. This later rise is probably asphyxial in origin, though the blood in the arterial cannula was not always obviously poorly aerated till later, when the blood pressure was falling. In most of the experiments Traube curves were present in the tracing when the blood pressure was high, just before the dramatic fall of blood pressure which appeared to precede failure of respiration. We have not been fortunate enough to get a good plethysmograph record during the period in which the Traube curves were characteristically present (figure 4 C).

During the final fall the bowel volume was diminished, the plethysmograph record following the blood pressure passively. Protocol 7 is a very good example of the high level of blood pressure reached before the final rapid fall. The plethysmograph record showed vasoconstriction with the rising pressure, and only followed the blood pressure passively in the last 3 or 4 minutes of the experiment.

Protocol 7. Rabbit, 2·70 kilos. Ether anaesthesia; carotid and trachea cannulized; loop of bowel enclosed in a plethysmograph.

4.3 p.m. Blood pressure 66 mm. Hg. Vagi divided. Blood pressures rises to 89 mm. Hg.
4.13 p.m. Blood pressure 90 mm. 5·0 mgm. venom injected. Blood pressure rises to 95 and falls quickly to 80 mm. Hg.
4.16 p.m. Respirations 72 per minute forcible. Blood pressure 93 mm. Hg.
4.17 p.m. Blood pressure 91 mm. Hg.
4.24 p.m. Blood pressure 102 mm. Hg.
4.27 p.m. Blood pressure 106, still rising. Respirations 67 per minute.
4.30 p.m. Blood pressure 120 mm. Respirations 68. Blood still bright in arterial cannula.
4.37 p.m. Blood pressure 140 mm., rising to 150 mm. Hg.
4.42 p.m. Blood pressure 140 mm., falling.
4.43 p.m. Blood pressure 90 mm., falling abruptly to 8 mm.
4.45 p.m. Respiration ceased.

In the last stages of several of these experiments the sciatic and phrenic nerves were stimulated with a faradic current, and brisk contraction was obtained in the innervated muscles.

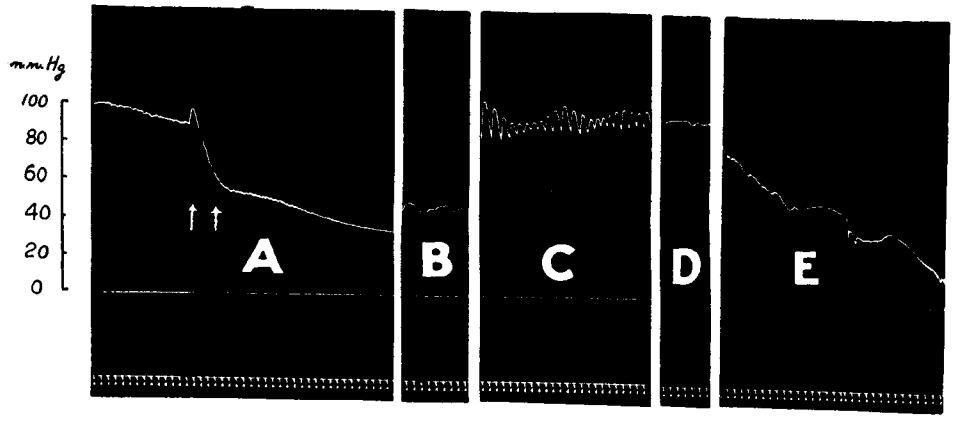

Figure 4. Blood pressure record of a rabbit which received 8.0 mgms. of the venom of *Pseudechis scutellatus* intravenously. At A, injection of venom. B, tracing at 3.1 p.m. (see Protocol 5). C, tracing at 3.12 p.m., showing Traube curves. D, tracing at 3.15 p.m. E, fall of blood pressure, showing effect of terminal convulsive movements.

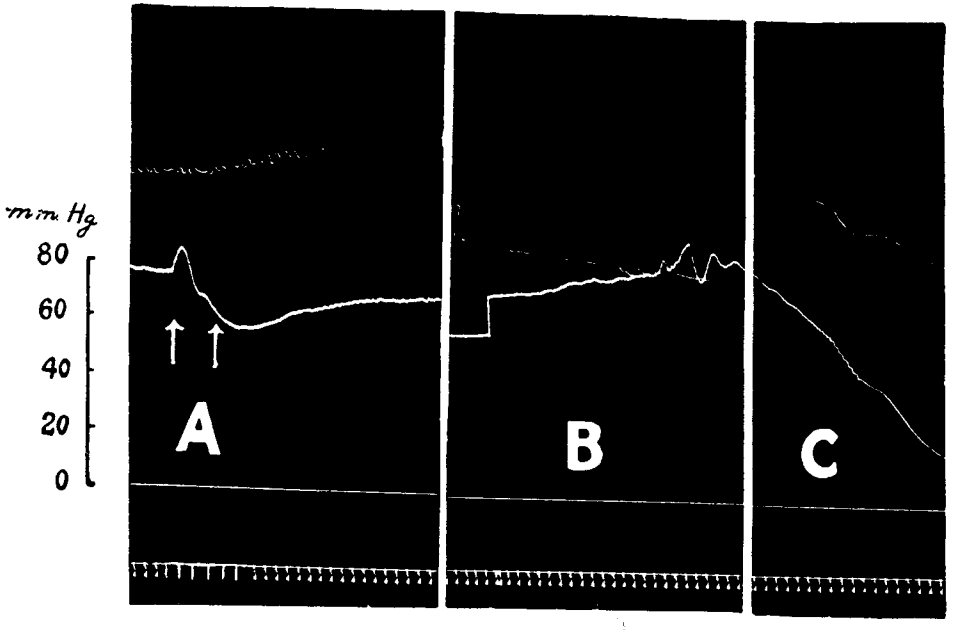

Figure 5. Blood pressure and plethysmograph records from a rabbit following the intravenous injection of the venom of *Pseudechis scutellatus*. The upper tracing is from the bowel plethysmograph. At A, 8.0 mgm. of venom injected. B shows part of the tracing at 12.53 and 12.57 p.m. (Protocol 6). The plethysmograph tracing point is placed a little behind that of the manometer. C shows the final fall of blood pressure; the plethysmograph writing point has been raised by blowing air into the system so that it records above the blood pressure tracing after crossing it in B.

Convulsive movements were not infrequent as a terminal event, and in figure 4 they produce the irregularities in the final fall of blood pressure. We are inclined to the belief that the asphyxial picture produced by this venom is due to intravascular haemolysis interfering with the oxygenation of the bulbar centres, since the animal goes on breathing vigorously, the ventilation of the lungs appearing to be excessive, and the blood in the arterial cannula remaining bright in colour. Respiration ceases when the blood pressure has already fallen to a very low level.

The effects of the venom upon the circulation in this dosage are probably wholly indirect, its first action being the sudden liberation of adrenaline, and its later effect being due to anoxaemia. There is no evidence of neurotoxic action in these rapid deaths, which appear to be due to the haemolytic action of the venom. These conclusions must be regarded as provisional only. Further experiments are in progress for the analysis of the action of this and other Australian venoms on the circulation.

The Stimulant Action of the Venoms on Plain Muscle.

Like all the other Australian elapine venoms so far tested, the two venoms discussed in the present paper have the stimulant action on plain muscle recently described by one of us (Kellaway).[2] On the plain muscle of the guinea-pig the reaction closely resembles that of the anaphylactic response of a sensitive uterus. The peculiar feature of the reaction is that having once responded to a dose of a venom, the plain muscle can no longer respond either to the same dose of that venom or to an equivalent stimulant dose of another venom of the group.

We have made no attempt to determine the limiting dose which with either of these venoms causes a maximal contraction of the isolated guinea-pig uterus. The venom of *Oxyuranus maclennani* is more potent than that of *Pseudechis scutellatus*. With the former a maximal response was obtained with 1 in 500,000; with the latter there was no response with 1 in 1,000,000, but a maximal response with 1 in 250,000. The denatured sample of *Oxyuranus* venom also caused a maximal response in 1 in 250,000.

CONCLUSIONS.

1. We have investigated three samples of snake venom, two unquestionably from *Oxyuranus maclennani* Kinghorn, and one which is almost certainly the venom of *Pseudechis scutellatus* Peters.

2. The venom of *Oxyuranus* injected subcutaneously is lethal to monkeys in doses of the order of 1·0 mgm. per kilo, to rabbits in those of 0·5 mgm. per kilo, to guinea-pigs in those of 0·08 mgm. per kilo, and to rats in doses of the

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order of 0·6 mgm. per kilo. Injected intravenously it is lethal to rabbits in doses of the order of 0·1 mgm. per kilo.

3. The venom contains "thrombase," and causes intravascular coagulation when injected in sufficient dosage. It coagulates citrated plasma *in vitro*.

4. It is only feebly haemolytic, but is powerfully neurotoxic, death in most animals being due to this activity of the venom.

5. It has a slight immediate depressor effect on the blood pressure, and finally causes death by respiratory failure. It diminishes the output of the heart.

6. The venom presumed to be that of *Pseudechis scutellatus* injected subcutaneously is lethal to rabbits in doses of the order of 2–3 mgm. per kilo, to guinea-pigs in doses of from 1·5–2·0 mgm per kilo, and to rats of 4–8 mgm. per kilo. Injected intravenously in rabbits it kills in doses of about 0·7 mgm. per kilo.

7. The venom has powerful anticoagulant and haemolytic activities, but is only feebly neurotoxic.

8. Its effects on the circulation in rabbits are in the main secondary to its haemolytic action.

9. Both these venoms have a stimulant action on isolated plain muscle.

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