

# The Effect of the Venom of the Papuan Black Snake (*Pseudechis Papuanus*) on Blood Coagulation

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THE Papuan black snake (*Pseudechis papuanus*) (Peters and Doria) is believed to be a very common, highly venomous snake in Papua (Worrell 1961). A clinical bleeding tendency due to a defibrination syndrome was thought to follow the bite of this snake and as a consequence the venom was believed to have a strong coagulant action (Champness 1966; Campbell 1967).

The present study was carried out to investigate the action of the venom on blood coagulation *in vitro* and also *in vivo* in dogs, there being little published work on this venom. It is known that it contains a high concentration of direct and indirect haemolysins (Doery and Pearson 1961) and, like several other Australian snake venoms, it contains a heat-stable anti-coagulant (Kaïre 1964).

## MATERIALS AND METHODS

### *In Vitro Studies*

**Papuan black snake venom.** Desiccator-dried venom was obtained from the Australian Reptile Park, Gosford, New South Wales, Australia. A stock solution of 10 mg/ml was made in 0.85 per cent (w/v) NaCl. This was stored in aliquots at  $-20^{\circ}\text{C}$ . The effect of the venom was studied using ten-fold dilutions ranging from 10 mg/ml to  $10^{-7}$  mg/ml in 0.85 per cent (w/v) NaCl.

**Normal human plasma.** Blood from three normal donors was collected into sodium citrate 3.8 per cent in the proportion of 9 parts blood to one part of citrate. The citrated blood was centrifuged at 3000 rpm for 10 minutes and the plasma separated, pooled, and kept at  $4^{\circ}\text{C}$ .

**Whole blood clotting time** (Lee and White 1913). Each estimation was carried out in quadruplicate. 1 ml of whole blood was dispersed in a pre-warmed glass tube containing 0.1 ml of saline or venom saline solution and mixed rapidly. The test was concluded if clotting did not take place within 60 minutes.

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**Prothrombin consumption test** (Merskey 1950). This test was carried out on the clotted and unclotted blood samples following the whole blood coagulation time.

**Plasma recalcification time.** The technique described by Biggs and MacFarlane (1962) was modified by the addition of 0.1 ml of venom solution to the plasma, followed immediately by recalcification. The tests were concluded if there was no clot formation at 600 seconds.

**Thrombin clotting time** (Rothnie and Kinmonth 1960). 'Fibrindex' (Ortho Diagnostics) was used in a concentration of 5 units/ml in 0.85 per cent saline. Saline or venom saline solution, 0.1 ml, was added to 0.1 ml of plasma at  $37^{\circ}\text{C}$ , followed immediately by 0.1 ml of thrombin solution and 0.1 ml of calcium chloride.

**Prothrombin time** (Quick 1942). Rabbit brain liquid thromboplastin (Hyland) was used. 0.1 ml of plasma and 0.1 of thromboplastin were mixed and incubated at  $37^{\circ}\text{C}$  for 3 minutes. 0.1 ml of saline or venom saline was added, immediately followed by 0.1 ml of calcium chloride.

**Partial thromboplastin time with kaolin** (P.T.T. & K.) (Biggs and MacFarlane 1966). 'Thrombofax' (Ortho Diagnostics) was used as platelet substitute. The test was performed in two ways: first by incubating 0.1 ml of the venom saline solution with 0.1 ml of plasma, 0.1 ml of kaolin and 0.1 ml of 'Thrombofax' for 3 minutes, and then adding 0.1 ml calcium chloride; second, by adding the venom to the incubating mixture immediately prior to the addition of calcium chloride.

**Thromboplastin generation test** (Biggs and Douglas 1953). The test was performed in the usual way with the addition of 0.3 ml of saline or venom saline solution to the incubation mixture. The decay of formed thromboplastin was assessed after adding 0.3 ml of venom (1 mg/ml) to the incubation mixture after the formation of thromboplastin and continuing the test for a further 8 minutes. This gave a

final venom concentration of 200 ug/ml of incubation mixture. The test was repeated using 0.3 ml 0.85 per cent NaCl as a control.

**Fibrinolytic assay.** Fibrin plates were prepared by the method of Nilsson and Olow (1962) as modified by McNicol and Douglas (1964). 100 ul of venom was put on each plate and these were incubated for 18 hours at 37° C.

**Fibrinogenolytic assay.** Plasminogen-free fibrinogen (Behringwerk) in 0.85 per cent (w/v) NaCl and venom saline were mixed in equal quantities to give a final concentration of 1 mg/ml of fibrinogen and 0.25 mg/ml of venom. The mixture was incubated at 37° C and periodically 0.1 ml aliquots were clotted with a thrombin solution (50 units per ml) over a 30 minute period.

**Caseinolytic assay.** This assay was carried out using the method of Remmert and Cohen (1949) as modified by Alkjaersig *et alii* (1959). 0.5 ml of venom saline solution was used in a concentration of 5 mg/ml and 1.5 ml of phosphate buffer was substituted for the acid, alkali and streptokinase solutions. The test was repeated using 0.5 ml plasminogen (Behringwerk) in 0.85 per cent (w/v) NaCl, 1 ml of phosphate buffer substituted for the acid and alkali and 0.5 ml of streptokinase. Finally the test was performed using 0.5 ml of plasminogen, 1 ml of phosphate buffer and 0.5 ml of venom (replacing the streptokinase)

### *In Vivo studies*

Mongrel dogs were sedated heavily with acetylpromazine and paraldehyde administered intramuscularly. Further sedation was given where necessary during the study period. A control dog received sedation only over a 5 hour period. Three dogs were given injections of venom subcutaneously, receiving 0.7 mg, 1 mg and 2 mg/kg respectively. The latter two were lethal doses. Blood samples were obtained by jugular vein puncture using a plastic syringe with a 19 gauge needle. These samples were dispensed into glass tubes for whole blood clotting time and three plastic tubes, one containing EDTA for full blood count, a second containing sodium citrate 3.8 per cent (9 parts blood to 1 part citrate) and a separate citrate tube containing trasylol, 100 kallikrein inhibitor units/ml blood, for fibrinogen estimation. Following centrifugation at 3000 rpm for 10 minutes the citrated plasma samples were stored at -20° C before performing the following coagulation studies: partial thromboplastin

time with kaolin, prothrombin time, plasma plasminogen (Alkjaersig *et alii* 1959), fibrinogen (Ratnoff and Menzie 1951), and fibrin plate assay.

## RESULTS

### *In Vitro Studies*

#### *Whole blood clotting time*

The mean results of the clotting times obtained on two separate occasions are shown in Fig 1. At a venom concentration of 10 ug/ml of blood, no clot was formed at 60 minutes. However, with a concentration of 100 ug/ml of blood there was firm clot formation in three tubes at a mean time of 53 minutes and the remaining five tubes contained considerable clot at this time. At a concentration of 1 mg/ml the blood was again incoagulable.

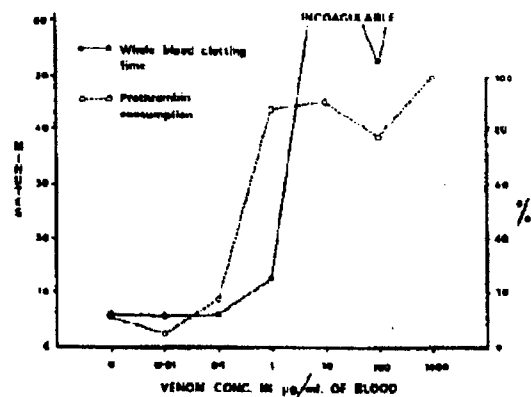


Figure 1.—The changes in whole blood clotting time and prothrombin consumption brought about by increasing concentrations of venom

#### *Prothrombin consumption*

A biphasic effect was again observed. The mean results of two experiments on two separate occasions are shown in Fig 1.

#### *Plasma recalcification time*

A similar effect was observed with the recalcification time (see Fig 2). Following a prolongation in clotting time with increasing venom concentrations, the plasma became incoagulable with a venom concentration of 3.3 ug/ml of clotting mixture. With increasing concentrations of venom the plasma clotted once again and at the highest concentrations became incoagulable.

#### *Thrombin clotting time*

There was no significant prolongation of the thrombin clotting time with increasing concentrations of the venom.

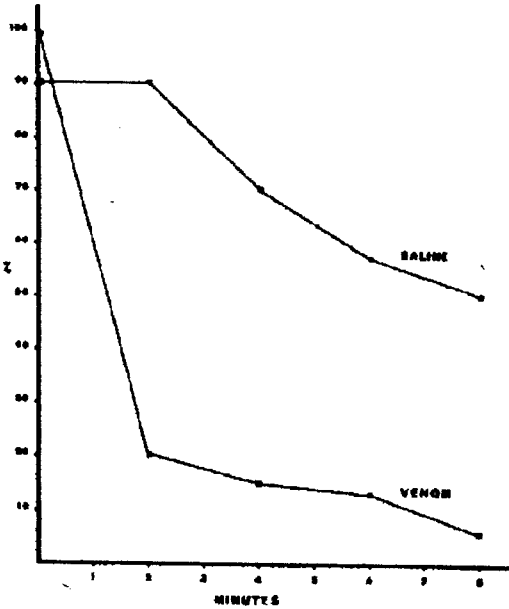


Figure 6.—The decay of formed thromboplastin with venom and saline added to the incubation mixture

### Proteolytic studies

Venom in concentrations up to 10 mg/ml produced no lysis of fibrin. However, incubation of venom with fibrinogen resulted in a prolongation of the clotting time of the fibrinogen which became incoagulable after 20 minutes (Fig 7).

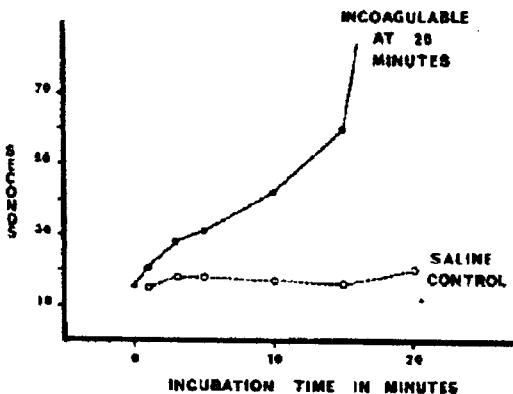


Figure 7.—The effect of the clotting time of a pure fibrinogen solution brought about by incubation with venom at 37°C

In the caseinolytic system, venom in a concentration of 1 mg/ml of incubation mixture produced a similar proteolytic activity to 2.5 casein units of plasminogen. On the other hand, the venom did not appear to have any

activator activity when combined with plasminogen in the test system.

### In Vivo studies

Abnormalities of the blood coagulation system were produced within the dosages of venom employed in these studies. The whole blood clotting time and partial thromboplastin time became prolonged and these changes appeared to be dose-dependant (Fig 8). There were no significant changes in plasma fibrinogen or plasminogen levels and no evidence of fibrinolysis on the fibrin plate assay.

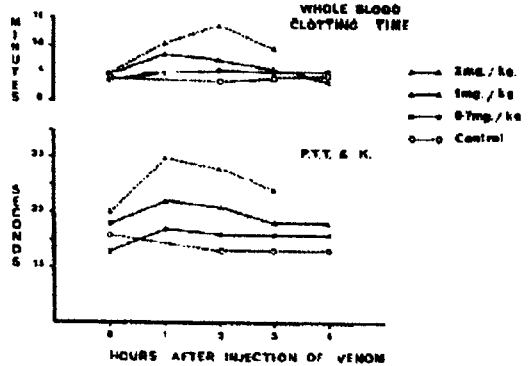


Figure 8.—The changes in the whole blood clotting time and the partial thromboplastin time with kaolin (P.T.T. & K.) of dog's blood following the subcutaneous injection of different doses of venom to three dogs and the subcutaneous injection of 0.85 per cent (w/v) NaCl to one control dog

### DISCUSSION

*In vitro* this venom has a potent anticoagulant action. The most significant interference with coagulation is observed in the thromboplastin system where generation of thromboplastin is inhibited by low concentrations of venom. It is possible that this action may be due, at least in part, to degradation of thromboplastin as it is generated. The conversion of prothrombin to thrombin by rabbit brain thromboplastin is also inhibited by the venom, but in the concentrations tested it does not interfere with thrombin-fibrinogen interaction. These anticoagulant properties are similar to those ascribed to the venoms of the mambas, *Dendroaspis angusticeps*, *D. polylepis* and *D. jamesoni* (Mackay *et alii* 1968), the Palestine viper, *Vipera xanthina palestinae* (de Vries and Gitter 1957) and the puff adder, *Bitis arietans* (Forbes *et alii* 1956). The venom has fibrinogenolytic and caseinolytic properties but does not lyse fibrin or bring about plasminogen activation. The three mamba venoms have similar actions (Mackay *et alii* 1968).

The unusual biphasic effect observed in the determination of whole blood clotting time, plasma recalcification time and prothrombin consumption suggests the presence of a coagulant component. This evidence is strengthened by the observation that incubation of the venom with the platelet substitute, kaolin and plasma caused less prolongation of the partial thromboplastin time than when the venom was added just before recalcification. The precise mechanism of this coagulant action cannot be assessed without fractionation of the venom.

The prolongation of the whole blood clotting time and partial thromboplastin time produced by the venom in dogs were findings consistent with the *in vitro* results obtained.

These observations are at variance with some aspects of the clinical picture ascribed to Papuan black snake bite by Campbell (1967). However, that study of thirteen patients only included one patient who was bitten by a positively identified snake. This patient, who was only mildly envenomated, did, however, develop a slightly prolonged whole blood coagulation time and a positive rabbit anti-fibrin test (Ferreira and Murat 1963) in his serum.

### SUMMARY

Venom of the Papuan black snake (*Pseudochis papuanus*) is a powerful anticoagulant *in vitro*, primarily due to its effect on the thromboplastin system and prothrombin conversion to thrombin. It is fibrinogenolytic and caseinolytic but does not cause fibrinolysis or activation of plasminogen. There is evidence to suggest that the venom contains a coagulant component, although the overall effect *in vivo* in dogs is anticoagulant.

These experimental findings are at variance with previous clinical observations which have suggested that the venom has a coagulant action *in vivo*, causing a bleeding diathesis due to defibrination.

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" . . . many nations simply cannot afford the luxury of having one physician for every 1,000 persons. This really should be self-evident, but it is not. Unfortunately, the proliferation of medical schools has become a status symbol for developing nations. A past Health Minister of Nigeria stated in an address in Edinburgh that he planned to train 1,000 medical graduates per year in Nigeria. The country could not conceivably support such numbers of doctors. They would migrate to countries where the doctor shortage is expressed in economic need rather than biological need . . . unless countries can afford to pay something close to the world market price for physicians, or adopt restrictive migration policies, they will not keep the physicians that they have trained. Developing nations cannot afford to invest their scarce resources in training doctors for rich nations. An obvious solution to this dilemma lies in increased and more effective use of paramedical personnel."

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