Clinical Features of Twig Snake (*Thelotornis capensis*) Envenomation

P. M. ATKINSON, B. A. BRADLOW, J. A. M. WHITE, H. B. W. GREIG, M. C. GAILLARD

SUMMARY

A case of disseminated intravascular coagulation in a 13-year-old-boy caused by a bite from a twig snake (*Thelotornis kirtlandii capensis*) is described. The coagulant enzyme was found to be c single-chain molecule with a molecular weight of 56 000. It was able to activate prothrombin and factor X as well as weakly accelerating fibrinolysis. Its action on synthetic chromogenic substrates is described. Boomslang antivenom was unable to block twig snake venom, and the implications of this as regards treatment are discussed.

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Thelotornis kirtlandii capensis is known in South Africa under three names, the vine, twig and bird snake. It is a back-fanged member of the *Boiginae* and is known to be poisonous. Found only in Africa, it inhabits the continent south of the tropical region and in southern Africa it is found mainly in Natal, the Transvaal lowveld and the northern half of SWA.[1] The twig snake is closely related to the boomslang *(Dispholidus typus)*, but can be distinguished from it by a more slender build and a characteristic keyhole-shaped rather than round pupil. Bites in man are extremely rare because the snake is not aggressive and the fangs are situated far back in the mouth, making an effective bite difficult. In 1975 a well-known herpetologist died after being bitten by a *Thelotornis*, the clinical course being that of disseminated intravascular coagulation.[2] In another case described by Beiran and Currie[3] the patient was given polyvalent antiserum to no avail, and recovered after a transfusion of 6 units of blood. Although no fatal cases have been documented in South Africa, at least 1 patient is known to have died after being bitten on the tongue (F. Muller (1968) — personal observation).

Biochemical studies on the venom and its mechanism of action are few. Kornalík and Táborská[2] demonstrated the ability of the venom to clot citrated plasma and activate prothrombin. They also demonstrated a relative *in vitro* heparin resistance and showed in experiments on rats that the venom causes a consumptive coagulopathy.

The present study records a further case of twig snake envenomation as well as observations on its coagulant action and biochemical properties and the effect on it of boomslang antivenom.

Department of Haematology, School of Pathology, University of the Witwatersrand and South African Institute for Medical Research, Johannesburg

P. M. ATKINSON, M.B. B.CH.
B. A. BRADLOW, M.D., F.R.C. PATH., B.SC., DIP. PATH.
M. C. GAILLARD, B.SC. HONS

Departments of Surgery and Haematology, University of Natal, Durban

J. A. M. WHITE, M.B. CH.B., F.R.C.S.
H. B. W. GREIG, M.B. CH.B., F.C.P. (S.A.)

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CASE REPORT

A healthy 13-year-old boy was admitted to Addington Hospital soon after being bitten on the back of the left index finger while attempting to catch a snake in a bush. Physical examination showed three tiny puncture wounds with slight surrounding erythema, but there was no evidence of swelling, bleeding or lymphadenopathy and the bite was not painful. The child was hyperventilating and complained of crampy abdominal pain and paraesthesiae in the hands. The cardiovascular, respiratory and central nervous systems were normal. After 5 hours the haematological values were normal except for a mild leucocytosis of 12,6 × 10^9/l; however, the bleeding time was prolonged at 13 minutes and the blood was completely incoagulable. Urine examination was negative. A slow infusion of 400 ml of freeze-dried plasma was given. After 20 hours the blood was still incoagulable but no signs of bleeding could be detected. After 29 hours the patient had another episode of hyperventilation with crampy abdominal pain and paraesthesiae. Coagulation studies were carried out 40, 80 and 112 hours after admission (Table I). The child was kept under observation until 6 days after the bite, but no further plasma infusions were given. Serial urine and stool examinations showed no sign of bleeding, serum electrolyte and urea values were normal, and there was no sign of jaundice; he was discharged after platelet levels had returned to near-normal values on the 6th day.

The snake had been captured and was positively identified as a *T. capensis* by Mr Fritz Muller, a herpetologist.

MATERIAL AND METHODS

Crude venom was obtained by dissecting out the venom glands of three freshly decapitated snakes kindly provided by the Transvaal Snake Park. The glands, which are situated superficially just behind and below the eye (Fig. 1), were crushed in a tissue-grinder and the venom was extracted into saline (8,5 g/l), freeze-dried, and stored in this form at −20°C.

TABLE I. SUMMARY OF THE PATIENT'S COAGULOGRAMS

	Hours after bite			Normal values
	40	84	112	
Prothrombin time (s)	12,7	12,8	25,4	11,1 - 12,1
Kaolin partial thromboplastin time (s)	31,4	34,0	54,5	26,4 - 34,2
Thrombin time (s)	12,3	10,4	60	10,5 - 15,6
Platelet count (X $10^3/\mu l$)	198	144	21	150 - 400
Fibrinogen titre	1/128	1/64	1/4	1/128
Protamine sulphate test	—	+	+	—
Ethanol gelation test	—	+	+	—
Fibrinogen degradation products (μg/ml)		10	80	10
Factor V (%)	95	60	29	50 - 150
Factor VIII (%)	118	100	45	50 - 150
Factor X (%)	78	66	56	50 - 150

Fig. 1. A dissected venom gland of an adult twig snake. The characteristic keyhole-shaped pupil can be seen.

Partial purification was achieved by anion exchange chromatography on a DEAE cellulose (Merck) column using a tris HCl saline gradient (Fig. 2) by gel filtration on a Sephadex G 100 superfine column (Pharmacia). The column was calibrated with cytochrome *c*, thrombin, albumin and prothrombin.

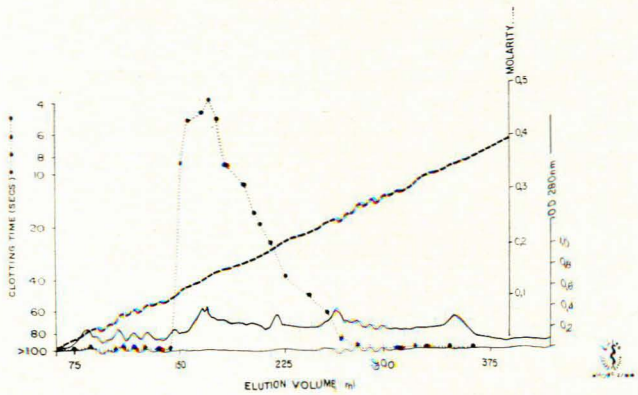

Fig. 2. Anion exchange chromatography of crude twig snake venom on DEAE cellulose using a tris HCl saline gradient.

Human prothrombin was purified from 2 litres of fresh human plasma according to the method of Esnouf and Jesty,[4] fibrinogen was purified according to the method of Atencio *et al.*,[5] and polyacrylamide gel electrophoresis (PAGE) was carried out according to the method of Fairbanks *et al.*[6] using sodium dodecyl sulphate (SDS) 2 g/l and 8M urea. Disulphide bonds were reduced with mercaptoethanol. Dalton Mark VI molecular weight markers (Sigma) were used to determine molecular weights from the PAGE runs.

Chromogenic substrates were supplied by Ortho Diagnostics Inc., Raritan, NJ, USA. Alumina plasma was prepared by absorption with aluminium hydroxide gel (British Drug House). Fibrin agar plates were prepared by the method of Holmström.[7] Plasma deficient in clotting factors was obtained either from patients with congenital deficiencies or from Diagnostic Reagents Ltd. Di-isopropyl fluorophosphate (DFP) was obtained from Merck. All the other chemicals used were of analytical grade and obtained from Merck, British Drug House or Sigma. The boomslang antivenom was a generous gift from Dr Price of the South African Institute for Medical Research.

RESULTS

The average weight of six venom glands was found to be 38,7 mg, roughly half the weight of an average boomslang venom gland (average weight of forty glands 76,6 mg). An average of 3,3 mg of crude twig snake venom protein could be extracted from each gland.

The crude venom was able to clot citrated plasma but not plasma taken into ethylenediamine tetra-acetic acid (EDTA). The venom was able to clot plasma deficient in factors VII, VIII, IX and X but could not clot pure fibrinogen, alumina plasma or plasma congenitally deficient in prothrombin. It was extremely potent (Fig. 3), and boomslang antivenom was not able to block it (Fig. 4). Purified human prothrombin was cleaved in three positions by the enzyme (Fig. 5). The prothrombin fragments released corresponded in molecular weight to prothrombin fragment 1, prethrombin 1, and prethrombin 2. The splitting of the molecule therefore appeared to be the same as that caused by boomslang venom.[8-10] The venom was able to clot the plasma of patients receiving cou-

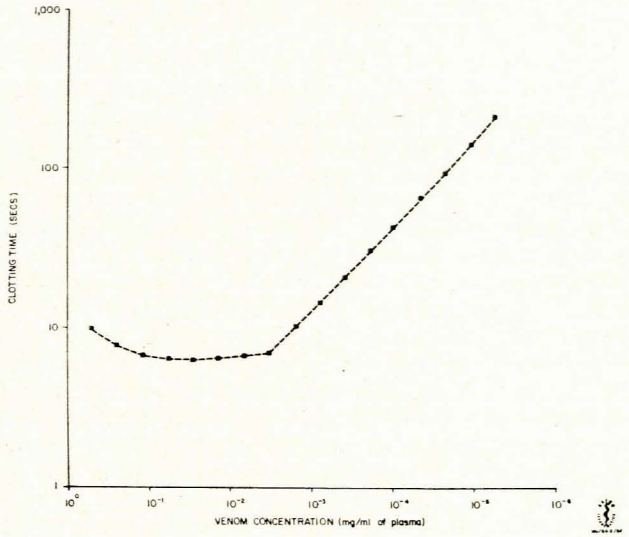

Fig. 3. The clotting of citrated human plasma at 37°C by an equal volume of crude twig snake venom in decreasing concentrations.

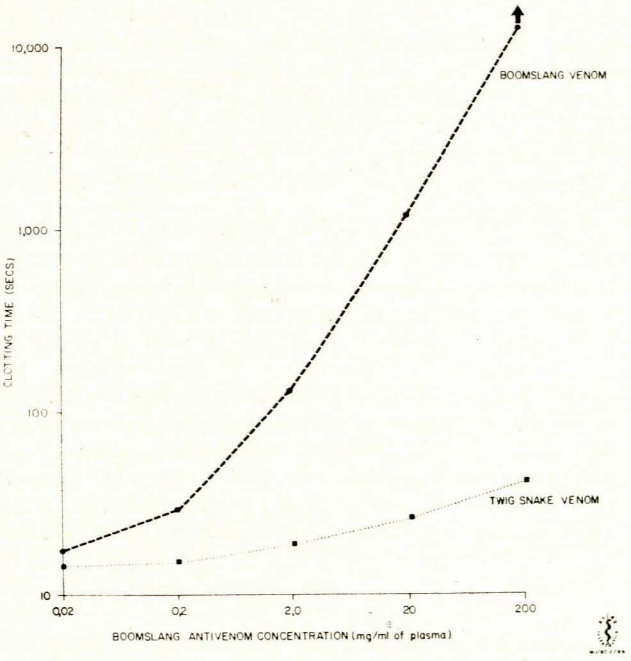

Fig. 4. The inability of boomslang antivenom to block the clotting action of twig snake venom — 1,0 ng of crude twig snake venom and 1,0 ng of crude boomslang venom were added to 0,1 ml of plasma and 0,1 ml of boomslang antivenom. The final antivenom concentration, expressed in mg/ml plasma, is shown on the graph.

marin anticoagulants in the same time as that of normal persons, as was the case with boomslang venom.[11]

The venom produced a small zone of lysis on an unheated but not on a heated fibrin agar plate. It was, however, unable to lyse a clot produced from the clotting of purified fibrinogen by thrombin.

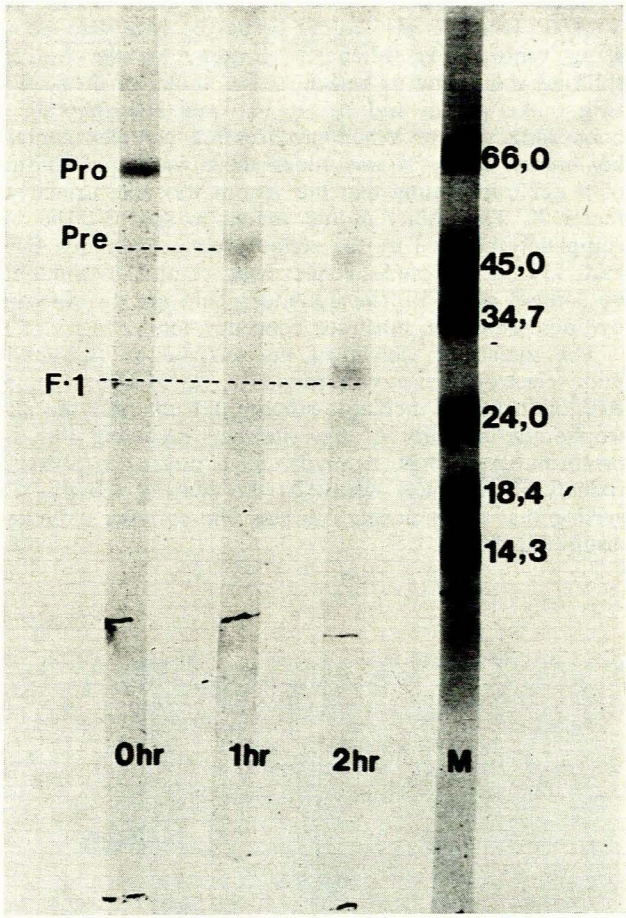

Fig. 5. The initial splitting of human prothrombin by twig snake venom. Prothrombin 1 mg/ml was incubated at 37°C with 100 ng of twig snake venom. The reaction was stopped at time intervals by heating at 60°C for 5 minutes (Pro = prothrombin; Pre = prethrombin 1; F.1 = prothrombin fragment 1).

The venom was found to be stable in the freeze-dried form at −20°C but was unstable at room temperature in buffer. Under the latter conditions no clotting activity remained after 2 weeks. Heating the enzyme for 5 minutes at 60°C inactivated it. The enzyme was able slowly to hydrolyse 6 of the 7 chromogenic substrates tested (Table II). There was minimal splitting of the factor X_a substrate S 2222.

TABLE II. AMIDOLYTIC ACTIVITY OF TWIG SNAKE VENOM USING DIFFERENT CHROMOGENIC SUBSTRATES

Substrate (1 mg/ml)	Peak optimal density /min (× 10^{-6})
S 2238	**127**
S 2302	**91**
S 2160	**85**
S 2444	**60**
S 2266	**51**
S 2251	**47**
S 2222	**24**

Crude twig snake venom cleaved S 2238 faster than S 2160. This was also found to be the case with boomslang venom. The Michaelis constant of the S 2238 splitting was found to be $8{,}8 \times 10^{-4}$ mol/l in the case of twig snake venom and $7{,}5 \times 10^{-4}$ mol/l in the case of boomslang venom. When prothrombin complex containing human factor X was added there was rapid splitting of S 2222, indicating that the venom was able to activate factor X. The ability of the venom to split S 2160 was completely blocked by the serine protease inhibitor DFP, and 150 μg of crude venom was completely inhibited by 5 nmol of DFP. The inhibitor could not be overcome by increasing the substrate concentration.

The molecular weight of the enzyme on polyacrylamide electrophoresis was found to be 56 000 (Fig. 6). Reduction with 6-mercapto-ethanol did not alter the electrophoretic mobility of the enzyme, indicating that the prothrombinase was a single-chain molecule; however, reduction completely destroyed the clotting activity, suggesting that the molecule contains one or more interchain disulphide bonds.

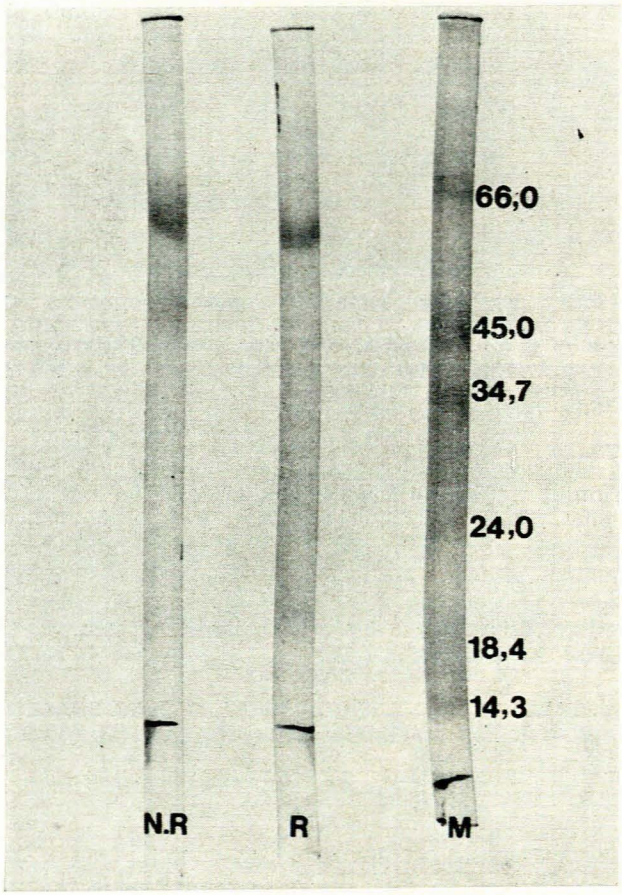

Fig. 6. SDS-polyacrylamide gel electrophoresis of the semipurified twig snake venom's active coagulant enzyme (N = non-reduced; R = reduced; M = molecular weight marker).

DISCUSSION

The twig or bird snake has many features in common with the boomslang. Both are back-fanged snakes capable of causing death through disseminated intravascular coagulation (DIC). The venom appeared to have the same mechanism of action as that of the boomslang in that it was able to activate prothrombin as well as factor X. Kornalík and Táborská[2] failed to demonstrate factor X activation using a mixture of normal and factor X-deficient plasma in various proportions. A possible explanation for the discrepancy between their results and ours is that any activation of factor X would probably be overwhelmed by thrombin produced from prothrombin in the system. We have demonstrated on SDS-PAGE that *D. typus* venom can activate highly purified factor X. No direct fibrinolytic activity was associated with the venoms but they appeared to accelerate fibrinolysis by activating plasminogen. This effect was mild and probably of minor importance. The ability of both venoms to split the chromogenic substrates S 2160 and S 2238 could be completely blocked by DFP, suggesting that they may be serine proteinases. Twig snake venom splits S 2238 more rapidly than S 2160, as is the case with boomslang venom, and in both cases the active enzyme was found to be a single-chain protein. The molecular weight was also found to be about the same.[11] Twig snake venom appears to have at least one interchain disulphide bond that is necessary for function because reduction completely blocked its clotting ability. Despite the many similarities, twig snake venom is not blocked by boomslang antivenom.

From the practical point of view the fact that boomslang antivenom does not inactivate twig snake venom means that this avenue of therapy will be of no avail. Furthermore polyvalent antivenom will probably be of no value either, as was the case with Beiran and Currie's[3] patient. This leaves transfusion of fresh plasma and platelets as the only practical therapy available at present. The use of heparin in consumptive coagulopathies caused by coagulant venoms such as those of the boomslang and *Echis carinatus* is still controversial. Evidence from animal[12] and clinical[13] studies suggests that heparin is not beneficial, but further studies are in progress. On the basis of current knowledge it seems unlikely that heparin will benefit patients with twig snake envenomation.

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Mucocutaneous Lymph Node Syndrome in a Young Adult

A Case Report

NORMA SAXE, A. HORAK, J. GOLDBLATT

SUMMARY

The mucocutaneous lymph node syndrome, first noted in 1961 by Kawasaki in Japan, is an acute, febrile mucocutaneous condition accompanied by cervical lymphadenopathy, which affects infants and young children. More recently it has been recognized in other countries, but not before 1979 in South Africa.

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The mucocutaneous lymph node syndrome (MLNS) or Kawasaki disease is a syndrome of unknown aetiology. The syndrome was first noted in Japan in 1961 and reported in the English-language literature in 1974.[1] It was later diagnosed in Hawaii,[2] Canada,[3,4] South Korea,[5] Australia,[6] the USA[2,7] and the UK,[8] but had not been encountered in South Africa before 1979.* The disease is commoner in males and usually affects children under 5 years.

We initially considered both the age of our patient (18 years) and the fact that it was one of the first cases in South Africa to be unique aspects worth reporting. However, 3 cases have recently been reported in young adults[9-11] and 1 suspected case has been reported in a 27-year-old person.[12]

Departments of Dermatology and Medicine, University of Cape Town and Groote Schuur Hospital, Cape Town
NORMA SAXE, M.B. CH.B., F.F. DERM. (S.A.)
A. HORAK, M.B. CH.B.
J. GOLDBLATT, M.B. CH.B.

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Reprint requests to: Dr N. Saxe, Dept of Dermatology, Groote Schuur Hospital, Observatory, 7925 RSA.
* See letter by Kibel *et al.* on p. 6 of the *SAMJ* of 5 July 1980.

CASE REPORT

Ten days before admission to Groote Schuur Hospital, Cape Town, an 18-year-old White man presented with malaise, weakness, sore throat, fever, mild headache and difficulty in opening his mouth. He had vomited for 2 days and had a non-productive cough. His doctor found tender, bilateral cervical lymphadenopathy and treated him for tonsillitis with amoxycillin (Amoxil) 250 mg three times daily. This dosage was increased to 500 mg three times daily after 2 days. At that stage his white blood cell count (WBC) was 16 000/μl, the erythrocyte sedimentation rate (ESR) 80 mm/h and the Paul-Bunnell test negative. He was examined by an ENT surgeon who altered his medication to cephridine (Cefril), without clinical improvement. He was then referred to hospital.

On clinical examination he had a temperature of 38,7°C, flushed facies, mild palmar erythema and oedema, marked conjunctival congestion and bilateral, tender cervical lymphadenopathy. An erythematous macular rash was present on the trunk, and the oropharyngeal mucosa was diffusely erythematous, with small, superficial palatal ulcers. He had a strikingly red 'strawberry' tongue (Fig. 1) as well as redness of the external urethral meatus and anterior urethra.

His temperature continued to vary between 38°C and 40°C. The pulse rate was 110/min, the jugular venous pressure not elevated, and the blood pressure 140/90 mmHg. A 3rd heart sound could be heard. Examination