12. Herrmann, R. B., *Bull. Seismol. Soc. Am.*, 1980, **70**, 447–468.
13. Nair, G. J., Some Seismic Results of Rajasthan Explosion of 18 May 1974, AG224, Procurement Executive, MOD, UK.
14. Bath, M., *Handbook on Earthquake Magnitude Determinations*, Seismological Institute, Uppsala, Sweden, 1969.
15. Dahlman, O. and Israelsson, H., *Monitoring Underground Nuclear Explosions*, Elsevier, Amsterdam, 1977.
16. Chidambaram, R. and Ramanna, R., Proc. Tech. Committee on Peaceful Nuclear Explosions IV, Vienna, IAEA, 1975, pp. 421–435.
17. Chidambaram, R., Sikka, S. K. and Gupta, S. C., *Pramana*, 1985, **24**, 245.
18. Marshall, P. D., Springer, D. L. and Rodean, H. C., *Geophys. J. R. Astron. Soc.*, 1979, **57**, 609–638.
19. Bache, T. C., *Bull. Seismol. Soc. Am.*, 1982, **72**, S131.
20. Evernden, J. F. and Marsh, G. E., *Phys. Today*, August 1987, 37–44.
21. Barker, B. *et al.*, *Science*, 1998, **281**, 1967–1968.
22. Wallace, T. C., *Seismol. Res. Lett.*, 1998, **69**, 386–393.
23. Evernden, J. F., *Phys. Soc.*, 1998, **27**, 10–11.

ACKNOWLEDGEMENTS. We thank Dr Vijai Kumar for some preliminary analysis of regional data which were useful for the present study, and our colleagues at Gauribidanur seismic array station and IMD, New Delhi for providing the transcript of digital data. We also thank the unknown referee for his valuable comments.

Received 7 September 1999; revised accepted 21 october 1999

In vitro haemorrhage-like activity of Russell's viper (*Vipera russelli russelli*) venom from Eastern India with mice organs

Kausiki Datta and Debasish Bhattacharyya*

Division of Protein Engineering, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Jadavpur, Calcutta 700 032, India

***Vipera russelli russelli* (Indian subspecies of Russell's viper) envenomation causes severe haemorrhage in different organs of the victim's body. Application of crude venom extracted from Russell's viper of Eastern Indian origin on sliced tissues of BALB/c mice shows that among the target organs like lungs, kidneys, heart, brain, eye balls, etc. liver is most potent in terms of release of haemoglobin and hence blood. Spectral and size-exclusion HPLC analysis indicate that it is only haemoglobin that gives absorption of the extract at 540 nm. Release of haemoglobin follows second-order kinetics depending on both concentration of venom and amount of tissue incubated. The reaction is completely inhibited by venom antibody indicating absence of artifacts in the process. Approximately 20% of the release of blood may be accounted for fibrinolytic and haemolytic activity of the venom. Treatment of different inhibitors with venom shows that in cases where *in vitro* release of blood was partly or fully inhibited, good correlations were observed with *in vivo* haemorrhage and also with lethality. Thus the protocol may be developed for an emperical assay system to assess antihaemorrhagic property of a substance.**

SNAKE envenomation is a WHO identified occupational hazard for paddy farmers in South-east Asian countries. Incidents of snake bite leading to death are common in many tropical countries during or after the rainy season because of increased human settlements at the natural habitats of snakes. *Vipera russelli russelli* (Indian subspecies of Russell's viper) is one of the four major classes of snakes that causes death in the Indian subcontinent. Its venom consists of many active compounds that cause coagulopathy, necrosis, renal failure, neurotoxicity, myotoxicity, cardiotoxicity, convulsions, hypotensive, anticoagulation, etc. Among these, haemorrhage from the site of bite, unhealed wounds, liver, lung, kidney, intestine, eye ball, brain, etc. of the victim is severe and is a major manifestation of envenomation though renal failure is considered as the primary cause of death[1].

The observed haemorrhage may be the combination of three major factors like rupture of capillary membranes carrying blood, fibrinolytic activity to melt clots and inhibition of one or more blood clotting factors. Whatever may be the contribution of each factor, it is relatively easy to measure haemorrhage among other pathophysiological factors of venom because of ease and quantitation of released haemoglobin. It may be noted that the only treatment available in the market for snake envenomation is application of antivenom and its application is continued as long as the blood clotting time of the victim falls within the normal range.

Therefore, inhibition of haemorrhagic potential of the venom toxins by certain compounds may be considered as a preliminary index of its antivenom character. However, there is an intriguing variation in the clinical manifestation of envenoming by Russell's viper from neighbouring countries like Sri Lanka, Burma, Thailand, China and Taiwan[1,2]. Though haemorrhage is common, some pathological effects are either variable or questionable or absent altogether. Even venom composition of Russell's viper from different regions of the Indian subcontinent was found to be distinctly different as revealed by sodium dodecylsulphate-polyacrylamide gel electrophoresis[3]. Moreover, haemorrhagic toxins purified from Russell's viper from different geographical locations have shown organ specificity in test animals – e.g. the most basic phospholypase A_2 of Southern Indian

*For correspondence. (e-mail: iichbio@giasel-01.vsnl.net in)

venom has shown lung haemorrhage but no dermal haemorrhage[4], while two other phospholypase A_2 have shown different potency of lung and liver haemorrhage[5,6]; a protease from the same source has shown haemorrhage in the eyes, lungs and liver only[7]; another haemorrhagin isolated from eastern Indian has been shown to induce severe lung and liver haemorrhage but no dermal haemorrhage[8]. So testing with a purified toxin, say for screening of antivenoms, may lead to erroneous results. Here we report the effect of crude Russell's viper venom (RVV) on different test animal tissues in terms of quantitative release of haemoglobin. Subsequently, a good correlation between *in vitro* release of haemoglobin from mice liver and *in vitro* toxicity was observed.

Desiccated shinning yellow crystals of RVV were obtained from a licenced venom supplier (from Calcutta). Approximately 70% of the dry weight of the venom was found to be protein after assay with Bio-Rad protein assay reagent. A 10 mg/ml solution of venom in PBS was kept at 4°C and after centrifugation to remove fibrous materials, was used as the stock. Bivalent antiserum (against *Vipera russelli russelli* and *Saw scaled viper*) was a gift from Serum Institute of India, Pune. All fine chemicals e.g. phenylmethyl sulphonyl fluoride (PMSF), 2,4,6-trinitro benzenesulfonic acid (TNBS), EDTA, iodoacetic acid, leupeptin, aprotinin, 1,10-phenanthroline, haemoglobin, thrombin, fibrinogen, etc. were from Sigma Chemicals. Urokinase was a product of M/s Korea Green Cross Corporation, Seoul, Korea. All other reagents were of analytical grade.

All test organs were from BALB/c mice maintained at an in-house facility. Mice were sacrificed by a chloroform chamber and organs were removed immediately. They were washed, cut into small pieces, softly pressed using tissue paper and weighed – they are usually between 0.2 and 0.5 g. All tissue pieces were initially incubated separately with 0.5 ml of PBS for 15 min at 37°C to remove adhering, occulted and releasing blood. Tissues were again washed with PBS before application of RVV. Preincubation of the organs with PBS for a longer time continued release of haemoglobin but the amount was low and has been accounted for as control. 5 to 50 μg of venom was then applied to the preincubated tissues in 2 ml of PBS at 37°C for 0–180 min. For estimation of release of haemoglobin at stipulated intervals, 1 ml of the supernatant was removed, centrifuged and absorbance at 540 nm was read. The supernatant after absorbance measurement was quantitatively returned to the original incubation mixture as quickly as possible. All experiments were completed by 3 h to minimize proteolysis.

To measure the molecular weight of the coloured material of the liver extract, the solution was dialysed against 20 mM K-phosphate buffer, pH 7.0 for a short time and its elution profile from a Waters Protein Pak 300 size-exclusion HPLC column (78 × 1.3 cm, fractionation range 20–400 kDa) was monitored at 540 nm at a flow rate of 0.5 ml/min. The column was precalibrated with the following marker proteins: BSA, 66 kDa; ovalbumin, 43 kDa; myoglobin, 17 kDa and cytochrome *c*, 12.5 kDa. A linear dependence of log (molecular weight) vs elution volume was observed. Dermal haemorrhage after application of the toxin on mice was measured semiquantitatively from post mortem of the animals after Kondo *et al.*[9]. Fibrinolytic activity was measured from lysis of a fibrinogen plate made after polymerizing 166 mg of fibrinogen with 2.5 U of thrombin in 1 ml of 20 mM K-phosphate buffer, pH 7.4 at 37°C for 3 h (ref. 10). RVV (1–2 mg/ml, accurate quantitation was done by gravimetric analysis) in 50 mM K-phosphate buffer, pH 7.5 was incubated with inhibitors of concentration 10–20 mM at 37°C for 30 min. Reactions were terminated by dilution with PBS and 15 μg of venom was injected into mice (20 ± 2 g) (n = 4–6) intra-venously and lethality was observed for 18 h. The carried over reagents in any case did not cause any toxicity in the animals which was tested separately.

The following organs of mice are known to be mostly affected by RVV in terms of haemorrhage at sublethal dose: liver, kidneys, lungs, eye balls, brain and intestine[2]. Preincubation of the chopped tissues of these organs with PBS for 15 min at 37°C followed by incubation with venom showed that optical absorbance of the incubate at 540 nm increases in a time-dependent manner which was mostly completed by 3 h. The wavelength was selected assuming haemoglobin as one of the components released from the organs. Results after equating with tissue weights show that the release of haemoglobin was maximum in liver (4.45 mg/ml/g tissue) followed by lung (3.93) and kidney (1.52). Haemoglobin release was not detectable from the small intestine and eye balls. Therefore, in all subsequent experiments the liver was taken as the target organ for measuring the *in vitro* sensitivity of the toxin.

The 540 nm absorbing material released from the liver was indeed haemoglobin as confirmed by the following experiments: (a) the 540 nm absorbing material was completely precipitated from the liver extract by 10% trichloroacetic acid (TCA), e.g. an o.d. of 0.435 of the supernatant was reduced to 0.003 after TCA precipitation where a control PBS had also the same absorption of 0.003 against water. This suggests that, the absorbing material was not an acid soluble organic molecule and possibly proteinous in nature; (b) the absorption spectra of the liver extract between 340 and 800 nm show three maxima at 414, 540 and 576 nm having relative intensities of 1:0·10:0·09. This may be compared with haemoglobin showing absorption maxima at 414, 541 and 576 nm having relative intensities of 1:0·11:0·116; the corresponding molar extinction coefficients being 125,000, 13,500 and 14,600 (ref. 11). While calculating

the absorption parameters of the liver extract, corrections were done for low intensity scattering of light observed in the experiments possibly due to high concentration of other proteins; and (c) only one symmetrical peak of the liver extract appeared in a precalibrated Protein-Pak 300 SE-HPLC column while monitoring the elution at 540 nm which corresponds to Mw of 68 kDa compared to native Mw of haemoglobin being 64 kDa. These three evidences are fairly good indicators of release of haemoglobin as the only detectable 540 nm absorbing material in the venom incubate. They also suggest that the sensitivity of the measurement could be raised by more than 5-fold by measuring absorbance at 414 nm instead of 540 nm.

Overnight storage of the supernatant under frozen condition leads to proteolytic degradation of haemoglobin as a number of 540 nm absorbing peaks appear in the SE-HPLC. Overnight storage at 25°C leads to disappearence of the 540 and 560 nm absorbing bands but not the 414 nm band (known as Soret Band). Lyophilized haemoglobin, as sold by Sigma, also fails to show the higher wavelength bands.

Release of haemoglobin from liver was a time-dependent phenomenon which was usually followed for 3 h (Figure 1). The reaction followed a second-order kinetics as evidenced by linear dependency of the inverse of $A_{540\,nm}$ of the product against inverse of time (Figure 1, upper inset). The reaction profiles with varying venom concentration converge at a single point corresponding to infinite time, indicating validity of the kinetics. Using the molar extinction coefficient of haemoglobin as the product, a rate constant of $0.04 \times 10^3\,M^{-1}s^{-1}$ was derived while incubating 0.35 g of liver with 25 μg of venom in a volume of 2 ml at 37°C. Log *k* values of the reactions were also found to be linearly dependent on the amount of venom applied between 0 and 50 μg/2 ml of PBS under the specified conditions (Figure 1, lower inset). Moreover, the rate of the reaction was found to be linearly dependent on the amount of liver incubate between 0.065 and 1.2 g in 2 ml PBS (result not shown). It is interesting to note that though RVV contains a number of haemorrhagins acting on the liver and more than a number of factors may help release of blood, the product formation shows a rather simple monophasic kinetics. Temperature dependency of the reaction between 15 and 50°C shows an optimum rate at 37°C. Higher temperature leads to coagulation of the products. pH dependency of the reaction between 4.0 and 10.5 shows an optimum at 7.5.

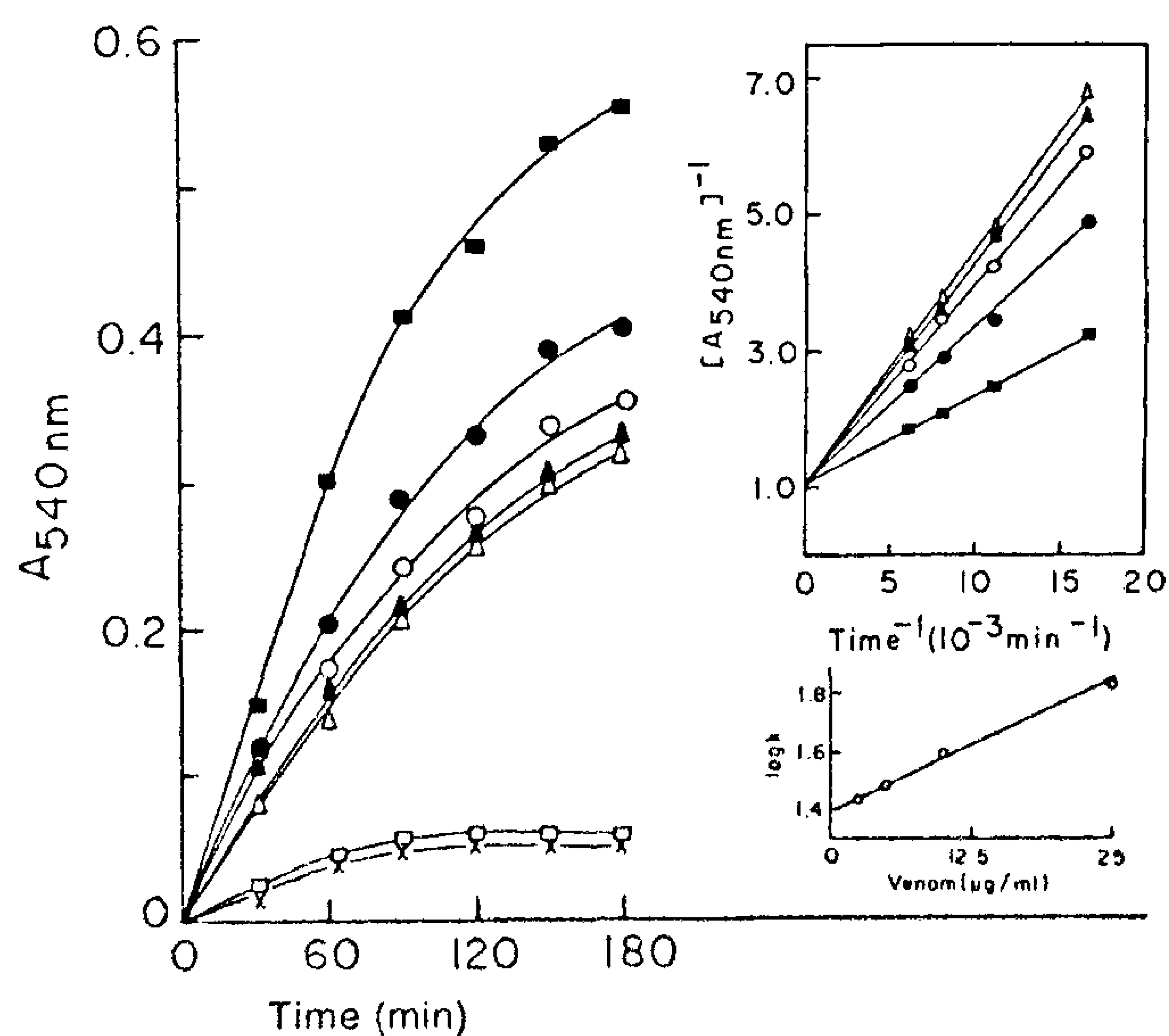

Figure 1. Time course of release of haemoglobin from mice liver tissue by varying amounts of Russell's viper venom; Δ, 5 μg; ▲, 10 μg; O, 15 μg; ●, 25 μg; ■ 50 μg of venom; X, PBS as control and □, 50 μg of venom preincubated with antivenom. (Upper inset), Double reciprocal plot of the reaction profiles. (Lower inset), Dependance of log*k* of the reactions on concentration of venom.

The release of haemoglobin from the liver may originate from the fibrinolytic and haemolytic activity present in RVV apart from haemorrhagins. Fibrinolytic activity of varying amounts of venom (0–50 μg) was compared with a fixed amount of urokinase by observing lysis on fibrin plates under conditions identical with incubation of venom with tissues. An equivalency of 25 μg of crude venom with 10 μg of commercial urokinase was drawn by observing a 5 mm diameter lysed zone. The said amount of urokinase could release about 10% of haemoglobin when compared to the venom equivalance.

It is difficult to distinguish between straying of RBCs from the vessels due to haemolysis and haemolysis followed by RBC release caused by haemorrhagins; yet some quantitations have been done which might be extrapolated with approximations. Measuring haemolysis under previously described conditions on 1% mice RBC shows that 100 μg of crude venom in PBS causes 8.3% haemolysis compared with a control using distilled water as diluent taken as 100%. PBS under similar conditions shows a background of 4.5% of haemoglobin release.

An attempt was also made to quantitate release of haemoglobin from the tissue in distilled water to assess maximum haemolysis in the absence of haemorrhagins. The experiment was unsucessful as the pH of the supernatant dropped from 7.4 to 5.8. Alternately three sets of experiments were done with equal weight of tissue slices with different compositions of the incubate: PBS as control, 20 mM K-phosphate buffer, pH 7.4 as a hypotonic solvent which stabilizes pH, and PBS containing 15 μg of venom. After background correction it was observed that approximately 12% of the haemoglobin released may be accounted for haemolysis.

To check whether higher release of haemoglobin from the liver when compared to other organs is due to higher

circulation of blood, RBC counts in the lungs, liver and kidneys of mice were measured using a haemocytometer. RBC count/mg for these organs was 10×10^3, 67.1×10^3 and 14.2×10^3, respectively. Thus it shows that there is no direct correlation between erythrocyte count/unit weight of a tissue and haemoglobin release by the venom.

Crude RVV was treated with a number of reagents like group specific amino acid modifiers, metal chelators, specific protease inhibitors and venom antibody. The modified venom was tested for its ability to release haemoglobin from the liver *in vitro* and also for haemorrhage *in vivo* including lethality. The results have been summarized in Table 1 which shows that the venom antibody completely inhibits release of haemoglobin, indicating that the venom-induced reaction had biological relevance and was not merely an artifact. Similar to inhibition by antibody, physical denaturation of the active venom components by heat also inhibited hemoglobin release. Amino acid modification reagents and metal chelators partly inhibited the reaction but protease inhibitors tested so far were found to be ineffective. The haemorrhagic potency of the modified venoms when tested *in vivo* for inducing dermal haemorrhage in mice, was found to be very similar to *in vitro* experiments. A (+) and (–) sign in Table 1 indicate 80–100% of the test animals either died or survived after modified vemon treatments. Thus the lethality of the modified venom tested so far ran in parallel with its haemorrhagic potency observed *in vitro* except the case of leupeptine where *in vitro* haemoglobin release was observed but dermal haemorrhage and lethality were prevented.

Table 1. Effects of modifiers of RVV on *in vitro* release of blood from mice liver and *in vivo* haemorrhagic activity

Inhibitors*	% of haemoglobin release (*in vitro*)	Remarks on haemorrhage[a]	Lethality[b]
Crude venom (RVV)	100	Severe dermal haemorrhage	+
PBS (control)	10	No dermal haemorrhage	–
Aprotinin	100	Severe dermal haemorrhage	+
Iodoacetamide	100	Severe dermal haemorrhage	+
Leupeptin	91	No dermal haemorrhage	–
PMSF	80	Severe dermal haemorrhage	+
1,10-phenanthroline	75	Severe dermal and ocular haemorrhage	+
TNBS	37	Dermal haemorrhage less compared to the crude venom	–
EDTA	34	Severe ocular bleeding, no dermal haemorrhage	–
Crude venom after thermal inactivation	15	No dermal haemorrhage	–
Antibody	10	No dermal haemorrhage	–

*15 µg of RVV was incubated with different inhibitors of concentration 10–20 mM at 37°C for 30 min before measuring the *in vitro* and *in vivo* activities (n = 4–6).
[a]Haemorrhage observed after 18 h of intra-dermal injection.
[b]Lethality observed within 18 h of intra-venous injection.

While working with haemorrhagic toxins of RVV of Eastern Indian origin we felt the requirement of an assay system which could quantitatively estimate the toxicity. Kondo's method of dermal haemorrhage[9] to measure toxicity failed in the case of VRR-22, one of the strong haemorrhagic components which showed severe lung and liver haemorrhage. The Lung Dye Diffusion method as developed for VRR-22 (ref. 8), failed in the case of VRR-73, another haemorrhagic toxin from the same source, which had specificity towards muscle and skin (unpublished observation). Therefore no single organ could represent total haemorrhagic activity of the venom. Yet as a working solution of this problem, mice liver was found to be best suited for *in vitro* studies. Spectrophotometric quantitation of the released blood at 540 nm could be done easily and far more accurately than post mortem quantitation of haemorrhage in animals. Moreover reaction parameters for different sets could be controlled precisely. This was reflected in the general characterization of the reaction as shown in Figure 1. It has also been demonstrated that the reaction depends primarily on the haemorrhagic toxins and not on fibrinolytic or haemolytic activities.

The mechanism by which snake venom haemorrhagic toxins act *in vivo* has not yet been settled. It has been demonstrated that at least for some of the haemorrhagic toxins the pharmacological and catalytic sites are different[6,12,13]. But rupture of blood capillaries is an obvious process where proteolysis is strongly suspected[14,15]. Fibrinolytic activity and inhibitors of blood clotting factors present in RVV help in the continuation of haemorrhage but they cannot initiate it. Proteases like trypsin and chymotrypsin which have no fibrinolytic activity can also induce *in vitro* release of haemoglobin though quantitation was difficult due to turbidity of the extract. For a multicomponent system, it is difficult to correlate the final reaction kinetics with the reaction profile of individual components; whether each haemorrhagin acts on the liver by the same mechanism is not clear now.

The biological significance of this study is that though *in vitro* blood releasing capacity of the venom in native or modified conditions from mice liver emperically represents its haemorrhagic potency, it corresponds quite well with *in vivo* toxicity in test animals at sublethal dose. This is also reflected in the lethality of the venom applied (Table 1). The method has been extended in testing some 'folk' medicines, locally known as 'antivenoms' and the correlation, both success and failure, was found to be good. Whether the protocol could be applied with confidence for RVV of other geographical origins remains to be seen.

1. Warrell, D. A., *Trans. R. Soc. Trop. Med. Hyg.*, 1989, **83**, 732–739.
2. Warrell, D. A., in *Handbook of Clinical Toxicology of Animal Venoms and Poisons* (eds Meier, J. and White, J.), Oxford Clendron Press, 1995, pp. 493–594.
3. Jayanti, G. P. and Gowda, T. V., *Toxicon*, 1988, **26**, 257–264.
4. Kasturi, S. and Gowra, T. V., *Toxicon*, 1989, **27**, 229–237.
5. Vishwanath, B. S., Kini, R. M. and Gowra, T. V., *Toxicon*, 1988, **26**, 713–720.
6. Jayanti, G. P., Kasturi, S. and Gowda, T. V., *Toxicon*, 1989, **27**, 875–885.
7. Jayanti, G. P. and Gowda, T. V., *Toxicon*, 1990, **28**, 65–74.
8. Chakrabarty, D., Bhattacharyya, D., Sarkar. H. S. and Lahiri, S. C., *Toxicon*, 1993, **31**, 1601–1614.
9. Kondo, H., Kondo, S., Ikezawa, H., Murata, R. and Ohasaka, A., *Jpn. J. Med. Sci. Biol.*, 1969, **13**, 43–51.
10. Astrup, T. and Mullertz, S., *Arch. Biochem. Biophys.*, 1952, **40**, 346–351.
11. Iorio, E. E. D., *Methods Enzymol.*, 1981, **76**, 57–72.
12. Kasturi, S. and Gowda, T. V., *Toxicon*, 1990, **28**, 91–99.
13. Vishwanath, B. S. and Gowda, T. V., *Toxicon*, 1987, **25**, 929–937.
14. Ohsaka, A., Just, M. and Habermann, E., *Biochim. Biophys. Acta*, 1973, **323**, 415–438.
15. Bjarnason, J. B. and Fox, J. W., *J. Toxicol – Toxin Rev.*, 1988–89, **7**, 121–209.

ACKNOWLEDGEMENTS. Research was supported by the Indian Council of Medical Research, New Delhi (grant No. 58/9/94-BMS-II). KD was supported by the ICMR project. Dr S. C. Lahiri, from ICMR provided academic help in this study.

Received 19 July 1999; revised accepted 22 October 1999

Structural aberrations in fluorosed human teeth: Biochemical and scanning electron microscopic studies

A. K. Susheela*^,‡^, Madhu Bhatnagar*, N. Gnanasundram† and T. R. Saraswathy†

*Fluorosis Research and Rural Development Foundation, C-13 Qutab Institutional Area, New Delhi 110 016, India
†Madras Dental College, Chennai 600 018, India

The present investigation was carried out to provide biochemical and ultrastructural evidences on the aberrations that appear in teeth in human Dental Fluorosis (DF), a condition caused by excess intake of fluoride. Human fluorosed teeth were obtained from the OPD of Madras Dental College, Chennai. Normal tooth samples were also collected from patients who opted for denture. The samples were investigated for fluoride and calcium contents, besides the tooth surfaces were examined under scanning electron microscope to assess the morphological aberrations. An increase in fluoride content and decrease in calcium content in fluorosed human teeth were observed when compared to the control. The scanning electron micrographs of the enamel surface of fluorosed human teeth show pitted, uneven and rough surfaces. Cracks and fissures were also observed on the enamel surface of fluorosed teeth. The present study provides evidence to suggest that pitting, perforation and structural alterations in DF are the result of impaired enamel mineralization.

DENTAL fluorosis (DF) is a hypomineralization of tooth enamel caused by continuous ingestion of excessive amount of fluoride during tooth development. This results in a variety of pathological changes in the structure of the teeth[1,2]. DF is characterized by opaque, lusterless white patches in the enamel which may become striated, pitted and discoloured followed by the breakdown of mineralized layer of the enamel shortly after eruption[3,4]. As revealed by studies in humans[5–7] and several other mammalian species[8–13], these alternations arise from fluoride effects on both secretary and maturation stages of amelogenesis. Studies on various animal models[10,13] and in human[14] support the view that the early maturation stage is the most critical developmental period for DF, but sufficiently high concentration of fluoride might effect the enamel at all stages of its formation[9,15].

In addition to diet, modern sources of ingestion of fluoride included a variety of dental products, some of which have been identified as risk factors for fluorosis[16,17]. A highly significant association was found between the estimated fluoride ingestion from toothpaste and fluorosis[18]. In fluoridated toothpaste users, especially children in the age group 5–10 years and 10–14 years, even after rinsing their teeth satisfactorily with water fluoride level in circulation was enhanced within a few minutes[19]. It has been reported that DF and dental caries, diseases of different etiology, co-exist in the same individual at fluoride levels ranging from 0.5 to 5.0 mg/l and above[20]. The calcium content is found depleted in fluorosed teeth and the tooth matrix becomes demineralized[20,21]. Studies conducted on fluorosed human tooth matrix molecules revealed that one of the sulphated isomers of glycosaminoglycans, i.e. dermaten sulphate accumulates as a result of fluoride ingestion and thus results in demineralization of tooth matrix. It is also a fact that the demineralized loci in teeth is unlikely to get remineralized due to the presence of dermaten sulphate in the matrix[22]. Embery *et al.*[23] have shown that in rat incisor teeth on exposure to fluoride, dermatan sulphate accumulated and provided evidence to suggest that fluoride ingestion in high amounts causes a major imbalance to the ground substance components of mineralized tissues. In the animal model it has also been

‡For correspondence. (e-mail: susheela@ndf.vsnl.net.in)