

According to our findings and those reported by other investigators^{4,7,8}, dibekacin does not produce a non-depolarizing blockade, because a non-depolarizing agent should be antagonized by neostigmine⁹.

The inability of neostigmine to reverse the neuromuscular blockade produced by dibekacin suggests an impaired acetylcholine release from the motor end-plate in response to nerve impulses. On the other hand, the similarities of the blockades produced by EDTA and dibekacin, the ability of calcium to restore the neuromuscular transmission, as well as the protective action of calcium against the neuromuscular blocking activity of dibekacin, lead to the assumption

that aminoglycoside antibiotics are involved in the process of acetylcholine release by nerve impulses, antagonizing calcium ions. The ability of calcium ions to antagonize the neuromuscular blocking activity of dibekacin is related to their ability to inactivate the antimicrobial action of aminoglycoside antibiotics¹⁰.

The clinical importance of the neuromuscular blocking activity of the aminoglycoside antibiotics lies with the respiratory depression and/or prolonged apnoea which is faced when the above antibiotics are administered concomitantly with non-depolarizing muscle relaxant agents^{5,6}.

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Myotoxic phospholipases A from snake venom, *Pseudechis colletti*, producing myoglobinuria in mice

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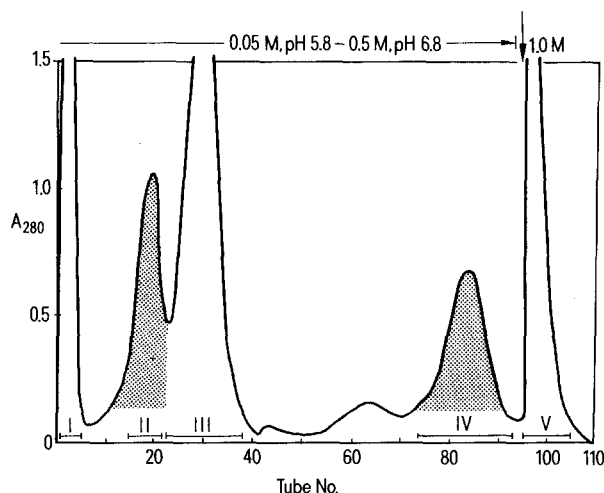
Summary. 2 proteins producing myoglobinuria in mice were isolated from the venom of the Australian elapid snake *Pseudechis colletti* and identified as phospholipases A showing close similarities in amino acid composition to a similarly acting enzyme from a sea snake venom (*Enhydrina schistosa*).

Myoglobinuria is the most conspicuous symptom in sea snake¹ (*Enhydrina schistosa*) as well as in several Australian Elapidae snake envenomations^{2,3}; it has often been mistaken for haemoglobinuria. The active principle in sea snake venom producing this effect has been characterized as a basic phospholipase A⁴. This paper reports the isolation of 2 phospholipases A from an Australian snake venom, *Pseudechis colletti* which causes myoglobinuria in mice.

Materials and methods. The crude venom was purchased from Australian Venom Suppliers, Turrumurra, NSW (Australia). Fractionation was performed by chromatography on CM-Sephadex C-25 using a linear gradient of 0.05 M ammonium acetate buffer pH 5.8 to 0.5 M, pH 6.8 for elution. Lethality of the fractions collected was assayed by s.c. injection into mice (4 male mice for each dose), myoglobinuria was observed by placing the mice on filter paper which was stained red by myoglobinuric urine. After elution of the paper with distilled water the extract was lyophilized; myoglobin was identified by gel filtration on a calibrated Sephadex G-75 column⁵ (125 × 1.5 cm, 0.1 M ammonium acetate buffer pH 6.8) and by spectrophotometry before and after reduction using Na₂S₂O₄. Phospholipase A activity was determined according to the method of Marinetti⁶ measuring the clearing of an egg yolk solution at 925 nm. Amino acid analysis was performed after 24, 48, 72 h hydrolysis of the protein samples in 5.7 N HCl at 110 °C; tryptophan was estimated after methane sulfonic acid hydrolysis for 24 h.

Results and discussion. By ion-exchange chromatography on CM-Sephadex C-25 the venom of *Pseudechis colletti* was

separated into 5 main fractions. All exhibited high phospholipase A activity when tested on an egg yolk solution and all fractions, except I, showed lethal properties producing paralytic symptoms when injected s.c. into mice. But only fractions II and IV caused myoglobinuria after a lag period of 1–2 h; only myoglobin was found in the urine,



Chromatography of *Pseudechis colletti* venom (160 mg) on a CM-Sephadex-C-25 column (8 × 1 cm). Elution was carried out using a linear gradient of 0.05 M ammonium acetate buffer pH 5.8 to 0.5 M, pH 6.8 followed by stepwise elution with 1.0 M buffer. Fractions of 5 ml were collected at a flow rate of 30 ml/h. The dotted peaks (II and IV) produce myoglobinuria in mice.

haemoglobin was absent. Both fractions proved to be homogeneous in disc electrophoresis at pH 4.3. For all fractions the following lethal activities were determined by s.c. injection into mice:

| | Acute toxicity approximate LD ₅₀ mg/kg | Minimum dose for myoglobinuria mg/kg |
|-----|---|--|
| I | — | — |
| II | 4.5 | 0.5 |
| III | 0.8 | — |
| IV | 4.3 | 2.9 |
| V | 1.2 | — |

Besides acute toxicity characterized by respiratory paralysis within 2–3 h, fractions II and IV (to a lesser extent fraction III, probably due to contamination with fraction II) produce myoglobinuria at a lower dose level. In the latter case the mice die in an emaciated state after 3–4 days which might be due to muscle degradation and renal failure as a result of massive myoglobin excretion. Whereas both fractions exhibited nearly the same LD₅₀-value in acute toxicity, fraction II, which was less basic, was 6 times more active in causing myoglobinuria, indicating that there is no strict relationship between basicity of the protein and muscular damaging effect. Furthermore, quite different pathogenic mechanisms, paralysis and myoglobinuria, seem to be associated with the same molecule.

The amino acid composition of fractions II and IV shows close similarities to that reported for *Enhydrina schistosa* myotoxin⁴ considering the number of Asx, Ala, Gly, Lys and the 12 (II) and 14 (IV) half-cystine residues.

Australian snake venoms show a great variety of phospholipases A⁷ which may have high neuromuscular blocking activity like taipoxin⁸ (from *Oxyuranus scutellatus* venom) and notexin⁹ (from *Notechis scutatus* venom), or being less toxic (in terms of LD₅₀) may directly affect muscle tissues, resulting in a massive release of myoglobin. Other toxic phospholipases A of minor specificity in action (sometimes of postsynaptic blocking activity) are also present.

Phospholipase A is, perhaps, the most versatile enzyme, having a primarily digestive function and evolving to toxins with high affinity for various membrane structures, i.e. those of nerve and muscle.

Amino acid composition of fractions II and IV from *Pseudechis colletti* and of the myotoxin from the sea snake *Enhydrina schistosa*⁴

| | Fractions II | IV | Myotoxin |
|----------------|-----------------|--------|----------|
| Asx | 22 | 17 | 21 |
| Thr | 8 | 8 | 4 |
| Ser | 5 | 7 | 5 |
| Glx | 10 | 6 | 6 |
| Pro | 1 | 7 | 3 |
| Gly | 12 | 12 | 9 |
| Ala | 11 | 11 | 10 |
| 1/2 Cys | 12 | 14 | 14 |
| Val | 4 | 4 | 6 |
| Met | 2 | 3 | 2 |
| Ile | 4 | 4 | 3 |
| Leu | 5 | 5 | 4 |
| Tyr | 8 | 7 | 12 |
| Phe | 4 | 4 | 2 |
| His | 2 | 2 | 2 |
| Lys | 10 | 14 | 10 |
| Trp | 2 | 2 | 0 |
| Arg | 5 | 3 | 7 |
| Total | 127 | 130 | 120 |
| Formula weight | 14,170 | 14,200 | 13,500 |

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The induction of chromosome aberrations in human lymphocytes by negative π -mesons under conditions of anoxia and oxygenation

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Summary. After a dose of 3.0 Gy in the peak position of the pion depth-dose curve, the ratios between observed chromosome aberration yields under conditions of oxygenation and of anoxia were obtained for lymphocytes exposed at 3 depths in a plastic phantom. These ratios were 3.7, 1.9 and 1.3 in the plateau, peak and post-peak positions, respectively, suggesting a corresponding decrease in the oxygen enhancement ratio.

Calculations by Fowler and Perkins² showed that negative π -mesons (pions) possess several advantages from the point of view of radiotherapy. Their ideal depth-dose profile and advantageous LET distribution combine to give a very useful distribution of biological damage within the treatment volume. Low LET radiations do have the advantage of allowing normal tissue to recover during the interval between treatment fractions but with the disadvantage that the oxygen enhancement ratio (OER) for this type of radiation is of the order of 3. Thus anoxic tumor tissue may remain resistant to the levels of radiation dose achievable without causing intolerable damage to adjacent healthy tissues.

Pion induction of chromosome abnormalities was first examined by Richman et al.³ working with *Vicia faba* root meristem cells. Gnanapurani et al.⁴ analyzed chromatid aberration in the same material and estimated the OER of peak radiation at 1.8. Further OER estimations range from 1.35 for *Vicia faba* root growth⁵ to 2.2 for V79 Chinese hamster cell survival⁶.

The characteristics of the pion beam available from the NIMROD accelerator have been examined using a variety of biological systems⁷. An OER of 1.8 was obtained for *Vicia faba* root growth compared with a value of 3 with cobalt-60 γ -rays⁸. OERs of the order of 1 were obtained for mouse testis weight loss and spermatogonial cell survival in