Crotales rober rober venom contains several different professes, and the proteolytic activity of the crude venom is 6-15 times greater in adult than in juvenile venom. Venom samples were assayed for proteolydc, phosphodiesterase, t.-amino acid oxidase and dastinaae-tike activities and were subjected to gel filtration on BioGel P-100. Two major size classes of professes were resolved (mol. wt 67,000 and 20,300). EDTA, N~thyhnaleimide (N-EM) and 1,10-phenanthrotine inhibited proteolytic activity of crude venom, and EDTA, ZnN and Cu" inhibited proteolydc activity of the fradionsted venom.

THE RED DIAMOND RATTLESNAKE (Crotalus ruber) is a large species whose chaparral habitats are increasingly encroached upon by human activity in southwestern California. Though behaviorally inoffensive, its large size (to 1.5 m; KLAUBER, 1972), high venom yield (GLENN and STRAIGHT, 1982) and evidence of severe tissue damage on envenomation (RUSSELL, 1%9; LYONS, 1971) combine to make this snake of clinical concern within its restricted range. However, with few exceptions, very little is known about the chemical nature of this venom (e.g. Tu et al., 1966; DuRKIN et al., 1981).

Initial investigations in this laboratory of crotalid proteolytic enzymes indicated high activity levels in C. ruber venom. Proteolytic enzymes are most prevalent among crotalids and viperids (Tv et al., 19C~ and the severe tissue damage associated with rattlesnake envenomation results chiefly from the action of professes and related enzymes (OWNBY, 1982)

azure (lot No. 810279) and casein yellow (lot No. 610029) were obtained from Ca1BioChem. BioGel P-100 (100-200 mesh) was purchased from Bio-Rad Laboratories. Molecular weight protein standards and other biochemicals (analytical grade) were obtained from Sigma Chemical Co.

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Crude venom was assayed for proteolytic activity (STEYN and DELPIERRE, 1973), L-amino acid oxidase activity (essentially the method of WEISSBACH *et al.*, 1961; reaction terminated by the addition of 10% (w/v) trichloracetic acid), elastinase-like activity (SIMPSON and TAYLOR, 1973) and phosphodiesterase activity (BJÖRK, 1963). The effects of phenylmethylsulfonyl fluoride (PMSF), 1,10-phenanthroline, *N*-ethylmaleimide (*N*-EM) and EDTA at three concentrations (1, 10 and 100 μg/ml) were evaluated using hide powder azure as substrate. Venom (30 μg), inhibitor and 0.05 M *N*-2-hydroxyethyl-[illegible]

room temperature (21 – 23°C) for 30 min. Proteolytic activity was then assayed as above. All assays were run in duplicate and compared to control hydrolysis.

Adult *C. ruber* venom (150 mg) was fractionated on a 2.8 × 96 cm column of BioGel P-[illegible]

to fractionation, the column was calibrated with albumin (mol. wt 67,000), ovalbumin

III
Venom: 150 mg (Specimen 1)
Buffer: 0.05 M Ammonium acetate
Sample volume: 3.0 ml
Column dimensions: 96 x 2.8 cm
Flow rate: 4.8 ml/hr
2.0
1.5
SE ACTIVITY
DASE ACTIVITY