

isoenzymic forms were investigated by starch-gel electrophoresis.

The authors acknowledge a Science Research Council Studentship to D.G.T. The work was supported by grants from the National Kidney Research Fund and the Medical Research Council.

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### The Toxic Proteins of an Australian Jellyfish *Chironex fleckeri*

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The venom of the box jellyfish *Chironex fleckeri* from Australian waters resembles that of the Atlantic jellyfish *Physalia physalis* (Lane, 1968) in its toxic action, except that it is more potent. Tentacle extracts of *Chironex* exhibit the same pharmacological activities (Turner & Freeman, 1969) as the venom. From such extracts, two lethal factors have been separated on Sephadex G-200 (Crone & Keen, 1969) in positions corresponding to molecular weights of 70 000 and 150 000. Both toxins have cardiovascular effects and one of them (mol.wt. 70 000) is a strong haemolysin (Keen & Crone, 1969a).

The resolution of the two active fractions has been improved by ion-exchange chromatography on CM-Sephadex C-50. The non-haemolytic toxin, which was eluted first, was then chromatographed on Sephadex G-200 and was recovered in the position corresponding to 150 000 mol.wt. The lability of the haemolysin prevented further chromatography of this toxin.

The tentacle extracts were also chromatographed on thin layers of Sephadex G-200, and the position of haemolytic activity was detected by the use of filter paper soaked in a suspension of erythrocytes in 150 mM-NaCl-10 mM-phosphate, pH 7.4. The behaviour of the haemolysin was markedly dependent on the ionic strength and pH of the eluting buffer and on the degree of loading of the chromatogram. The adsorption of the haemolysin on the Sephadex matrix was strong at low salt concentration but was much less under the conditions used for column chromatography. The effect is sufficient,

however, to make electrophoresis on support media difficult.

These results support the view that there are at least two toxic proteins present in *Chironex* extracts. The larger, non-haemolytic, toxin appears to be more stable and may prove to be easier to isolate than the haemolysin, which rapidly loses activity under the mildest conditions. Since tentacle extracts also contain a dermatonecrotic agent which is antigenic (Keen & Crone, 1969b), there is possibly a third toxic protein in the extracts, but dermatonecrosis has not been related to a specific protein fraction. The possibility that the active compounds are small molecules adsorbed strongly to proteins in the extracts cannot be totally excluded, but the present results make this very unlikely.

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### Tyrosine Metabolism in the Blowfly Larva, *Calliphora erythrocephala*

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Tyrosine metabolism in dipterous insects has received considerable attention, because the metabolic products of this amino acid play essential roles in the hardening and darkening of the larval cuticle at the time of puparium formation (Karlson & Schmid, 1955; Karlson & Schweiger, 1961; Karlson & Wecker, 1955; Karlson & Sekeris, 1962; Sekeris & Karlson, 1962). In haemolymph from third-instar larvae of *Phormia regina*, Levenbook (1966) found 4.59  $\mu\text{mol}$  of tyrosine/g of larva, and in the whole larva at the 'rounded-off' stage 24  $\mu\text{mol}$ /g of larva was found (Levenbook & Dinamarca, 1966). In the present work with *Calliphora*, free tyrosine was determined by the method of Udenfriend (1957). In late third-instar larvae (7-8 days old) 20  $\mu\text{mol}$ /g of larva was found in the fat-body, 1.4 in the haemolymph, 0.5 in the muscle and 0.2 in the cuticle. Thus 90% of the tyrosine in 7-8-day-old larvae is present in the fat-body. In the 4-day-old larva there is 0.1  $\mu\text{mol}$  of tyrosine/fat-body, the amount rising to 1.0  $\mu\text{mol}$  in 6-day-old larvae and finally reaching 1.4  $\mu\text{mol}$  in 7-8-day-old larvae.

In the early stages of pupation, the larva rounds off but remains white. During this stage, which lasts less than 1 h, the tyrosine content of the fat-