isoenzymic forms were investigated by starch-gel electrophoresis.

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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 μmol of tyrosine/g of larva, and in the whole larva at the 'rounded-off' stage 24 μmol /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 μmol /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 μmol of tyrosine/fat-body, the amount rising to 1.0 μmol in 6-day-old larvae and finally reaching 1.4 μmol 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-

body falls but there is still about $0.8 \mu\text{mol}$ of tyrosine left in the fat-body 24h later when the larval cuticle has tanned to a golden brown. In 4–8-day-old larvae the amount of tyrosine in the haemolymph remains fairly constant at $1 \mu\text{g}/\mu\text{l}$, but during the 'rounded-off' stage the concentration doubles, only to fall again within 24h to a value lower than that found earlier in the instar.

Since the content of tyrosine in the fat-body falls at the same time as that in the haemolymph rises, this strongly suggests that the fat-body is the source of some, if not of most, of the tyrosine which is transported via the haemolymph for use in the reactions resulting in the sclerotization of the larval cuticle. Furthermore, the fact that the content of tyrosine in the haemolymph suddenly rises during the 'rounded-off' stage suggests that during this period the rate at which tyrosine is released by the fat-body is faster than the rate at which it is taken up by the epidermal cells. Since these changes are taking place during or just after the critical period of ecdysone release (Shaaya & Karlson, 1965) it may well be that the release of tyrosine from the fat-body is under the control of this hormone.

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Species Differences in the Formation of 4-Amino-3-biphenyl β -D-Glucosiduronate *in vitro*

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Exposure of man or experimental animals to 4-aminobiphenyl generally results in the development of neoplastic disease (Melick, Escue, Naryka, Mezera & Wheeler, 1955; Walpole, Williams & Roberts, 1954; Bonser, 1952), the site of tumour formation depending on the species. In man, dog and rabbit tumours of the urinary bladder develop

whereas in rats and mice intestinal tumours or hepatomas are produced. Guinea pigs appear to be refractory to the carcinogenic action of this type of compound.

4-Aminobiphenyl is hydroxylated on the 3- or 4'-position of the nucleus or on the nitrogen atom (Bradshaw & Clayson, 1955; Uehleke, 1963), but the routes leading to the initiation of the carcinogenic process are at present unknown. The failure to find 2-amino-3-biphenyl glucosiduronate as a metabolite of the non-carcinogenic amine 2-aminobiphenyl (Gorrod & Carey, 1970) suggested that the glucosiduronic acid conjugate of 4-amino-3-hydroxybiphenyl may be implicated in carcinogenesis as suggested by Clayson, Jull & Bonser (1958) and Boyland (1963).

Condensation of potassium 3-hydroxy-4-nitrobiphenyl with methyl (tri-*O*-acetyl- α -D-glucopyranosyl bromide)uronate in alcoholic solution gave methyl (4-nitro-3-biphenyl) tri-*O*-acetyl- β -D-glucopyranosiduronate. Reduction of this material with hydrogen in the presence of platinum oxide yielded methyl (4-amino-3-biphenyl) tri-*O*-acetyl- β -D-glucosiduronate, which on methanolysis with sodium methoxide gave sodium (4-amino-3-biphenyl) glucosiduronate. This material was identical in chromatographic properties and response to detection reagents with the compound formed on incubating 4-amino-3-hydroxybiphenyl with UDP-glucuronic acid in the presence of a hepatic microsomal preparation.

4-Amino-3-biphenyl glucuronide was assayed by diazotization followed by coupling with 1-*N*-naphthylethylenediamine in acid media. The coloured pigment formed had an absorption maximum at 572nm and gave a linear relationship up to $0.5 \mu\text{mol}$ under the conditions used.

The 4-amino-3-hydroxybiphenyl-UDP-glucuronate glucuronyltransferase activity was assayed in hepatic microsomes obtained from various species. Activity decreased in the order guinea pig > rabbit > dog > hamster > mouse > rat.

Very little activity was found in the cat and no activity was detected in human foetal liver.

The results do not give a clear correlation between glucuronide-forming ability and susceptibility towards the carcinogenic action of 4-aminobiphenyl. It may be that involvement of β -glucuronidase releasing the 4-amino-3-hydroxybiphenyl from its conjugate will be a further factor in deciding species sensitivity to this compound.

This work was carried out while the author was a Research Fellow of the Royal Commission for the Exhibition of 1851.

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