These were variegatic acid and xerocomic acid, and two new pulvinic acids, only one of which exhibited the blueing reaction. Of taxonomic value is the presence or absence of the polyphenol oxidase responsible for the blueing. In this respect, a species of the *Boletus bicolor* group did not turn blue although variegatic acid was present. Extracts of two *Suillus* species contained four pigments, which are considered to be polyhydroxydiphenylbenzoquinones on the basis of their spectral properties and colour reactions.

The genus *Paxillus* has morphological affinities with both Boletineae and Agaricineae. A blueing *Paxillus* species afforded variegatic acid and a new non-blueing pulvinic acid. *Paxillus atrotomentosus* elaborates a tetrahydroxydiphenylbenzoquinone, atromentin (Kögl & Postowsky, 1925). The co-occurrence of the benzoquinone and pulvinic acids in *Paxillus* suggests that this is a chemotaxonomic link between the sub-orders.

The diphenylbenzoquinones are readily oxidized to pulvinic acids and the biological oxidation is probably catalysed by a pyrocatechase-type enzyme. Pyrocatechase oxidizes catechol to *cis-cis*-muconic acid (Knox, 1961). A biosynthetic scheme accounting for the production of blueing and non-blueing pulvinic acids will be presented.

Paxillus involutus elaborates involutin, a polyhydroxydiphenylcyclopentenone (Edwards, Elsworthy & Kale, 1967). The biosynthesis of this metabolite may be explained in terms of a by-pass from the pulvinic acid pathway and appears to involve a decarboxylation of an intermediate muconic acid derivative, cyclo-dehydration and reduction.

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The Use of Zonal Centrifugation in the Preparation of Glomeruli and Tubular Fragments from Rat Kidney

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A number of methods have been used for the isolation of glomeruli and of tubular fragments from a variety of animals. Greenspon & Krakower (1950) separated glomeruli from a cortical mash of

dog kidney by means of a sieve of known mesh size. Fong & Drummond (1968) used a similar procedure for rat kidney, and van Breda Vriesman & Willighagen (1968) digested dog kidney with collagenase before sieving. Collagenase has also been used in the preparation of rabbit renal tubules (Burg & Orloff, 1962). These preparations have been used in the production of antisera, in the preparation of basement membranes and in studies on active transport. Mattenheimer, Pollak & Muehrcke (1970) isolated different regions of the human nephron by micro-dissection and compared their enzyme content. The development of a simplified procedure for the simultaneous preparation of large quantities of glomeruli and tubular fragments would allow a detailed study of the enzymic content and isoenzyme forms present in normal and experimentally damaged kidneys.

Male Sprague-Dawley rats (200 g) were killed by a blow on the head, and the renal arteries and veins were exposed and clamped. Then 5 ml of a 0.05% collagenase solution in 0.9% NaCl (adjusted to pH 8.5 with 0.25 M-NaOH) was injected into each renal pelvis. After 10 min the kidneys were dissected out, the renal capsules removed and the cortices separated from the medullae. A known weight (4–16 g) of cortical preparation was then minced to a paste with a razor blade and made up to a 5% suspension in the collagenase solution. The suspension was forced through a no. 1 Luer needle and incubated at 25°C with constant stirring. After 1 h the suspension was filtered through two layers of muslin and the filtrate was washed three times by centrifugation at 5000 g-min on an MSE 18 ultracentrifuge and suspension of the pellet each time in 100 ml of 0.9% NaCl (pH 7.2). The final pellet was suspended in 50 ml of 40% (w/w) sucrose solution (pH 7.2).

The suspension was fractionated by zonal centrifugation in an MSE type A zonal rotor at 5000 rev./min for 45 min at 4°C. A continuous density gradient of 40–60% (w/w) sucrose was used and fractions (35 ml) were collected. Four bands were apparent in the rotor and the fractions corresponding to them were characterized by light-microscopy. A sharp band at 50–51% (w/w) sucrose contained decapsulated glomeruli and was distinct from a larger band, containing tubular fragments, at 45–48% (w/w), and both were well separated from erythrocytes and cell debris. Glomeruli and tubular fragments were recovered from these fractions by centrifuging in an MSE 18 ultracentrifuge at 500 000 g-min, and the resulting pellets were suspended in a small volume of 0.45 M-sucrose solution.

Acid and alkaline phosphatase, leucyl- β -naphthylamidase and *N*-acetyl- β -glucosaminidase activities were assayed in the two preparations and their

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-