PREDATION

Unpalatable Legumes

from our Plant Ecology Correspondent

MANY species of plant and animal have responded to intense predation pressure by developing toxic metabolites which render them unpalatable. The development in plant seeds of insecticidal compounds has highly selective value for ensuring survival and ultimate germination. A biochemical survey of various legume seeds, some of which are conspicuously free from insect predation, has now been carried out by Rehr, Bell, Janzen and Feeny (*Biochem. Systematics*, 1, 63; 1973).

In Central America and the southern United States, where the survey took place, legume seeds suffer attacks from a variety of Lepidoptera larvae and also from Coleoptera of the family Bruchidae. One abundant lepidopteran is the southern armyworm, *Prodenia eridania*, a polyphagous species which is known to be capable of consuming a wide variety of food plants, including many containing insecticidal toxins. These toxins are apparently detoxified by *Prodenia*. Its catholic appetite makes the southern armyworm an ideal test organism for use in bioassays of seed toxicity and palatability.

Rehr *et al.* ground a variety of legume seeds and offered them incorporated into an artificial diet to *Prodenia* larvae. Many of the seeds which did not suffer severe predation in the field contained high concentrations of unusual amino acids; these were extracted and then fed to the armyworms. This provided a means of identifying precisely the repellent compounds involved in seed protection.

Seeds of some legume species, for example *Cassia grandis*, which are heavily preyed upon in the wild, were found to contain no uncommon free amino acids. A diet containing ground *Cassia* seeds proved quite acceptable to the armyworms and produced negligible mortality. It is worth noting that this species produces large quantities of seeds, which presumably compensated for losses by predation.

Entada polystachya suffers moderate predation from insects in the field and the seeds of this species were found to contain an unidentified amino acid. A 5% ground diet of this species caused some pupal deformity in *Prodenia*, in which ventral abdominal segments were soft. Deformed pupae either died or produced adults incapable of copulation.

Legume species which were observed to be fairly free from predation, for example *Gymnocladus dioica* and *Albizia julibrissin*, contained high concentrations of unusual free amino acids, in these cases β -hydroxy- γ -methylgluta-

mic acid and albizziine respectively. A 5% *Gymnocladus* seed diet caused heavy mortality among the armyworms. They were strongly repelled by material containing 4% of β -hydroxy- γ -methylglutamic acid, which is the concentration found in *Gymnocladus* seeds. Normal L-glutamic acid at this concentration was neither repellent nor harmful to *Prodenia* larvae.

Evidently there is a highly selective advantage, in terms of seed survival, in the production and accumulation of certain amino acids. The protective system is most highly developed and most effective in plants such as *Gymnocladus* where toxin concentration is sufficiently high to be repellent to the predator even before consumption.

NEUROTOXINS

Receptor Function

from our Neurochemistry Correspondent

NATURALLY-OCCURRING toxins continue to be fashionable and powerful weapons in the armoury of molecular pharmacologists bent upon the analysis of receptors and factors influencing their formation and function.

α -Bungarotoxin has recently been used to "label" cholinergic receptors in cultured neurones from chick embryo sympathetic ganglia (Greene, Sytkowski, Vogel and Nirenberg, *Nature*, 243, 163; 1973). But the use of another toxin, histrionicotoxin, derived from a Colombian arrow-poison frog, has suggested that α -bungarotoxin may bind to a second site at the synapse in addition to the cholinergic receptor itself (Albuquerque, Barnard, Chiu, Lapa, Dolly, Jansson, Daly and Witkop, *Proc. natn. Acad. Sci. U.S.A.*, 70, 949; 1973).

The excitatory action of acetylcholine (ACh) at receptors on skeletal muscle involves two stages: first, combination with receptor sites, followed by an increase in the permeability of the post-synaptic membrane to sodium and potassium ions, resulting in a transient depolarization. As yet little is known about the increase in ion conductance, and how this is triggered by combination of ACh with its receptors. Albuquerque *et al.* now report, however, that the perhydro derivative of histrionicotoxin reversibly blocks ACh-induced potential changed at endplates in normal muscles as well as at extra-junctional sites in chronically denervated muscles, apparently by preventing the potassium conductance change, and, to a lesser extent, the sodium conductance change. Moreover, during successive ACh applications, the toxin becomes completely ineffective only after the first contact with ACh, suggesting that its action is dependent on an initial binding of ACh at the re-

ceptor. Thus, H_{12} -histrionicotoxin may be extremely valuable in the investigation of the "ionophore", although the ideal "label" would be an irreversible binding agent.

α -Bungarotoxin has previously been thought to affect neuromuscular transmission more or less irreversibly. But Albuquerque *et al.* report a partial recovery of the endplate potential after washout of α -bungarotoxin from poisoned diaphragm endplates. These results together with results from binding studies using a tritium-labelled derivative of α -bungarotoxin indicate two classes of binding sites, one occupied preferentially by curare, at which α -bungarotoxin binds irreversibly, and the other occupied preferentially by histrionicotoxin, at which the binding of α -bungarotoxin is reversible. Both sites are involved in synaptic excitation by ACh, and are equated by the authors with, respectively, the receptor site and the "ionophore".

More recently, Giacobini, Filogamo, Weber, Boquet and Changeux (*Proc. natn. Acad. Sci. U.S.A.*, 70, 1708; 1973) report how receptor blockade with a toxin from cobra venom affects the development of neuromuscular contacts in embryonic chick muscles. In their system toxin binding is confirmed to be specific for the ACh receptor site by its almost complete abolition by a variety of known cholinergic ligands. Chick embryos survive high doses of toxin and are therefore convenient for the study of the development of innervated muscle in the presence of toxin. In normal conditions the development of the motor innervation takes place in three main stages. First, from the fourth to the sixth day of incubation, motor nerve fibres invade the limb-bud muscles, and the first myotubes appear, with a diffuse localization of acetylcholinesterase. From the sixth to the twelfth day a massive formation of myotubes occurs, and acetylcholinesterase becomes restricted to the myotendon junctions. Finally, from the twelfth day onwards, muscle fibres replace the myotubes, and the nerve endings differentiate into motor endplates with a selective localization of acetylcholinesterase; at this stage action potentials can be seen, and the ACh sensitivity becomes restricted to the endplate region. Embryos injected with toxin were found to become atrophic with a marked reduction in the growth and differentiation of skeletal muscles—in 16-d embryos which had been treated with toxin on the 3rd, 8th and 12th day, the majority of contractile elements were still present as myotubes. Moreover, very few motor endplates could be seen, and the sciatic nerve, spinal roots and sensory ganglia appeared atrophic. It is unlikely that the toxin affects impulse propagation,