INVERTEBRATE PHARMACOLOGY[1,2]

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Introduction

Of the approximately one million species extant in the world today, about 95 per cent are invertebrate.

Much physiological and pharmacological experimentation is carried out on vertebrates, particularly mammals. This is a reflection, legacy, and necessity of the medical sciences since it is generally assumed (sometimes erroneously) that results obtained using other mammals will have direct application to man. The whole field of biological science received its initial impetus from medicine.

The study of the pharmacology of invertebrates may have a different *raison d'être*. Knowledge of similar processes in a wide variety of animals has intrinsic value and indicates the plasticity of living tissues during evolutionary history. Furthermore, as tools for the understanding of fundamental processes, the astonishing range of the invertebrates has much to offer.

This feature has been recognised by an ever increasing number of workers in comparative physiology, and one of the most interesting developments in the investigation of basic phenomena has been the use of certain invertebrate preparations with peculiar morphological features. In particular, workers are now at last able to probe the functioning of single, identified, and repeatedly found cells and to test the effects of drugs directly applied to the membranes of such cells. The muscle receptors of crustaceans, the giant neurones of gastropods, the giant fibres of squids are all so individual as to permit experiments on the same cell in different preparations, thus improving the chances of satisfactory replication of results, and of demonstrating the differences between non-identical cells. This is a major advance over the blunderbuss and bathwater approach of only a few years ago when wholesale perfusion of thousands of cells was necessary and individual characterisation impossible.

Another advantage offered by some invertebrates for pharmacological work is the high concentration of certain active substances in certain tissues

[1] The survey of literature for this review was concluded in June 1967.

[2] Abbreviations: ACh (acetylcholine); BOL (2-brom-D-lysergic acid diethylamide); CNS (central nervous system); DOPA (3,4-dihydroxyphenylalanine); e.p.s.p. (excitatory postsynaptic potential); GABA (γ-amino butyric acid); i.p.s.p. (inhibitory postsynaptic potential); LSD (lysergic acid diethylamide); m.e.p.p. (miniature excitatory postsynaptic potential); UML (1-methyl-D-lysergic acid butylonamide); 5-HT (5-hydroxytryptamine); 5-HTP (5-hydroxytryptophan).

of some species. For instance, acetylcholine (ACh) is found in extremely high concentrations in the octopus brain. This fact was exploited by Bacq & Mazza (1) when they chemically identified ACh in nervous tissue for the first time. Other substances found in large quantities in nervous, and other tissues of certain species include 5-hydroxytryptamine (5-HT) and DOPAmine. The high concentrations of these amines in tissues, which are also suitable for detailed electrophysiological and biochemical studies, have promoted work to determine their functions and modes of action in these locations. At present, research on 5-HT in invertebrates is in the vanguard of studies to elucidate the biological importance of this amine. The preponderance of DOPAmine over noradrenaline in certain locations suggests that in such tissues, the view that DOPAmine has a role other than that of precursor to noradrenaline may be tested.

Experiments with extracts from invertebrate sources have led to the discovery of new biologically active substances. One of these is the peptide eledoisin which was discovered in the octopod *Eledone* (2). Eledoisin has potent vasodilator and depressor effects on vertebrates. Other active peptides peculiar to invertebrates include wasp and hornet kinins, the gamete shedding factor of starfish, and various crustacean and insect neurosecretory substances. Studies on the nature of cholinomimetic agents in hypobranchial gland extracts from gastropod molluscs led to the discovery of several new choline-esters (3). Many other active substances of unknown composition, such as Substance X and various toxic agents, are reported in the literature. Many of these substances will undoubtedly be of general value in biomedical work when isolated and identified.

Finally, there is another ever widening aspect of invertebrate pharmacology. The pressures upon the human population of the world are growing everyday, and nowhere is the pressure felt more than in the necessity for increased food production. More efficient land use is leading to the production of many new insecticides and drugs which are applied to the soil. The control of insect populations by luring with pheromones is a direct result of the above. Effective chemotherapy of animal parasites *in situ* leads to advances in veterinary practice, and perhaps a knowledge of natural invertebrate repellents can be applied to protect precious livestock and products.

We have not attempted to review certain aspects of Invertebrate Pharmacology, such as insect pheromones, insecticides, or the pharmacology of parasitic invertebrates. Since space limitations necessitate selectivity, we have restricted our attention to those aspects of the field in which we have particular interest and which we believe may lead to discoveries of general importance.

5-Hydroxytryptamine

One of the first suggestions concerning the role of 5-HT in invertebrates was that the substance acts as a cardio-excitatory transmitter in molluscs (4). This theory is supported by the following findings: 5-HT and its immediate precursor 5-hydroxytryptophan (5-HTP) are present in molluscan

nervous tissue; enzymes for conversion of 5-HTP to 5-HT and for the subsequent inactivation of 5-HT have been detected in molluscan tissues; excitation of the *Mercenaria* heart following nerve stimulation is blocked with 5-HT antagonists; immersion of the *Mercenaria* heart in reserpine results in loss of the excitatory response to nerve stimulation, whereas sensitivity to applied 5-HT is unaffected; *Mercenaria* hearts, which are tachyphylactic to 5-HT, are unresponsive to stimulation of the cardio-excitatory nerves.

However, Florey (5) considered that a transmitter role for 5-HT had not been clearly demonstrated in either molluscs or other invertebrates, because not all the criteria proposed for transmitter action had been adequately satisfied. Obstacles to acceptance were (*a*) lack of evidence of an action of 5-HT on the postsynaptic membrane; (*b*) lack of unequivocal evidence for the localization of 5-HT in neurones rather than in glial cells; (*c*) the need to demonstrate 5-HT release from nerves during stimulation; (*d*) lack of confirmation that 5-HT antagonists block the effects of stimulating proposed serotonergic nerves. Recent research has provided evidence on all these points.

Mode of action and effects.—Responses of molluscan neurones to application of 5-HT to entire ganglia have been studied by Kerkut & Walker (6) and Gerschenfeld & Tauc (7). However, only recently have iontophoretic techniques been used to localise the application of the drug to individual neurones, and thus obviating the possibility of effects mediated via surrounding neurones. Gerschenfeld & Stefani (8, 9) have shown that one type of neurone in ganglia of the gastropod *Cryptomphallus aspersa* is responsive to iontophoretic application of 5-HT. These neurones were the CILDA cells, so called because they show a prolonged inhibitory response to presynaptic stimulation (10). 5-HT depolarised these cells which frequently resulted in action potential firing. Repeated application of 5-HT produced intense desensitisation similar to that seen with ACh receptors on cells known to receive cholinergic input. As would be expected, if 5-HT acts as a transmitter on the CILDA cell, the amine altered membrane conductance. This effect was at least somewhat specific, since the amine did not alter membrane characteristics of hyperpolarised (H) cells (neurones with an inhibitory cholinergic input). CILDA cells responded to minute amounts of 5-HT which were antagonised by BOL and UML, both of which appeared to act as competitive antagonists. Morphine also antagonised the 5-HT effect. Another interesting observation was that the CILDA cells were also depolarised by ACh which, however, was slightly less potent than 5-HT. Atropine blocked the response of the cells to both ACh and 5-HT. The response of the CILDA cell to presynaptic stimulation could also be blocked with BOL. However, since BOL has a strong action on nerve conduction in *Aplysia* (11), its importance in blockade of the postsynaptic response in terms of 5-HT release is dubious. Nevertheless, Gerschenfeld & Stefani consider that their studies support a neurotransmitter role for 5-HT in the gastropod CNS.

The nonspecificity of neurone membranes towards active compounds is now generally accepted. Thus, 5-HT and ACh can both affect CILDA cells,

and in the same way. Asher, Gerschenfeld & Tauc (14) have also shown that single neurones in *Aplysia* receive two types of excitatory input mediated by different transmitters but with the same end result. Bailey & Laverack (15) showed that the natural input from the peripheral sense organs (osphradium) of the gastropod, *Buccinum*, may provoke a combination of responses in central ganglion cells, and these presumably are mediated via axons releasing different transmitters on the same cell.

Cooke (16), from studies to determine the site of action of 5-HT, as well as active factors in pericardial organ extracts on the decapod crustacean heart, concluded that 5-HT acts directly upon the cardiac ganglion, driving the burst frequency to a higher rate, and increasing duration of the bursts. The effects of 5-HT may result from a specific action on the pacemaker mechanism in the ganglion. Cooke also considers that the amine may affect neuromuscular transmission between the cardiac ganglion neurones and heart muscle fibres. An effect on cardiac dendrites seems unlikely. A facilitatory action of 5-HT on the crayfish neuromuscular junction has been demonstrated by Dudel (17).

There have been many recent studies on the pharmacological effects of 5-HT and LSD derivatives on molluscan heart preparations (18–26), glochidia and veliger larvae (27–30), and squid chromatophores (31).

The experiments of Twarog (32) on the anterior byssus retractor muscle (ABRM) of *Mytilus* suggest an alternative, intracellular site of action of 5-HT. This preparation has been studied for many years because of the dual nature of its response to electrical stimulation. Tonic contraction (or "catch") of the muscle is not dependent on continuous nervous activity. A cholinergic system appears to be involved in the dynamic contractile events and 5-HT relaxes the muscle. Twarog has shown that resistance to stretch during tonic contraction is inversely proportional to 5-HT concentration over the range 10^{-8} to $5 \times 10^{-7} M$. Furthermore, 5-HT greatly affected the electrical response of muscle cells to excitor nerve stimulation. It lowered the threshold to nerve stimulation, increased the amplitude and occurrence of spike potentials, and enhanced recovery of the preparation. It has been proposed that 5-HT promotes intracellular calcium binding and that catch is dependent on high concentrations of free calcium ions in the cells. Previously, Twarog (33) had shown that relaxation resulting from 5-HT application is not accompanied by any obvious changes in membrane potential. Thus, the possibility of an intracellular action of 5-HT on the anterior byssus retractor muscle is raised. BOL and UML do not antagonise 5-HT effects on the muscle (Twarog, personal communication, 1967). Histochemical studies on the muscle and innervating nerves should be conducted to determine the cellular location of 5-HT in this system.

Inactive phosphofructokinase isolated from *Fasciola hepatica* can be activated by 5-HT as well as cyclic adenosine 3′,5′-phosphate (33a). These findings are consistent with the view that the effect of 5-HT on this enzyme, in homogenates, is mediated through the production of the cyclic nucleotide. These studies and previous observations by Mansour and co-workers [see

(33a)] implicating 5-HT in the regulation of glycolysis in *Fasciola* are of considerable interest, especially in relation to the putative intracellular action of the amine considered by Twarog.

Cellular and subcellular localization.—The histochemical localization of 5-HT within cells of the nervous system of invertebrates followed the development of the para-formaldehyde fluorescent technique by Falck & Hillarp (34, 35). Molluscan neurones were studied by Dahl, Falck, Lundquist & von Mecklenburg as early as 1962 (36). A more recent publication by the Swedish group (37) fully describes the histological localization of 5-HT as well as catecholamines in ganglia of *Anodonta piscinalis*, *Helix pomatia*, and *Buccinum undatum*. Similar studies on ganglia of *Anodonta cygnea* have been reported, though with different interpretations of results, by Zs-Nagy (38). The same technique has also been used on representatives of several other phyla including crustaceans (39) and annelids (40–42). Cells containing 5-HT have also been reported in two species of coelenterates and a sponge by means of different histochemical methods (43, 44).

The yellow fluorescence characteristic of 5-HT was observed in neurones of ganglia of the lamellibranch and gastropod species studied (37). Small fluorescent yellow granules, sometimes arranged in rows and suggesting the presence of nerve fibres with varicosities, were observed in neuropile regions of several ganglia (cerebral, visceral, and pedal). The varicosities are thought to be nerve terminals similar to those of adrenergic systems of vertebrates. The distribution of fluorescence within individual neurones indicated a high concentration of amine within the cell body, the proximal axon, and the nerve terminal, but not along most of the length of the axon. This may indicate synthesis in the soma, followed by transport along the axon to presynaptic storage sites at the terminals.

In *Astacus* (decapod crustacean), neurones containing 5-HT occur in small numbers in the protocerebrum, medulla externa, medulla interna and ventral nerve cord (39).

In the polychaete *Nephthys*, Clark (40) found neurones containing 5-HT in the cerebral ganglion and the ventral nerve cord of the CNS, in the segmental nerves, and in the intestinal wall. Longitudinal fibres of the ventral nerve cord had but few varicosities. Small aggregations of fluorescent granules occurred in the basement membrane where the muscle fibres were inserted near the intersegmental groove. Clark suggests that in the annelids these 5-HT bearing axons are motor, but the longitudinal fibres could be association fibres running from ganglion to ganglion. Rude (41) considers that at least some of the yellow fluorescent cells in the ventral nerve cord of *Lumbricus terrestris* may represent interneurones.

Walker, Sedden & Kerkut (42, 45) have found 5-HT within the cytoplasm of the two segmental giant neurones (Retzius cells) of the ganglia of *Hirudo medicinalis*. These spontaneously active neurones were inhibited by 5-HT in the bathing medium.

The histological localization of 5-HT in motor nerves of annelids may be correlated with the observations of large amounts of the amine in the nervous

system of several annelids (46), and with the effect of 5-HT on annelid muscle. The dorsal muscle of the leech, long used for assay of ACh, is relaxed by 5-HT, which also antagonises the ACh effect (47). Electrical stimulation of the ventral nerve cord leads to loss of tone in the muscular pharynx of *Nereis virens*, and this response is mimicked by 5-HT (48).

Welsh (49) demonstrated that 5-HT is not freely soluble in the cytoplasm of molluscan nerve cells, but is associated with sedimentable particles. More recently, Zs-Nagy et al. (50) showed that about 50 per cent of the total 5-HT was sedimented in several fractions prepared by differential centrifugation of homogenate of *Anodonta cygnea* ganglia (Max R.C.F. was 10^5g for 30 min). Studies with *Mercenaria mercenaria* ganglia showed that about 70 per cent of the total 5-HT in homogenates prepared in 1.1 *M* glucose could be sedimented when centrifuged at 5×10^4g for 30 min (51). The discrepancy between the two studies is almost certainly due to the release of 5-HT from its binding particles in the experiments of Zs-Nagy et al. since a hypotonic solution was used in one stage of their preparation procedure. Thus, it would appear that at least 70 per cent of the amine is particle-bound in the bivalve CNS. Whether the 30 per cent of the amine found in solution represents the proportion of the amine normally freely dissolved in the cytoplasm of neurones or is an artefact of homogenisation and centrifugation is not known.

The subcellular entities with which 5-HT is associated have not yet been definitely identified. However, experiments with *Mercenaria* ganglia have shown that particles binding 5-HT differ from those binding ACh, because they behave differently when centrifuged through a sucrose gradient (51). The sedimentation properties of the particles from *Mercenaria* ganglia more closely resembled those of 5-HT particles from the dog duodenal mucosa (52) than those of the mammalian brain (53, 54). Nevertheless, 5-HT was released in a quantitatively similar fashion to ACh when *Mercenaria* particles were subjected to changes in pH, tonicity, and increased temperature. Thus, there appear to be some similarities in the binding mechanism of the two substances in this situation.

From the results of an electron microscopic study of different homogenate fractions of *Anodonta*, Zs-Nagy et al. concluded that 5-HT is not associated with dense-cord vesicles, as some workers had tentatively suggested. Rather, they speculated that the amine was associated with endoplasmic reticulum vesicles. Cottrell & Maser (55) also concluded that 5-HT is not associated with large dense-centered vesicles of dimensions 1000–2000Å in diameter. However, electron micrographs of fractions containing 5-HT particles in a considerably purified form did not provide any evidence that the amine is associated with large endoplasmic reticulum vesicles as suggested by Zs-Nagy et al. Cottrell & Maser consider that the subcellular localisation of 5-HT in molluscan neurones is still an open question.

Release of 5-HT during nerve stimulation and effects of blocking agents.— 5-HT has been detected both chromatographically and spectrofluorometrically in heart perfusates of *Helix pomatia* following stimulation of the cardio-

excitatory nerves (56), but was not detected in perfusates from unstimulated hearts. The amounts of amine collected ranged between 5 and 10 μg for ten periods of stimulation of three minutes duration. The quantity seems particularly high in view of the observations that gastropod ganglia rarely contain more 5-HT than 10 μg/g tissue (46, 57). In the heart of *H. aspersa*, the concentration amounts to only about 3.0 μg/g wet wt. It seems probable, therefore, that 5-HT is synthesized on demand by the cardio-excitatory nerves. Rózsa & Perényi (56) also observed a second substance which may be derived from arginine in heart perfusates. However, this substance was also detected in perfusates of non-stimulated hearts and it was not considered further as a transmitter.

Previously, Rózsa & Graul (58) had shown that cardio-excitation resulting from stimulation of the intestinal nerve is chemically mediated. This point was demonstrated with an arrangement of donor and recipient hearts, similar to that devised by Loewi. More evidence in favour of 5-HT being the transmitter released by the cardio-excitatory nerves was based on the finding that BOL and chlorpromazine not only antagonised the effect of applied 5-HT, but also antagonised neuronally evoked excitation. The results obtained with BOL are in good agreement with those obtained in 1963 by Loveland (59) on the *Mercenaria* heart. However, since BOL affects nerve conduction in *Aplysia*, the value of the drug in testing for serotonergic transmission in molluscs needs to be re-examined. The possibility that other derivatives of LSD also interfere with nerve conduction casts doubts on the value of UML for such experiments.

During the last two or three years most of the major obstacles for acceptance of 5-HT as a neurotransmitter in molluscs have been overcome and it appears now that this amine serves a similar function in annelids. Nevertheless,
feld (60), a transmitter role for 5-HT has not been unequivocally proved, even in the molluscs. However, the value of these criteria may be questioned since the evidence required to fulfill some of them is not only unavailable, but possibly not obtainable under any foreseeable circumstances (61) for the majority of suspected transmitter compounds. Kerkut (62) has reviewed the animal kingdom for distribution of suspected transmitter substances and also concludes that we have no reason to exclude substances because they do not fit a vast range of different criteria. Another consideration is that not all transmitter substances may act identically to the one clearly defined transmitter, ACh. For instance, is it unreasonable to suppose that substances released from nerve endings could act in the same way as a transmitter on elements very close to its release site and also act as a neurosecretory substance on distant cells? Also, how should we classify a substance which is released similarly to a transmitter and causes similar effects by acting intracellularly as do some hormones, on the post-synaptic cell instead of on the postsynaptic membrane? Clark (63) has recently pointed out that there "is always a danger that we may forget that our systems of classification are invented, arbitrary, and provisional."

5-HT in non-nervous tissues.—The occurrence of 5-HT in comparatively and sometimes extremely high concentrations in non-nervous tissues of some invertebrates suggests that it has functions in these animals other than mediating nerve impulses. 5-HT was discovered in the toxin-producing salivary glands of *Octopus vulgaris* in 1953 (64, 65), and it has since been detected in the venom and venom apparatus of a wide variety of species (66). It has also been found in excretory organs [green gland of crabs (46)], in the protozoans *Crithidia* and *Tetrahymena* [at concentrations of 0.13 μg and 0.64 μg/g wet wt, respectively (67)], and in the spermatophore sac of *Octopus vulgaris* (68). Mann (68) found between 120 and 1960 μg/g of the amine in the glandular region of the spermatophore sac, the amount depending on the age of the animal. Thus, 5-HT may play a role in the ejaculatory process during mating, either in aiding discharge of spermatophores from the sac or in assisting passage of sperm to the site of fertilization by stimulating contractions of the male or female reproductive tracts.

Fowler & Goodnight (69) have indicated a cyclic production of 5-HT in pooled samples of gut and brain tissue of the opilionid arachnid *Leiobunum logipes*. The maximal concentration of 5-HT occurs at 0200 hrs each day, while maximal locomotory activity occurs at 2200 hrs. There is some correlation between the rate of increase of the concentration of the amine and the onset of increased activity. However, the reliability of the assay method utilised in this study seems doubtful.

Catecholamines

Catecholamines have been detected in representatives of several invertebrate phyla (Table 1). DOPAmine, noradrenaline, and adrenaline have all been found. The very high concentrations of DOPAmine in nervous tissue of certain molluscs are particularly noteworthy. High concentrations of one or more catecholamines have also been reported in the entire insect body at certain developmental stages, in insect eggs, and in the radial nerves of a starfish and a sea urchin.

Data on the presence of noradrenaline and adrenaline in gastropod and lamellibranch molluscs are conflicting. For instance, Dahl, Falck, von Mecklenburg, Myhrberg & Rosengren (37) were unable to detect either amine in ganglia of *Anodonta piscinalis*, whereas Puppi (70) claimed levels of 0.38 μg/g of noradrenaline and 4.0 μg/g of adrenaline in the visceral ganglion of *Anodonta cygnea*. Recent chromatographic studies indicate that noradrenaline is a constituent of *Spisula solida* ganglia and also the brain of *Eledone cirrhosa* (71). DOPAmine, however, is the major catecholamine in each case.

Effects and possible modes of action.—The effects of catecholamines on molluscan neurones have been demonstrated independently by Kerkut & Walker and by Gerschenfeld and co-workers. Individual neurones of *Helix aspersa* responded to comparatively low concentrations of the amines in the medium bathing the ganglia. Generally, adrenaline increased firing rate of neurones at concentrations of 10^{-7} g/ml or above, while noradrenaline de-

TABLE I

CATECHOLAMINES IN INVERTEBRATE SPECIES[a]

Group and Species	Tissue	DOPAmine	Noradrenaline	Adrenaline	Ref.
Protozoa					
Crithidia fasciculata	Whole		0.1–0.2	0	85
Tetrahymena pyriformis	Whole		0.25–0.35	0.13–0.15	85
Annelida					
Lumbricus terrestris	Ganglionic chain		0.32	1.4	83
	Whole		0.015	0.003	90
Mollusca					
LAMELLIBRANCHIA					
Anodonta piscinalis	Cerebral ganglia	8.12–11.64			36, 37
	Visceral ganglia	18.70–19.21			36, 37
	Pedal ganglia	47.16			37
Anodonta cygnea	Cerebral ganglia		0.56	5.36	70
	Visceral ganglia		0.38	4.0	70
Mercenaria mercenaria	Pooled ganglia	137–405			91
Modiolus modiolus	Pooled ganglia	35–118			91
Ensis directicus	Pooled ganglia	31–49			91
Mya arenaria	Pooled ganglia	96			91
Mytilus edulis	Pooled ganglia	35			91
Aequipecten irradians	Pooled ganglia	60–88			91
Spisula solidissima	Pooled ganglia	26			91
Spisula solida	Pooled ganglia	80–100	10–12		71
GASTROPODA					
Melongena corona	Pooled ganglia	51–82			91
Lunatia heros	Pooled ganglia	12–38			91
Busycon canaliculatum	Pooled ganglia	6–22			91
Buccinum undatum	Cerebral ganglia	17.3			37
Helix pomatia	Pooled ganglia	12.4			87
	Cerebral ganglia	7.25			37
Helix aspersa	Pooled ganglia	5.5			86
CEPHALOPODA					
Octopus vulgaris	Posterior Salivary glands		1–3		92
Octopus apollyon	Posterior Salivary glands	Present	Present		93
Eledone cirrhosa	Brain	16–26	4–10		71
Arthropods					
CRUSTACEA					
Carcinus maenas	Pooled ganglia	7.3			86
	Pooled ganglia	1.5–3.0			71
INSECTA					
Tenebrio molitor	Whole larvae	10–15	1.3–2.2	0.021–0.061	83
Apis mellifica (workers)	Whole imagos	5–10	0.75	0.05	83
	Whole mature pupae	2–4	0.22	0.026	83
	Whole immature pupae	?	0.045	0.049	83
	Whole larvae	2–4	0.3	0.010	83
Pieris brassicae	Whole larvae		0.015		83
Teleogryllus commudus	Eggs	Present			80
Gryllus bermudiensis	Eggs	Present			80
Gryllodes sigillatus	Eggs	Present			80
Melanoplus bivittatus	Eggs	Up to 2.83/egg			80
Acheta domesticus	Eggs	Up to 93.5			79
Echinodermata					
Asterias rubens	Radial nerves	6–16	1–4		71
Echinus esculentus	Radial nerves	5–14	3–7		71

[a] Except where stated otherwise, amounts are expressed in μg/g weight of fresh tissue.

creased firing rate of most neurones studied although it excited some. On most neurones DOPAmine decreased the action potential firing rate. Great sensitivity towards DOPAmine was shown, inhibition sometimes being accomplished by as little as 10^{-11}g/ml (72).

Gerschenfeld & Tauc's studies (7) on *Aplysia depilans* neurones showed that both adrenaline and noradrenaline excited H cells and inhibited depolarised (D) cells (neurones which according to these workers' classification do not receive an inhibitory input). Noradrenaline was about ten times more active than adrenaline, and the effect of both amines was approximately ten times greater on the D cells than the H cells. More recently, Gerschenfeld (73) discovered a particular type of D cell (D inhi cells) which does receive a direct noncholinergic inhibitory input. Gerschenfeld & Chiarandini (74) found that the inhibitory postsynaptic potentials result from a selective increase in permeability to K. Of all the naturally occurring substances tested, DOPAmine was the most active in inhibiting the D inhi cell. The α-adrenergic blocking agents dibenamine and dihydroergotamine did not affect the inhibitory input. The response to DOPAmine after this treatment has not been described. In all of these electrophysiological studies, it is interesting that the one catecholamine definitely detected in the gastropod nervous tissue, DOPAmine, is more potent than either noradrenaline or adrenaline.

The eye of *Limulus* exhibits lateral inhibition (75) and perhaps self-inhibition (76). The pharmacology of these events has been investigated in a preliminary way by Adolf (77), who found that adrenaline, noradrenaline, and other agents excite electrical activity in the eye (eccentric cell) causing depolarisation and increased discharge frequency.

With regard to a possible transmitter role for catecholamines in insects, Smalley (78) has shown that neuronal excitation of the light organ of the firefly, *Photinus pyralis*, may be mediated by an adrenergic mechanism. Noradrenaline, adrenaline, and amphetamine induced glowing of the organs *in situ*. Pretreatment with reserpine or denervation of the organ blocked the response to amphetamine but not to noradrenaline. Electrical stimulation of the light organ resulted in a fast flash followed by a slow one. Only the slow flash was thought to be under direct neuronal control. The slow response to electrical stimulation was blocked by yohimbine and amphetamine, but reserpine blocked both responses. The effect of reserpine on the fast flash response to electrical stimulation was interpreted as an action of the drug on glycolysis in the light organ itself. Bioassays indicated the presence of a mixture of noradrenaline and adrenaline or another substance(s) with effects similar to those given by a mixture of the two on the rat uterus and colon preparations.

Involvement of DOPAmine in the tanning of the insect cuticle is discussed below. Furneaux & McFarlane (79, 80) have suggested that there may be a relationship between the occurrence of catecholamines in insect eggs and their ability to absorb water.

Cellular localization.—The neuronal localization of catecholamines has been demonstrated by Falck's fluorescence technique in molluscs (36, 37,

81); in sea anemones (81a); in annelids (40–42, 81); and in insects (82). On histological grounds, Dahl et al. concluded that at least some of the neurones containing catecholamines observed in molluscs are sensory in nature. Clark (40) and Rude (41) suggest the same for the annelids, *Nephthys* and *Lumbricus*. Fluorescence, characteristic of catecholamines in molluscan ganglia, has generally been assumed to represent the localization of DOPAmine alone. However, recent studies indicate that some fluorescence due to noradrenaline may have been observed in some of the histochemical preparations. Since noradrenaline has been clearly demonstrated in annelid nervous tissue (83), the primary catecholamine fluorescence of neurones in *Nephthys* and *Lumbricus* is probably at least partly due to this amine. However, Rude (41) has also recently reported the presence of DOPAmine, as well as noradrenaline, in *Lumbricus*.

A different histochemical method (normally used for adrenal medulla) has been used by Lentz (44) to show catecholamines in spindle-shaped, bipolar and multipolar cells in the mesenchyme of the sponge *Sycon ciliatum*. Adrenaline staining was blocked by pretreatment with α-methylDOPA but not affected by reserpine; noradrenaline staining was inhibited by reserpine but not α-methylDOPA. The specificity of the methods used, however, seems to warrant a further investigation with the fluorescence technique, particularly because it is uncertain whether sponges have a nervous system (84).

Metabolism of catecholamines and effects of drugs.—Studies on catecholamines in protozoans have recently been presented by Janakidevi, Dewey & Kidder (85). Adrenaline was found in one and noradrenaline in both species studied. Phenylalanine, tyrosine, and DOPA were metabolised to catecholamines in *Tetrahymena pyriformis*, but only the latter two were precursors in *Crithidia fasciculata*. In *Crithidia*, noradrenaline is metabolised to normetanephrine and 3-methoxy-4-hydroxymandelic acid. It has been suggested that the role of catecholamines in these protozoans is to regulate carbohydrate metabolism.

Reserpine depletes DOPAmine from nervous tissue of molluscs (37, 71, 86), and both DOPAmine and noradrenaline from starfish radial nerve (71). Catecholamine fluorescence disappears slowly from molluscan ganglia treated with relatively large doses of reserpine [*Anodonta* and *Helix* (37)].

Homogenates of *Helix pomatia* ganglia and hearts can convert DOPA to DOPAmine (87), and injection of DOPA into intact *H. aspersa* increases ganglionic levels of DOPAmine (86). In the latter case, it was also shown that α-methylDOPA reduces the content of DOPAmine in the ganglia. Thus, it appears that DOPAmine is formed from DOPA by decarboxylation. Little is known of the further metabolism of DOPAmine in molluscs. However, the monoamine oxidase inhibitor nialamide is reported to have little or no effect on the intensity of catecholamine fluorescence in cell bodies in *Anodonta* ganglia (whereas 5-HT fluorescence was increased) (37) and iproniazid did not increase the yield of DOPAmine formed from DOPA in the experiments of Cardot with snail ganglia and heart homogenates. Thus,

monoamine oxidase does not appear to be important in the metabolism of DOPAmine in these tissues.

The work of Sekeris, Karlson and colleagues (88, 89) with insects has shown that DOPAmine is a precursor of N-acetylDOPAmine, ultimately involved in the tanning of the cuticle. Apart from the active amines detected by Östlund (83), these workers also found N-acetylDOPAmine, tyramine, and N-acetyltyramine in various insect species. In young larvae, tyrosine was metabolised to tyramine or to *p*-hydroxyphenyl pyruvic acid, which in turn was eventually reduced to *p*-hydroxyphenyl propionic acid. Tyrosine could also be converted to tyramine and then to N-acetyltyramine in the last larval stages, but in these older larvae, tyrosine was mainly hydroxylated to DOPA and then decarboxylated to the amine.

DOPAmine was converted to its N-acetyl derivative and incorporated into the cuticle or conjugated to form N-acetylDOPAmine-4-O-glycoside. Another minor route of DOPAmine metabolism was to dihydroxyphenyl acetic acid. The formation of noradrenaline and adrenaline was not studied in detail, but there was some evidence to suggest that noradrenaline can be formed from DOPAmine in *Tenebrio*. Sekeris & Karlson suggest that the use of DOPAmine for tanning of insect cuticle accounts for the high DOPAmine content in this group and also for fluctuations in concentration of this amine during the developmental stage. It is also interesting that there are two pathways for forming DOPAmine in insects, from tyramine and from DOPA. Synthesis of DOPA decarboxylase in epidermis of larvae about to pupate is induced by ecdysone (89) (see below).

Acetylcholine

Bioassay and effects.—The value of the isolated molluscan heart, particularly that of the clam *Mercenaria mercenaria*, for the bioassay of ACh has been recognised for some time (94, 95). Details of the procedures used in the bioassay of ACh on hearts of *Protothaca* and *Tapes* have been described in great detail by Florey (96), but the use of *Mercenaria mercenaria* seems best documented. Such preparations have been used extensively in the United States over the last decade, but relatively infrequently in Great Britain, probably because of variability among British lamellibranchs and the lack of detailed information on any one species. However, recent experience has shown that the heart of *Mya arenaria* [used by Gaddum & Paasonen (97) for 5-HT estimation] is an extremely satisfactory preparation if carefully prepared and pretreated with BOL or ergometrine (98).

The overall effect of ACh is inhibitory to most bivalve hearts (99, 100). However, hearts from many of these species also show an excitatory response, usually of tone or amplitude of beat, to very high or very low doses of ACh, or in the presence of benzoquinonium. With a few species (*Mytilus galloprovincialis* and *Modiolus modiolus*) the excitatory effect predominates (100,101)

It has generally been considered that ACh is not involved in neuromuscular transmission in insects, because among other things ACh is ineffective on the neuromuscular junction of insect skeletal muscle (102). However,

McCann & Reece (103) have recently shown that although ACh has no effect when topically applied to muscle fibres of the fly (*Sarcophaga bullata*), ACh, as well as glutamic acid, causes depolarisation of the fibres when injected intra-abdominally. Eserine injected by the same route caused repetitive firing. Furthermore, Larsen, Miller & Yamamoto (104) have reported that *d*-tubocurarine caused flaccid paralysis in several insect species. Previous studies (102) suggest that these effects of ACh and curare are mediated at sites other than the neuromuscular junction. Although curare may act centrally, McCann (105) has concluded that curare can cause "vertebrate-type neuromuscular block" in flight muscle fibres of the fly, *S. bullata*, when the drug is injected intra-abdominally. This conclusion was reached on the basis of a decrease in size of the excitatory junction potentials (e.j.p.) in individual muscle cells following curare injection. Examination of McCann's published recordings shows that apart from e.j.p. diminution, action potential size was also decreased with curare, indicating perhaps an increase in membrane conductance. Thus, the effect of curare might be explained by an increased potassium permeability (assuming resting potential here is K-dependent) of the muscle-fibre membrane and not by a direct action on the receptors. Other sites of action are also possible. It would be very interesting to know more about the precise action of curare at the insect neuromuscular junction, because if curare is causing blockade as envisaged by McCann, it would mean that either ACh is the transmitter at these junctions or that curare can block non-cholinergic synapses.

Receptors.—The ventricle of the *Anodonta* heart recovers from inhibition brought about by ACh application within one to three minutes, even in the continued presence of ACh (106). This recovery phenomenon is thought to be due to an inactivation of the ACh receptors of the ventricle by the release of a substance(s) from the heart. Cholinesterase was not detected in the tissue so it seems unlikely that the ACh is hydrolysed. A similar mechanism of terminating ACh inhibition on the *Helix pomatia* heart has been proposed by Rózsa (107). Repeated stimulation of the cardio-inhibitory nerve led to gradual but dramatic diminution of degree of inhibition. Rózsa considers that such receptor changes may be induced by the action of a substance other than ACh. Small amounts of Substance X (57, 108) which do not cause immediate excitation of the heart of *Mercenaria mercenaria* can interfere drastically with ACh inhibition (51).

Specific differences in receptor sites are indicated by McLennan & York (109) in the stretch receptors of *Orconectes virilis* and *Procambarus leniusculus* These sensory cells are thought to possess a single type of ACh receptor on the membrane but the pharmacological properties of the receptor sites appear to differ in the two species.

Changes in the disposition of cholinoreceptors in skeletal muscle during the course of evolution have been postulated recently by Khromov-Borisov and Michaelson (110). On the basis of the effects of different cholinomimetic and cholinolytic drugs, with differing numbers of C atoms between charged parts of the molecules, it was suggested that the C-16 structure (anionic

points of different cholinoreceptors separated by a distance approximating the length of a chain of 16 C atoms) appeared earlier in the course of evolution than the C-10 structure. The C-10 structure may have appeared in conjunction with a tetrameric receptor complex (i.e. with the four anionic points of the individual receptors arranged at the corners of a square and with the cationic heads arranged along the diagonals of the square).

Occurrence and neuronal localization of ACh and cholinesterase.—Recent studies on the distribution of ACh and cholinesterase include those of Webb, Dettbarn & Brzin (111) on the squid, *Loligo pealii*, and those of Loe & Florey (112) on *Octopus dofleini*. In the squid, ACh and cholinesterase as well as choline acetylase were demonstrated in the giant axon, but much higher concentrations of all three substances were found in the distal (giant) synapses of the stellate ganglion. The squid retina also contains ACh. Very high concentrations of both ACh (236 μg/g wet wt) and cholinesterase were detected in the optic ganglia of the *O. dofleini*. Other ganglia of the CNS were also rich in these substances. There was a correlation between cholinesterase activity, which appeared to be due to a specific acetylcholinesterase, and ACh content of the different ganglia. Loe & Florey pointed out that the *O. dofleini* optic ganglia should be an excellent preparation for investigating the subcellular localization of ACh. The concentration of ACh in this organ is 50 to 200 times greater than that in the mammalian brain.

Florey & Winesdorfer (113) have been able to separate ACh-rich particles from *Octopus* brain tissues by means of ultracentrifugation. The ACh-rich fraction contained nerve terminals, which themselves contained synaptic vesicles. They were unable to isolate synaptic vesicles from the nerve endings. Although ACh was released under hypo-osmotic conditions, there was almost no disruption of nerve endings.

Particulate binding of ACh has also been studied in the ACh-rich nervous tissue of *Mercenaria mercenaria* (51, 55) and the starfish, *Asterias rubens* (114). In both cases, results were consistent with localization of ACh in synaptic vesicles. Particulate ACh from both sources was released into solution by heating or by lowering the osmotic strength or pH of the suspending medium, as with particulate ACh from mammalian brain (115). Neuropile areas of the starfish radial nerve with large numbers of small vesicles, similar in size and shape to synaptic vesicles of the mammalian brain (116), contained larger amounts of ACh than other areas with fewer vesicles. The same areas also showed relatively high levels of an acetylcholinesterase.

Holothurian and other echinoderm muscles are sensitive to applied ACh (117) and Ferguson (118) has indicated that ACh induces contractions in muscles of the digestive glands of *Echinaster spinulosus*, at low concentrations whereas γ-aminobutyric acid (GABA), adrenaline, noradrenaline, and 5-HT were ineffective.

Histochemical localization of nonspecific cholinesterase has been demonstrated in the ganglia of *Helix pomatia* by Zs-Nagy & Salánki (119). The enzyme, which was mainly concentrated in the neuropile of inactive animals, hydrolysed butyryl choline more rapidly than ACh. However, Dettbarn &

Rosenberg (120) have provided biochemical evidence for the presence of an acetylcholinesterase in *Aplysia*. A specific acetylcholinesterase has also been demonstrated in the intestinal juice of *Helix pomatia* and some of its properties have been investigated (121). Although Zs-Nagy & Salánki (119) were unable to obtain histochemical evidence for any cholinesterase activity in *Anodonta cygnea*, Salánki, Hiripi & Lábos (122) managed to detect specific cholinesterase, which hydrolysed acetylmethylcholine as well as ACh, in homogenates of ganglia of the same species.

Periplaneta americana (cockroach) ventral nerve cord is particularly rich in acetylcholinesterase (123). Exogenously applied ACh can be hydrolysed rapidly at the periphery of the cord (124). An eserine-sensitive cholinesterase was demonstrated in glial membranes at the periphery as well as in more central regions of ganglia. Smith & Treherne (125) suggest that the high level of cholinesterase at the periphery of the ganglia and also that associated with neurones of the ganglia, may explain the ineffectiveness of directly applied ACh in modifying electrical activity of insect ganglia (126). The connective tissue layer does not act as a simple diffusion barrier as was previously thought, because ACh can readily penetrate ganglia and other parts of the cockroach CNS in eserinised preparations (127). In two species of insects it has been shown that neuronal ACh is particle-bound (128, 129). Smith & Treherne (125) have observed a close association of small synaptic vesicles and an eserine-sensitive cholinesterase in synaptic areas of the neuropile of *Periplaneta* ganglia.

The role suggested for the cholinergic system in regulation of insect diapause (130) has recently been reinvestigated by Mansingh & Smallman (131). Electrical activity, cholinesterase, and choline acetylase activities were all detected in brains and ganglia throughout diapause. Furthermore, similar patterns of changes in activity of the enzymes during development were seen in both diapausing and non-diapausing forms of the same species of insects, as well as in non-diapausing species. It was, therefore, concluded that changes in the cholinergic system resulted from the general process of metamorphosis rather than being unique to diapause.

Certain non-neuronal tissues of insects contain appreciable, and sometimes extremely high, concentrations of ACh (123). It has recently been proposed that ACh may be a growth factor in early larval development of the silkworm (132). However, it was not made clear whether ACh or choline alone is required for growth stimulation. Most insects require choline for growth (133).

Mechanism of action.—The elegant studies on the mode of action of ACh on hyperpolarized (H) and depolarized (D) cells in the ganglia of gastropods by groups led by Tauc (134), Gerschenfeld (60), and Kerkut (62) have been carried further by Kerkut & Meech (135). Using chloride sensitive glass microelectrodes, they found that the Cl concentration of the cytoplasm of a D cell is more than twice that of an H cell in the parietal ganglion of *Helix aspersa*. This suggested that different responses to ACh of such neurones might be related to changes in membrane properties required to bring them

to chloride equilibrium potential. Replacement of chloride with acetate in the bathing medium of the ganglion had little effect on the D cell response, whereas in sodium-free media, ACh did not bring about depolarization. Thus, sodium rather than chloride ions appear significant in depolarization of D cells by ACh. This contrasts with the i.p.s.p. and ACh-induced hyperpolarization of the H cell where chloride is the major, if not the only, ion responsible for changes in the membrane potential (136).

Chloride-dependent hyperpolarization also follows application of ACh to a giant neurone in the esophageal ganglion of the nudibranch *Onchidium* (137). The same cell was also hyperpolarized with glutamate, but in this case the hyperpolarizing current was largely due to changes in K permeability. Thus, it appears that two different mechanisms of generating inhibitory potentials can exist in the same cell.

Conclusive evidence showing that different branches of one neurone can cause excitation at one synapse and inhibition at another has recently been presented by Kandel, Frazier & Coggeshall (138). Different specified neurones in the abdominal ganglion of *Aplysia californica* were impaled and recordings made simultaneously from two or more of such cells. Synchronous postsynaptic potentials of opposite sign were seen between certain combinations of cells, suggesting that the opposite synaptic actions were mediated by the same interneurone. When it was depolarized, hyperpolarizing potentials were recorded in some cells simultaneously with depolarizing potentials in another. Constant and short latency of the response, and the all-or-nothing nature of the potentials, strongly suggested that there were unitary monosynaptic connections between the interneurone and cells from which recordings were made. This scheme was confirmed anatomically. Cells which were synaptically hyperpolarized were also hyperpolarized with applied ACh, and ACh depolarized the cell which was depolarized synaptically. Furthermore, curare blocked both types of response resulting from activation of the interneurone. Therefore, ACh in one neurone can excite at one type of synapse and inhibit at another. This work is the first definite demonstration of this phenomenon. Whether it is a process restricted to nervous systems in certain invertebrates or is more generally encountered remains to be shown (139).

Histamine

A recent survey by Mettrick & Telford (140, 141) shows that histamine is present in at least one representative species from each of ten phyla studied. Concentrations of histamine comparable to those found in mammalian tissues, which are considered to be relatively rich in the amine, were found in the coelenterate *Aiptasia tagetes*, the trematode *Mesocoelium monodi*, and the echinoderm *Echinaster echiophorus*.

The parasitic forms are most interesting. *Mesocoelium* had the highest histamine content (58.3 μg/g body weight), and some histamine was found in all the parasites studied from the phyla, *Acanthocephala*, *Aschelminthes*, and *Platyhelminthes*. These parasitic forms possessed a significantly greater amount of histamine than corresponding free living species, and with one

exception, there was an inverse relationship between histamine content of parasite and host tissue.

The formative enzyme histidine decarboxylase was found in appreciable quantities in *Aiptasia* and *Mesocoelium*, and was active over a large pH range. Histamine in parasitic forms may be derived from the surrounding host tissues since histidine decarboxylase was detected only in *Mesocoelium* among the parasites. However, a high concentration of histidine is present in liver fluke (*Fasciola hepatica*) tissues (142).

Large amounts of histamine are also reported for the firefly, *Luciola italica* (average 210 μg/g body weight), the head containing more than the other parts of the body. N-acetylhistamine was also found in whole body extracts at a concentration of 10 μg/g (143).

An anomalous situation with regard to the presence of histamine in the crustacean *Carcinus maenas* has arisen as a result of the work of Kerkut & Price (144) and Belamarich & Clay (145). The former workers report a level of 1212 μg histamine /g cardiac tissue in this crab—the highest value in any invertebrate apart from certain tissues associated with venom production (66). Other organs said to have large amounts of histamine were the pericardial organs, while blood, muscle, gill, and other areas contained much smaller amounts. Belamarich & Clay, on the other hand, were unable to detect amounts of histamine greater than 1 μg/g in the hearts of *Carcinus maenas*. The reasons for this discrepancy may lie in the sources of the animals used. Kerkut & Price used animals from Southampton Water, England, and Belamarich & Clay, from New England, U.S.A.

An earlier report by Ungar, Ungar & Parrot (146) suggests that somewhat lower concentrations of histamine are present in the crustacean heart. These investigators also found relatively high levels of histamine in nervous tissue of *Octopus*. Bertaccini (147) reported 1.7 to 4.0 μg histamine/g of *Eledone moschata* nervous tissue.

Less is known concerning the occurrence, distribution, and metabolism of histamine in invertebrates than is known for mammals. More work is advocated since investigations of the comparatively rich sources available among invertebrates may help to elucidate the biological action(s) of this enigmatic substance.

Amino Acids

γ-Amino butyric acid (GABA).—Takeuchi & Takeuchi (148, 149) have shown that GABA exerts its inhibitory effect on presynaptic excitatory fibres similar to its action on muscle fibres (150, 151); that is, by increasing permeability to chloride ions. In chloride-deficient media the presynaptic inhibitory action of GABA, shown by e.p.s.p.'s in muscle of *Cambarus clarkii*, was reduced. The normal diminution of amplitude and time course of spontaneous junction potentials after GABA treatment was reversed in Cl-free media. The frequency of spontaneous e.p.s.p.'s was transiently increased. The GABA receptors are localized on the excitatory presynaptic terminals, not on the inhibitory terminals.

The possibility that GABA might exert direct inhibitory effect on crustacean muscle fibres by competing with L-glutamate for its receptors had been considered by Fatt & Katz (152), but Takeuchi & Takeuchi (148) have since shown that there is no appreciable competition for glutamate receptors between these two substances. It seems probable, therefore, that specific receptors exist for both substances on the crustacean muscle fibre.

Results of experiments by Usherwood & Grundfest (153) with GABA and picrotoxin on leg muscles of the locust (*Schistocerca gregaria*) and grasshopper (*Romalea microptera*) indicate that GABA acts as the transmitter substance released onto muscle fibres from peripheral inhibitory nerves in insects. Furthermore, a potentiating effect of picrotoxin on the response of locust muscles to stimulation of slow nerve fibres suggests that perhaps GABA also acts presynaptically on slow fibre endings in this group, as in crustaceans.

GABA antagonised the excitatory effect of L-glutamate on leg muscles of *Periplaneta americana* (154) but did not affect contraction resulting from application of ACh. Indeed, ACh could abolish the effect of GABA. The amplitude and frequency of miniature end plate potentials of cockroach leg muscle decreased when GABA was allowed to diffuse from micropipettes onto the muscle fibres (155). Whether either of these effects was due to a presynaptic action of GABA was not shown.

Thus, evidence continues to accrue that GABA is a transmitter released from peripheral inhibitory fibres of insects. Other evidence supporting this view is presented in tabular form by Kerkut & Walker (155). As yet, however, there has been no convincing demonstration that GABA is released from inhibitory nerves upon stimulation.

The specific sensitivity of neurones to GABA is well demonstrated by the responses of snail giant cells. Kerkut & Walker (72) showed that some ganglion cells of *Helix aspersa* are excited, and others inhibited by GABA. Gerschenfeld & Lasansky (156), working on *Helix pomatia* and *Cryptomphallus aspersa*, showed several different effects of GABA on various cells. Some cells were excited by glutamate and inhibited by GABA, whereas others were also excited by GABA. Others were excited by glutamate and unaffected by GABA, and yet others were inhibited by both GABA and glutamate.

It is apparent that every nerve cell must be investigated separately for its responses to topically applied pharmacological agents to determine the spectrum of its sensitivities. The concept of anatomical and chemical addressing developed by Horridge (157) gains support with such investigations.

Glutamic acid.—Glutamic acid is a commonly occurring metabolite in animal tissues. Recent work indicates that it may be of importance at certain nerve-muscle junctions.

The nervous tissue of arthropods contains large amounts of glutamic acid (102), as does the muscle of crustaceans [0.01–0.1 mg/g *Carcinus* (158)]. Enzymes which biotransform glutamic acid have also been detected in crustaceans and insects (159–161).

In relatively low concentrations, L-glutamic acid depolarizes leg muscle

preparations of crustaceans (162), and insects (155). In the crayfish, the neuromuscular junctional areas are most sensitive to locally applied glutamic acid (162).

The frequency of m.e.p.p.'s of insect muscle fibres is increased by glutamic acid (155, 163). Usherwood & Machili (163), working with very dilute solutions of the amino acid (10^{-12}–10^{-9} g/ml), did not observe any change in amplitude of m.e.p.p.'s with increased rate of discharge. They, therefore, suggested that the amino acid facilitated release of transmitter from the excitatory presynaptic terminals. With higher concentrations of glutamic acid, Kerkut & Walker (155) observed an increase in amplitude, as well as frequency, of the m.e.p.p.'s. Presumably, the amplitude effect reflected a facilitatory action of the drug on the postsynaptic membrane. Usherwood & Machili (163) treated a locust muscle preparation with glutamic acid decarboxylase and showed a decrease in nerve-evoked muscle contractions which was reversed by washing. The enzyme inhibitor phenylhydrazine potentiated mechanical responses of the muscle. Both observations favour the opinion that glutamic acid may be the excitatory transmitter in this preparation. The responses of insect muscle to motor nerve stimulation are affected by the perfusion of the preparation with glutamic acid, aspartic acid, and ACh (154). Each of these substances potentiated amplitude and duration of the contractions, with glutamic acid being most effective and the L-isomer being ten times more active than the D-isomer. Receptor sites for transmitter molecules are, therefore, specific for a particular compound and for particular isomers of that compound.

Glutamic acid also affects certain molluscan muscles and neurones. Contraction of the *Helix* pharyngeal muscle occurs when the circumesophageal ganglionic complex is stimulated, and the contraction is enhanced by the addition of L-glutamate (2×10^{-8} g/ml) (164). Aspartic acid also increases contraction but the threshold is much greater (165). Kerkut et al. (164) also showed that a ninhydrin positive substance was released in this preparation, as well as in crab leg and cockroach preparations, that was identified as glutamic acid. The amount released was proportional to the number of stimuli delivered. Small quantities of glycine were also detected in these perfusates. The evidence suggests that glutamic acid is important in nerve-muscle transmission though the amounts of substances available from this source suggest that release may take place from areas other than, or in addition to, nerve endings. Baker (166) has detected glutamic acid released from active nerves.

Neurones that respond to the application of glutamate have been reported in *Helix pomatia* and *Cryptomphallus aspersa* [threshold circa 10^{-7} g/ml (156)]. Some neurones were depolarised and excited, others hyperpolarised and inhibited. In *Onchidium* (137), one type of neurone was hyperpolarised by glutamate, as well as by ACh (see above).

The synapses of the stellate ganglion, between the giant axons from the brain and neurones that serve the various areas of the mantle, have been investigated by Miledi (167), Katz & Miledi (168), Miledi & Slater (169),

and Bloedel, Gage, Llinas & Quastel (170). The consensus is that transmission across these synapses is chemical, that packages of transmitter are released spontaneously from the nerve endings and that tetrodotoxin does not alter the quantal nature of transmitter release during depolarisation of the presynaptic ending, nor does it affect the postsynaptic response to transmitter. It has been tentatively suggested by Miledi (167) that L-glutamate may be the transmitter, but confirmatory evidence is still required.

Transport of glutamate occurs from the snail brain along nerve trunks to the vicinity of the pharyngeal retractor muscle (171). Movement of radioactive (C^{14}) glutamate from the CNS to muscle took only 20 minutes, while movement of labeled material in the opposite direction took several hours.

Active Peptides, Hormones, and Neurosecretory Substances

α-Ecdysone, the more active of the two forms of the insect moulting hormone, was the first insect hormone to be isolated and identified chemically (172, 173). The observations of Clever & Karlson (174) created great interest in the mode of action of this substance. These workers showed that small amounts of the hormone caused "puffing" in salivary gland chromosomes from *Chironomus tentans* larvae. Evidence indicates that changes in chromosomes are involved in the effects of the hormone on DOPA decarboxylase activity, which is involved in the process of sclerotisation and which is normally increased at pupation. These effects, and those on pupation, have been reviewed recently by Sekeris (175) and Karlson & Sekeris (89, 176). However, it has not been established that an interaction of ecdysone with the chromosomes represents the primary event in the sequence of events leading to pupation.

Kroeger (177, 178) believes that ion movements through the membrane of the cell are involved in the DNA response to ecdysone. He found that by changing the concentrations of inorganic ions of media bathing salivary glands, he was able to obtain chromosomal puff development. These effects are invoked by several different ions. Clever (179) on the other hand was unable to mimic normal puff patterns of giant chromosomes with ions during studies on another species.

Karlson & Sekeris (89, 176) do not favour Kroeger's theory since, for one thing, they found that ecdysone can double RNA synthesis in isolated nuclei. Previously, it had been shown (180) that tritiated ecdysone is preferentially taken up by nuclei of epidermal cells. Karlson & Sekeris prefer to consider the action of ecdysone to be a direct interaction of the hormone with DNA repressor molecules, resulting in unmasking of certain areas of the DNA molecules.

The parasitic complement of the guts of insects also seems to be influenced by ecdysone. As moulting draws near, gametogenesis occurs in the symbiotic flagellates of wood-feeding roaches. Gametogenesis also takes place under the influence of ecdysone administered to the host (181). The response of the flagellate population may be directly due to the insect hor-

mone, or to some intermediate metabolite formed during the insect response to ecdysone.

The corpora cardiaca of insects releases several different active substances. Three peptide-like substances were responsible for the effects of crude extracts on the heart and hindgut preparations of the cockroach (182). These compounds were associated with particles in homogenates of the corpora cardiaca. Other separate effects on the foregut, nerve cord, and blood trehalose are due to other compounds separable by chromatography. These substances are as yet uncharacterised, but 5-HT and an adrenergic substance, both of which occur in the organ, may account for some of the activity of extracts on the different preparations.

A substance which contracts the hindgut of cockroach has been detected in the nerves of this insect by Brown (183). This differs from the peptide-like compounds of the corpora cardiaca that affect the hindgut, as shown by proteolytic digestion experiments, and is most abundant in the nerves that supply the gut. It is associated with a light-particle fraction and is not ACh, adrenaline, noradrenaline, GABA, glutamate, or 5-HT. Freeman (184), however, has shown that low concentrations of DOPAmine contract the locust hindgut, and this requires further investigation.

The crustacean heart is excited by peptides obtained from the pericardial organs of crustacea (185, 186). Two active peptides from *Cancer borealis* are similar in size and amino acid composition (187). A molecular weight between 700 and 1500 is indicated and lysine, glycine, alanine, glutamyl, and aspargyl residues are probable components of the molecules.

Work continues on the crustacean eyestalk neurosecretory products, which cause a multiplicity of hormonal effects in this group. Most of the available data suggest that the hormonal effects are largely due to peptides (188). Efforts to determine the number of active compounds have shown that erythrophore contracting factor is a different compound from those causing movements of the distal retinal pigment of the eye and from the melanophore dispersing factor. Differences in the solubilities of the latter two factors suggest that these are also different substances. Rangarao (189) has shown that moult-inhibiting factor is a different compound from the chromatophoric hormones. Further data (188) indicate that the factor with diabetogenic activity differs from the moult-inhibiting hormone. Thus there appear to be five different hormone molecules in the crustacean eyestalk and there may be more.

The radial nerve cords of echinoderms (at least 20 species of asteroids) contain a substance that causes contraction of gonadal muscle and, hence, shedding of gametes from both intact muscle and isolated gonads (190, 191). The same substance promotes maturation of eggs within intact ovaries (190). It is probably also a peptide, the exact identity of which may be species-specific (191, 192). The compound from *Asterias forbesi* may contain between 10 and 15 amino acid residues.

Chaet (191, 193) has also detected the presence of a second factor, shedhi-

bin, that inhibits liberation of gametes. While the gamete-shedding substance remains at a constant concentration throughout the year (194), shedhibin concentrations fluctuate. The release of sexual products in natural conditions may be the reflection of lack of shedhibin.

An invertebrate insulin has recently been demonstrated by Wilson & Falkmer (195). This substance, isolated from the starfish *Pisaster ochraceus* increased deposition of glycogen in mouse diaphragm. The properties of starfish insulin are very similar, but not identical, to those of ox insulin. It is probable that the substance is involved in glycogenesis in the starfish, and similar compounds may be expected from many invertebrates. Preliminary evidence exists for the presence of insulin in the tunicate *Ciona intestinalis* and the cephalopod *Eledone cirrata.*

Tetrodotoxin

The great comparative value of certain invertebrate preparations has been demonstrated by the investigations on the mode of action of tetrodotoxin [or tarichotoxin (196)]. This nerve poison blocks the sodium-carried current of the action potential, but does not affect the potassium conductance as shown by voltage-clamp experiments on squid and lobster giant axons (197–199). When depolarizing currents greater than the sodium equilibrium potential were applied across the squid or lobster axon membrane in the presence of tetrodotoxin, outward movement of sodium ions was impaired, although sodium movement through the squid axon membrane was decreased to a greater extent than that of the lobster axon. Outward movement of sodium ions from a squid axon perfused internally with a high concentration of sodium was also blocked by tetrodotoxin (200).

Tetrodotoxin thus appears to block action potential propagation in nerves and muscles by a specific action on the sodium transference across active membranes. Externally applied tetrodotoxin is more effective than internally injected material (200, 201). Crustacean muscle preparations that are calcium-dependent are unaffected by tetrodotoxin (202, 203).

It has been generally believed that tetrodotoxin does not influence invertebrate sensory processes (204, 205), but Benolken & Russell (206) have recently shown that the graded transient responses of the eye of *Limulus* are reversibly blocked by tetrodotoxin.

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