COMPARATIVE PHARMACOLOGY[1,2,3]

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Otto Loewi liked to define a drug as "a chemical which when injected into an animal produces a scientific paper": this review is based on his definition. I have brought together some of the work on invertebrate animals which helps to explain how drugs act, and will also report some advances in invertebrate zoology which suggest new preparations suitable for pharmacological study.

Active Transport

Potassium.—An important new preparation for the study of active ion transport comes from the caterpillar of the giant silk moth *Hyalophera cecropia*. The caterpillar feeds on leaves and faces the problem of an overabundant supply of potassium. The midgut can be mounted like a frog skin for transport experiments. The midgut transports potassium from the blood into its lumen, and at least 83 per cent of the short-circuit current generated by the midgut represents potassium movement [Harvey & Nedergaard (1)]. Potassium transport is promptly inhibited by DNP, iodoactic acid, 50 per cent carbon dioxide, and by the carbonic anhydrase inhibitors sodium sulfide and ethoxzolamide [Haskell, Clemons & Harvey (2)]; the transport of hydrogen ion may be important for moving potassium. There is no inhibition by chlorthalidone, ouabain, or cholinergic drugs. The midgut seems an almost ideal preparation for the study of potassium transport.

Chloride.—Keynes (3) showed that the squid axon moves chloride from sea water into the axoplasm against an electrochemical gradient; the transport is blocked by DNP.

Sodium.—Kerkut & Thomas (4) injected neuron cell bodies into the central nervous system (CNS) of the snail *Helix* with NaCl at a rate calculated to raise the intracellular concentration by 4.4 m*M* per minute. Over the first ten minutes the resting potential of the cell membrane increased by about 30 mV. The hyperpolarization is prevented by pretreat-

[1] The survey of the literature pertaining to this review was concluded in July 1965.

[2] The following abbreviations will be used: ACh (acetylcholine); DCI (dichloroisoproterenol); DOPAmine (3,4-dihydroxyphenylethylamine); 5-HT (5-hydroxytryptamine); 6-HT (6-hydroxytryptamine); GABA (γ-aminobutyric acid); PCMB (*p*-hydroxymercuribenzoate); DNP (2,4-dinitrophenol).

[3] The preparation of this review was assisted by Grant 04874 from the Institute of Neurological Diseases and Blindness, Public Health Service.

ment with ouabain, PCMB, or by lowering the extracellular potassium concentration. There is no change in potential if the cells are microinjected with KCl. The hyperpolarization is produced by the active transport of sodium from the neuron.

Calcium.—The sarcoplasmic reticulum isolated from lobster muscle accumulates calcium by an active transport mechanism. Transport is inhibited by PCMB, DNP, and ethanol. The transport rate can be cut to 50 per cent of normal by anticholinesterases. This preparation may be suitable for finally establishing whether or not the action of anticholinesterases in reducing the rate of active ion transport really depends on the inhibition of cholinesterase [Van der Kloot (5, 6)].

Effectors

Last year's chapter in this series was exclusively devoted to the effects of drugs on nerve and muscle [Florey (7)], so this section will reflect only the most recent developments.

Nematocysts.—The pharmacology of the classic independent effector, the nematocyst of *Hydra*—a cell which is both sensory receptor and mechanical effector—was studied by Lentz & Barrnett (8). The nematocysts are normally discharged by mechanical stimuli, but chemicals can lower the threshold for stimulation. The most potent facilitating chemicals are fructose-6-phosphate and fructose-1,6-phosphate; their action can be blocked by NaF or by M-ethylmaleimide. A massive discharge of nematocysts is produced by ACh; the action of ACh is blocked by tubocurarine and hexamethonium, and is potentiated by physostigmine. There are four different types of nematocysts; one type (the holotrichousisorhizas) is discharged only by epinephrine, by 5-HT, or by histamine. Some of the main themes in pharmacology have run throughout the long evolution of multicellular animals.

Cilia.—The beat of the cilia on the mollusk gill may be regulated by the local production of 5-HT. The stimulating effect of exogenous 5-HT is potentiated by ATP; the ATP may work by increasing 5-HT uptake [Schor (9)]. If the tissue is fractionated, the 5-HT is found mostly in the endoplasmic reticulum, which calls to mind the role of sarcoplasmic reticulum in regulating muscle contraction [Zs-Nagy et al. (10)]. Aiello (11) found that the gills contain a polyphenol oxidase which can metabolize 5-HT. All three of these lines of evidence are consistent with the proposed role of 5-HT.

Action potentials without sodium.—Crustacean muscle fibers can generate action potentials in sodium-free solutions, as long as Ca^{2+} is present [Fatt & Katz (12); Fatt & Ginsborg (13); Hagiwara & Naka (14); Hagiwara, Chichibu & Naka (15)]. When in an ordinary Ringer's solution, most of the fibers do not produce a propagated action potential unless they are first exposed to one of a variety of organic bases, like

tetraethylammonium or procaine, or to Sr^{2+} or Ba^{2+}. Apparently, the inward current is carried by divalent cations instead of sodium. The action potential is not blocked by tetrodotoxin [Ozeki & Grundfest (16)], so this drug may be a highly specific inhibitor of the sodium mechanism. So far, we do not know how widespread this kind of mechanism may be in the Animal Kingdom. There are two reports that some of the neurons in the mollusk's central nervous system continued to generate action potentials for hours in sodium-free solutions as long as calcium was present [Oomura, Ozaki & Maeno (17); Gerasimov (18)].

Neuromuscular Transmission

Crustacea.—One of the preparations which helped make comparative pharmacology respectable is the crustacean neuromuscular junction, because of peripheral inhibition and the possibility that GABA is the inhibitory transmitter. The evidence continues to accumulate showing that the inhibitory axons contain GABA in extraordinary concentrations—as high as 0.1 *M* in lobster axons [Kravitz & Potter (19)], compared with less than 1 m*M* in the axons of excitatory neurons. The GABA content of the cell bodies of the inhibitory neurons in the central nervous system is also high [Otsuk, Kravitz & Potter (20)], while there is comparatively little GABA in internuncials or in sensory axons. The store of GABA in the inhibitory neurons is so high that it must be one of the most significant
classes of axons contain other substances in similar quantities.

Takeuchi & Takeuchi (21) applied GABA iontophoretically to crayfish muscle. Only certain circumscribed regions of the muscle responded to GABA. The responsive areas coincide with the points from which extracellular records can be made of inhibitory junctional potentials. As little as 4×10^{-15} *M* of GABA applied to the cell surface produces a change in the conductance of the postjunctional membrane; GABA applied intracellularly is without effect. Before concluding that GABA is the transmitter, it is essential to know that it is released from stimulated inhibitory nerves and that there are mechanisms for terminating GABA's physiological action.

As is now well known, GABA acts on the postjunctional membrane to increase chloride conductance, and at the same time acts on the ending of the motor nerve to decrease the number of quanta of the transmitter released with each nerve impulse. A similar dual action is shown by γ-aminohydroxybutyric acid and by β-alanine [Dudel (22)]. On the other hand, β-guanidinopropionic acid and other compounds have the presynaptic action but do not affect the postjunctional membrane. There is a clear difference in the pharmacological specificity of the pre- and the postjunctional receptors. Both pre- and postjunctional effects are blocked by picrotoxin.

Dudel (23, 24) also produced a brilliant study of the conduction of the impulse into the terminals of the motor nerve. His major tool was recording extracellular action potentials, using a digital averaging computer to raise the signal out of the background noise. As a recording electrode is moved down the motor nerve toward the terminals, the extracellular potentials change in a sequence which strongly suggests that the action potential is not conducted as far as the terminals. When the recording electrode is on a visible twig of the motor nerve, the record shows the typical triphasic extracellular action potential: (*a*) positive as the action potential approaches the recording electrode and local currents are flowing outward through the membrane; (*b*) negative when the membrane under the electrode is active and current is flowing inward; and (*c*) positive when the action potential has passed the recording site and current is again flowing outward through the membrane. When the recording electrode is moved further toward the nerve terminal, the potential change becomes diphasic, with the positive phase most pronounced. At the very terminal of the nerve, only a positive phase is recorded—the positive terminal potential. The interpretation is that the action potential is blocked before it reaches the terminal and that the terminal is depolarized electrotonically by local current flow from the activated regions.

When the inhibitory nerve is stimulated an instant before the motor axon, the positive terminal potential of the motor axon is reduced in amplitude, and so is the postjunctional potential. The same effects are produced by GABA. This change can be accounted for by assuming that the conductance of the nerve ending is increased, so that more of the local currents flow through the membrane adjacent to the point where the action potential is blocked and less current reaches the terminal itself.

Although the amplitude of the positive terminal potential is always reduced during inhibition, there does not seem to be a constant relation between the decrease in the terminal potential and the decrease in the postjunctional potential. Dudel therefore suggests that either the extracellular record is not a faithful mirror of the potential changes across the membrane of the nerve terminals, or that the potential change is not the only factor determining how many quanta of the transmitter are released.

Dudel (25) used the same powerful techniques to analyze the facilitation which occurs if the crustacean motor nerve is stimulated repeatedly. With facilitation, the positive terminal potential doubles in size, probably because the action potential is conducted to a point closer to the terminal. There is a good relation between the amplitude of the positive terminal potential and of the postjunctional potential during facilitation.

The excitatory postjunctional potentials become larger when the preparation is exposed to 5-HT. There is no change in the size of the quanta, only in the number of quanta released per stimulus [Dudel (26)]. The 5-HT increases conduction velocity in the ending in the motor nerve by at

least 10 per cent, and there is also an increase in the size of the positive terminal potential.

The depolarization of the terminals of the inhibitory nerve follows a similar pattern, but the events at the inhibitory ending are not influenced by GABA; i.e., the inhibitory transmitter does not feed back to inhibit its own release.

Transmission at the crustacean neuromuscular junction is potentiated by several of the ethonium ions, like 3-hydroxyphenyltriethylammonium, which produce similar effects on mammalian nerve-muscle preparations [Kuperman (*27*)]. If one of the ethyl groups in the onium center of the ions is replaced by a methyl group, then the compound depresses contraction. With the background of Dudel's studies, it would be most interesting to see how these drugs act on the terminals; it has long been suspected that they act at the terminals of mammalian motor nerves.

Insects.—Usherwood & Grundfest (*28*) showed that the locust and the grasshopper have inhibitory junctions on a small percentage of their muscle fibers [see also Hoyle (*29*)]. In order to show clearly the hyperpolarizing effect of inhibitory nerve activation, it is important to use a microelectrode filled with some salt other than the chloride. The effects of the inhibitory neuron are mimicked by GABA, and the effects of GABA and inhibitory stimulation are blocked by picrotoxin. The function of peripheral inhibition in the life of the intact animal is no better understood in the insects than it is in the crustacea.

Excitatory transmitters.—The question of excitatory transmitters in the arthropods remains unresolved. Perhaps the outstanding candidate is L-glutamic acid. Takeuchi & Takeuchi (30) applied glutamate to the muscle surface by iontophoresis. The regions which depolarize when glutamate is applied are sharply localized and coincide exactly with the points from which extracellular records of excitatory postjunctional potentials can be obtained. A detectable depolarization is produced by only 5×10^{-16} *M* of L-glutamate. It is worth pointing out again that the crustacean muscle is not sensitive to D-glutamate, so these effects differ from the rather generalized actions of D- and L-glutamate in the mammalian central nervous system.

In insects, the miniature postjunctional potentials, like the junctional potentials, are not affected by cholinergic drugs [Usherwood 31)]; 10^{-3} *M* 5-HT decreases the membrane potential and the amplitude of the miniatures, but has no effect on the frequency. Therefore, the entire matter of excitatory transmitters in the arthropods remains unresolved. No support has appeared for the idea that the transmitter is a compound containing nicotinamide [Van der Kloot (*32*)]; Armson & Horridge (33) were unable to demonstrate any difference in the uptake of ^{14}C-nicotinic acid by living and dead nerves.

Onychophora.—A significant departure from the usual approach was

shown by Florey & Florey (34) who used pharmacology as a tool to explore the evolutionary relationships of the Onychophora. This little known class of arthropods (according to some authorities, they are a separate phylum) has been regarded as a transition between the annelids and the arthropods. *Peripatus* is the best known genus. Their muscles proved to be cholinergic—like the annelids—and do not respond either to GABA or to glutamic acid. Pharmacologically, therefore, these strange beasts seem close to the annelids.

The trophic action of the nerve.—In the course of development, some cells are destined to multiply, others to die. For example, the abdominal intersegmental muscles of the wild silk moth break down shortly after the emergence of the adult moth. The breakdown of the muscles can be prevented by injecting the animal with pilocarpine or with eserine, and the protective action of the drugs is antagonized by atropine or by denervation [Lockshin & Williams (35)]. Therefore, it seems that the drugs act on the central nervous system and not directly on the muscles. In support of this view, electrical recordings show that the normal outflow of impulses in the motor nerves is sharply curtailed soon after the adult emerges. Chronic electrical stimulation of the motor nerves opposes or prevents the breakdown of the muscles. In short, there appears to be an excellent correlation between the impulse traffic and the trophic action of the nerve in supporting the existence of the muscle [Lockshin & Williams (36)].

Central Nervous System

Glia.—Over the past years a certain mystique has been generated about what the glia are doing in the central nervous system. Much of the mystery has been displaced by studies on the giant glia in the ganglia of the medicinal leech. There are only ten glial cells in each ganglion—six surround the nerve cell bodies, two are with the neuropile, and two envelop the thousands of axons running between the ganglia. The glial cells can be destroyed, and yet the neuron cells continue to function for hours [Kuffler & Potter (37); Nicholls & Kuffler (38)]. In this preparation, both glial cells and neurons can readily be penetrated with microelectrodes, and the effects of changes in the extracellular bathing solution can be followed. If the usual Ringer's solution is replaced with a high potassium solution, the resting potentials of neurons and glia go down synchronously; about 50 per cent of the extracellular sodium is replaced in nine seconds. If the ganglia are placed in a sodium-free Ringer, the neurons behave as if 50 per cent of the sodium were gone after 12 sec; during this period there is no change in the resting potential of the neuron or the glial cell. Therefore, it seems clear that ions penetrate the tissues solely by way of the extracellular space which makes up about 5 per cent of the total volume.

What then is the role of the glia? Kuffler & Potter (37) suggest that

they serve a trophic function. The glia are known to contain large amounts of glycogen which is depleted if the animals are made to swim until exhausted, presumably because substrates are passed along to the neurons. The question then is how the activity in the neurons signals the nearby glial cells to increase the supply of substrate. Kuffler & Potter suggest that electrical currents from the firing of the neurons flow locally through the glial membrane and cause activation.

In a similar vein, Wigglesworth (39) showed that in the last abdominal ganglion of the cockroach the glial cytoplasm penetrates deeply into the body of the neurons, forming a structure called the trophospongium. If the roaches are starved for three to four weeks, and then fed, glycogen appears first in the glia and can then be seen entering the neurons via the trophospongium.

The mollusk CNS.—Over the past years, no field has grown more rapidly than the study of neurons in the central nervous system of snails and seahares. This is a tribute to the remarkable ease with which the cells can be impaled with a microelectrode, and to the fact that many cells can be recognized by coloring, location, or physiological properties. The cells are monopolar and the cell is free of synaptic endings. So far, four pharmacologically distinct types of cells have been identified [Gerschenfeld & Tauc (40)]:

(*a*) Cells that are excited by ACh. They are depolarized to about 15 mV below the resting potential. These cells have no inhibitory inputs. The action of ACh and the excitatory postsynaptic potentials are blocked by hexamethonium, but not by physostigmine, tubocurarine, or atropine ("D-cells").

(*b*) Cells that are hyperpolarized and inhibited by ACh. Here the action of ACh is not touched by hexamethonium, but is blocked by atropine, tubocurarine, and physostigmine, as are the inhibitory postsynaptic potentials (IPSP's). These cells are excited by DOPAmine at 10^{-11} *M* ("H-cells").

(*c*) Cells that are excited by ACh just as type (*a*) cells are but are inhibited by DOPAmine. Neither the IPSP's nor the action of DOPAmine are affected by dibenamine, dihydroergotamine, picrotoxin, metrazol, tetanus toxin, or strychnine [Gerschenfeld (41)]. Adrenergic blocking agents of the β type cannot be used reliably because DCI blocks the nerve conduction; guanethidine also impairs interneuron firing ("DINHI cells").

(*d*) The final cell type that is excited by 5-HT [Gerschenfeld & Stefani (42)]. The response to 5-HT and to excitatory stimulation is blocked reversibly by morphine, atropine, chlorpromazine, and lysergic acid diethylamide. Irreversible block is produced by tryptamine and dibenamine. The monoamine oxidase inhibitor SKF *trans*-385b enhances the effects of 5-HT, but it is not certain whether the drug acts by inhibiting the enzyme or by mimicking 5-HT ("CILDA cells").

The effects of glutamic acid were also tested on the central nervous system of the snail [Gerschenfeld & Lasansky (43)]. D- and L-glutamic acids give the same effects, but the effect depends on the cell tested: some cells are depolarized and excited, others are hyperpolarized and inhibited. There is no relation between the direction of the response to glutamate and to ACh. Similarly, many cells respond to GABA—some cells are inhibited and others excited. To add further complexity, some of the cells excited by GABA are inhibited by glutamate, while others are excited by glutamate, and so forth. The data re-emphasized the widespread and often quite ambiguous responses to GABA and to glutamate found throughout the Animal Kingdom.

Chalazonitis & Nahas (44) studied the effects of 5 per cent CO_2 on synaptic transmission in *Aplysia*. Excitatory postsynaptic potentials which were below threshold for eliciting a spike, reached threshold when CO_2 was present; apparently, there is a slight depolarization of the membrane and also an increase in excitability.

I should re-emphasize that the cell bodies of these mollusk neurons are free of synapses, and yet the drugs produce their effects when they are applied to the soma. Frank & Tauc (45) voltage-clamped these neurons and measured the current flowing through a spot on the cell membrane about 20 μ in diameter. This small section of the membrane always responds to both depolarization and ACh, so that it is difficult to believe that there is any anatomical separation between a membrane which is chemically and one which is electrically excited. There is little occlusion between the conductance changes induced by ACh and by depolarization, which suggests that the two means of stimulation open up different pathways for ion movement through the membrane (of course, the same ions may move through two separate pathways). The cells which hyperpolarize in response to ACh depolarize instead if injected with anions smaller in size than acetate [Kerkut & Thomas (46)]—just as mammalian motor neurons do.

Coelenterates.—Barnes & Horridge (47) prepared an active extract from the marginal ganglia of the jellyfish *Cyanea,* which is capable of accelerating the rhythmic activity of intact ganglia. Similar extracts from non-neural tissues are inactive. The active extract has no effect on crustacean or clam hearts, so it presumably does not contain significant amounts of ACh, GABA, or 5-HT.

Learning.—Studies on the pharmacology of learning will obviously profit from the use of as simple a preparation as it is possible to find. Therefore, it is worth pointing out the work of Horridge (48) and Eisenstein & Cohen (49) who present convincing evidence for learning in the isolated prothoracic ganglion of the cockroach [also see Luco & Aranda (50)].

Heart and Circulation

Crustacea.—The heart rate in the crustacea is regulated by a neuroendocrine system, the pericardial organ. The organ is made up of a plexus of neuron endings which project into the pericardial cavity. Cooke (51) showed that the amount of excitatory substance released by an isolated pericardial organ is proportional to the rate at which the axons are stimulated—a direct demonstration of the relation between electrical activity and neurosecretion.

An excitatory extract prepared from the heart itself was shown by Kerkut & Price (52) to contain two active components: 6-HT and a mucopeptide.

Mollusks.—As far too little work has been done on the circulation in invertebrates, it is particularly pleasing to see the study of Johansen & Huston (53) who measured pressures at several points in the circulation of intact, undisturbed specimens of *Octopus.* Norepinephrine and epinephrine slow the systemic and branchial hearts and decrease peripheral resistance; 5-HT stimulates both hearts. Histamine is a powerful peripheral vasodilator.

Greenberg (54) gives a compendium of the actions of ACh on the hearts of 40 species of bivalve mollusks.

Insects.—A potent stimulator of the contractions of the heart and of the hindgut can be extracted from the cockroach corpora cardiaca, which are a pair of small endocrine glands lying behind the brain. Like the pituitary, the corpora cardiaca are bipartate, containing both intrinsic cells and the endings of neurosecretory axons. Davey (55, 56) showed that the substance from the corpora cardiaca acts on the target organs by an indirect route: it stimulates argentaffin cells in the heart or in the hindgut to release an indolalkylamine which, in turn, stimulates contractions by the muscles. The argentaffin cells can be blocked by treatment with ammonium carmine, and then the tissues no longer respond to the hormone from the corpora cardiaca. Davey (56) has investigated the mode of action of the hormones on the argentaffin cells. Drugs which block decarboxylases, like semicarbazide, prevent the action of the hormone. It seems likely that the hormone acts on the argentaffin cells to cause them either to produce more amino acid as a substrate to be transformed into indolalkylamine, or to unmask amino acids to the action of the decarboxylase.

Extracts of the cockroach corpora cardiaca also contain a substance which causes a rise in the blood sugar (trehalose), the activation of phosphorylase in the fat body, and a fall in tissue glycogen [Steele (57); Bowers & Friedman (58); Ralph & McCarthy (59)]. This active substance appears to be a polypeptide.

The motor nerves running to the phallic musculature of insects are

normally not firing, but are excited following the decapitation of the animal. The increase in motor outflow comes on gradually after decapitation and after 20 minutes there are six to eight bursts of motor activity per minute which cause ordered movements of the phallus. The effects of decapitation can be duplicated, for some reason, by concentrated extracts of the corpora cardiaca applied to the ganglia of the nerve cord [Milburn & Roeder (60)]; it is not known if there is any role for this system in the normal physiology of the animal.

Growth and Differentiation

Arthropods.—The growth of insects is regulated by two principal hormones. The first, ecdyson, is secreted by the thoracic glands and triggers the periodic molts which punctuate the insect's life [for reviews see Van der Kloot (61); Schneiderman & Gilbert (62)]. So far, a complete chemical identification of ecdyson has not been achieved, but the molecule can be hydrogenated to phenanthrene derivatives and is synthesized by insects from tritiated cholesterol, which indicates that it is a steroid [Karlson (63); Karlson & Hoffmeister (64)]. The empirical formula is $C_{27}H_{44}O_6$. As yet, no drug has been found to mimic the action of ecdyson. A bioassay method capable of measuring the ecdyson concentrations at different stages in the life cycle has been developed and put to use [Shaaya & Karlson (65, 66, 67)].

The effects of ecdyson are modulated by a second agent, the juvenile hormone, which is secreted by a pair of small glands (the corpora allata) which lie just behind the brain. The juvenile hormone serves to brake the progress in differentiation—it prevents the animal from moving onward in its program of development. C. M. Williams found that large quantities of this hormone can be extracted from adult male silk moths. Juvenile hormone activity is found in many places, including the feces of the mealworm *Tenebrio* [Karlson & Schmialek (68)]. The active substance in the feces proves to be the open chain terpine alcohol, farnesol:

$$CH_3 \cdot C(\cdot CH_3):CH \cdot CH_2 \cdot CH_2 \cdot C(\cdot CH_3):CH \cdot CH_2 \cdot CH_2 \cdot C(\cdot CH_3):CH \cdot CH_2OH$$

and its aldehyde, farnesal [Schmialek (69)]. These compounds act like the juvenile hormone in many test systems [Wigglesworth (70, 71)]; the all *trans* form has maximum activity [Yamamoto & Jacobson (72)]. Schmialek (73) reports that all the activity in the silk moth extract comes from farnesol and farnesal. However, further attempts to purify the extracts strongly suggest that a far more active compound is present [Meyer & Ax (74); Meyer, Schneiderman & Gilbert (75)]. Williams & Law (76) obtained a fraction by liquid gas chromatography in which activity was concentrated 50,000 times. The mass spectrographic analysis of the fraction suggested that it contained the epoxide of methylhexadeconoate—

but this compound was synthesized and found to be inactive. Apparently, the juvenile hormone was present only as an impurity in the active fraction. This means the juvenile hormone is at least 1000 times as potent as any of the active isoprenoid derivatives tested so far. Williams & Law suggest that it may be an epoxy-ester of a terpenoid acid.

Pheromones.—There are a number of examples of social hormones or pheromones, chemicals given off by one animal to affect the physiology or behavior of another. The secretion from the mandibular gland of the ant *Lascius* is the furan derivative of farnesol [Quilico, Piozzi & Pavan (77)], and the attractive substance in the Nassanoff glands of the honeybee is geraniol [Boch & Shearer (78)], which has two isoprene units in place of farnesol's three.

Another example of a pheromone is a secretion released by the male locust which promotes the maturation of other locusts—the effects can be mimicked by 2-chloroethanol [Carlisle & Ellis (79)].

The mode of action of ecdyson.—A major advance was the discovery by Clever & Karlson (80) that the injection of ecdyson into *chironomus* larvae promptly produces changes in the pattern of puffing in the giant chromosomes of the salivary gland—changes similar to those found at normal pupation. This suggests that the hormone might act by activating specific regions of the chromosome. The changes occur within 15 minutes after exposure to the hormone [Clever (81)]. The steps by which the hormone acts on the chromosome are by no means clear. The action of ecdyson on chromosome puffing can be copied by exposing the tissue to zinc, cadmium, chloroform, or urethane, or by changing the extracellular sodium-to-potassium ratio; all of these chemicals are without morphogenetic effect on the intact animal [Kroeger (82, 83)], which suggests that the activation of the chromosomes is still a secondary effect of the hormone's action. Laufer, Nakase & Vanderberg (84) showed that actinomycin D—which is assumed to act by inhibiting DNA-primed RNA synthesis—blocks the swelling at two parts of the salivary gland chromosome, but not at a third.

The idea that the action of ecdyson has something to do with DNA has been investigated further by taking advantage of the pupal diapause of giant wild silk moths. During the months of diapause, the insects have no significant secretion from the thoracic gland. When ecdyson secretion is resumed, adult development begins. Adult development is blocked by inhibitors of DNA synthesis like mitomycin C [Krishnakumaran & Schneiderman (85)], or by 5^1-fluoro-2-deoxyuridine [Wiliams (86)]. When the hormone is not present, only a few of the cell types in the animal incorporate tritiated thymidine [Bowers & Williams (87)]; the onset of development is marked by a widespread DNA synthesis. The nature of block in the synthesis of DNA in the absence of the hormone is still unexplained. The tissues from diapausing animals do contain low but

finite titers of thymidine kinase and thymidylate kinase; the activity of these enzymes increases twentyfold when ecdyson is secreted. The failure of DNA synthesis might come from a lack of DNA polymerase, the presence of a hitherto undetected inhibitor, or an *in vivo* feedback inhibition of the enzyme pathway [Brookes & Williams (88)].

Echinoderms.—Extracts of the radial nerves of starfish, when injected into intact animals, induce meiosis in the ovary and initiate the shedding of the eggs [Chaet (89); Kanatani (90)]. The shedding factor presumably comes from neurosecretory cells which are found in the radial nerve. The active substance seems to be a low molecular weight polypeptide [Kanatani (90)].

Coelenterates.—For many years it has been known that the *Hydra* contain an unspecialized cell type, the interstitial cells. It was thought that the interstitial cells might be totipotent, i.e., be able to differentiate into any of the other cell types found in the animal. This issue has been largely resolved by Diehl & Burnett (91) who found that the interstitial cells were selectively killed by a ten minute exposure to a 0.01 per cent solution of the nitrogen mustard, mechlorethamine hydrochloride. The animals survive for 40 to 50 days without interstitial cells; during this period the number of nematocysts goes down markedly; apparently they are no longer replaced by interstitial cells. On the other hand, the number of gland or mucous cells lining the digestive cavity remains unchanged, which indicates that they probably arise from some other cell lineage.

Protozoa.—Some species, like *Naegleria gruberi,* can exist in either of two distinctive forms: ameboid or flagellate. Pearson & Willmer (92) found that steroids like progesterone and deoxycorticosterone favor a change in body form when they are present in low concentrations. At higher concentrations, the steroids keep animals in the ameboid form. Progesterone acts at concentrations close to those found in the vertebrate body, which shows that the protozoan has a high sensitivity to these presumably unfamiliar molecules.

Conclusion

This review has been ruthlessly selective, some excellent and important papers are not cited; my guide was to favor papers which might be further from the reader's usual path. To do this, I have sacrificed any mention of such important topics as the pharmacology of nerve transmission, in which most of our information comes from squid and lobster giant fibers.

Comparative pharmacology has made excellent progress. We know as much or more about the action of drugs at the crayfish neuromuscular junction as at any vertebrate synapse; the cellular pharmacology of the molluskan central nervous system is better known than that of the cat; and the clues gained from the study of the action of insect hormones will

profoundly affect our view of biological control mechanisms. These achievements surely do not minimize the infinitely large number of unresolved problems and the wonderful opportunities for new researches.

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