PHARMACOLOGY OF POIKILOTHERMIC VERTEBRATES AND INVERTEBRATES[1]

R. FÄNGE

Institute of Zoophysiology, University of Oslo, Blindern, Norway

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I. ABBREVIATIONS

ACh	Acetylcholine
ChE	Cholinesterase
GABA	*gamma*-Aminobutyric acid
5-HT	5-Hydroxytryptamine (serotonin)
LSD 25	N,N-Diethyl-D-lysergic acid amide
MSH	Melanocyte stimulating hormone
TEA, TMA	Tetraethylammonium, tetramethylammonium

In the text, concentrations are in g per ml solution.

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II. INTRODUCTION

The growth of neuropharmacology in recent years has stimulated an interest in pharmacological investigations on lower animals (122, 150). The reason may be that many experimental problems are easier to attack in these animals than in the more highly evolved ones. Lower animals also provide valuable material for bioassays. It is natural that many animal species are more familiar to zoologists than to pharmacologists. The animal world comprises nearly one million different species, mostly invertebrates. Among the vertebrates, the majority are fishes (230).

This article will, it is hoped, give some hints on the usefulness for experimental purpose of organs from animals belonging to different systematic groups. The new and old literature has been reviewed, mainly with respect to the effect and distribution of biogenic amines. It has not been possible to include the vast number of interesting papers on actions of anaesthetics, phenols, opium alkaloids, strychnine, caffeine, veratrine, saponins, cardiac glycosides and many other substances. Neither has it been possible to consider all publications on insecticides, anthelmintics, molluscicides, protozoa-killing agents, *etc.* (see 40, 47, 49, 265, 269, 305, 322). Much of the literature has been surveyed previously (26, 122, 135, 157, 169, 273, 292, 364, 370, 377).

III. EFFECTS OF DRUGS ON CERTAIN ORGANS

A. Poikilothermic vertebrates (cyclostomes, fishes, amphibians, reptiles)

General remarks. The hagfishes (cyclostome) are among the most primitive of the true vertebrates. Their low position on the evolutionary scale is reflected in the composition of their blood, which compares closely in ionic distribution and concentration with sea water (247). In dogfishes and rays (elasmobranchs), the blood has a high content of urea. Different physiological saline solutions have been recommended for hagfish, dogfish, and bony fishes (82, 112, 227, 273).

1. Heart. Cyclostomes: Divergences from the usual pattern of pharmacological responses of the vertebrate heart are met with in the two groups of primitive vertebrates represented by the hagfishes and the lampreys.

In hagfishes (*Myxine*, *Polistotrema*) the heart does not react at all to ACh at 10^{-7} to 10^{-2} (17, 176a, 177). The isolated perfused heart is also insensitive to adrenaline and noradrenaline (277). Nevertheless, catecholamines may play a role in the physiological regulation of the *Myxine* heart. The activity of the heart can be depressed by dihydroergotamine (10^{-5}) or reserpine (2.5×10^{-6}). On such slowly beating or non-contracting hearts, noradrenaline (10^{-6}) has a marked stimulatory effect (42, 112). An acceleratory effect of extracts of hagfish heart prepared with ice-cold saline solution (177) might in part be explained as an effect of catecholamines, because this heart contains large amounts of adrenaline and noradrenaline stored in special cells (42). The myxinoid heart seems to lack completely any external innervation (17). Thus, the acceleration of the heart during mechanical distention cannot be due to reflex action (177). It seems possible that the acceleration is produced in part by release of catecholamines. The

remarkable insensitivity of the isolated hagfish heart to catecholamines may to some extent be explained by the circumstance that the heart is already under influence of catecholamines released from the heart itself because of mechanical distention during the perfusion.

The heart of lampreys (*Lampetra*, *Petromyzon*) is peculiar in being accelerated by low doses of ACh (10^{-8} to 10^{-7}). It is also accelerated by nicotine (10^{-6}), whereas catecholamines usually have a weak retarding influence (17). The excitation due to ACh is blocked by tubocurarine (10^{-4}) and by hexamethonium (10^{-4}), but is only weakly affected by atropine (5×10^{-5}) (17). The efficiency ratio, curare/atropine, is said to be the same for the heart and skeletal muscles in the lamprey, whereas in higher vertebrates the heart is much more sensitive to atropine than to curare. A similar curare/atropine ratio as for the lamprey heart has been observed in experiments with skeletal muscle in fish and denervated lymph hearts from the frog. From these observations is has been suggested that the lamprey myocardium is of a phylogenetically older type than the cardiac muscle of higher vertebrates (175). This might be true, but the unusual pharmacological behaviour of the lamprey heart may as well be due to the nature of its innervation (17).

Elasmobranchs (cartilaginous fishes): Catecholamines stimulate the hearts of rays and dogfish. Sometimes stimulation is preceded by brief inhibition, which can be prevented by atropine. Acetylcholine is inhibitory (112, 277).

Teleosts (bony fishes): Catecholamines stimulate and ACh inhibits the heart, but the sensitivity to these agents varies among species (112). In early embryonic stages of *Fundulus*, the heart has been reported to be insensitive to ACh (12).

Amphibians: Adrenaline is 10 to 20 times more active than noradrenaline in its stimulant effects on the isolated frog heart (277).

Reptiles: The ventricle of the turtle heart is in some cases insensitive to ACh (85). When tested on different atrial regions of the heart of a turtle (*Chrysemys*), catecholamines and nicotine give positive chronotropic and inotropic responses (81).

Cardiac innervation and response to ACh: There seems to be a correlation between the type of cardiac innervation and the way in which the heart responds to ACh. The heart of the lamprey (*Lampetra*), which is very richly provided with nervous elements, is accelerated by ACh (17, 175). This type of response, unusual for a vertebrate, does not necessarily mean that cardiac rhythmicity is neurogenic in the lamprey heart as it is in large crustaceans (292), but suggests that ACh acts indirectly *via* nerve cells. In the hagfish (*Myxine*) the heart is insensitive to ACh in all concentrations. The early embryonic fish heart (12) and the ventricle of the turtle heart (85), already mentioned as insensitive to ACh, share with the hagfish heart the property of being devoid of nerve fibres. Other examples of supposedly nerve-free heart tissue which does not respond to ACh are found in certain invertebrates (63, 137, 211, 213, 254).

2. Blood vessels. Elasmobranchs: Rise of systolic and pulse pressure and a decrease of heart rate have been observed after administration of LSD 25 (379).

Teleosts: Adrenaline (2×10^{-6}) and noradrenaline dilate gill vessels. It is of interest that the mammalian coronary vessels, which show the same response to catecholamines, develop embryologically from gill arteries (113, 193).

Effects of intravenous injections of adrenaline, histamine, ACh, and nicotine have been studied on the blood pressure of anaesthetized eels (264). The reactions observed were in general similar to those obtained in experiments with warm-blooded animals.

Amphibians: Most studies on vascular reactions in amphibians have been made on the perfused hindleg preparations (299) and the tongue. The arterioles of the frog tongue are constricted by adrenaline and dilated by histamine (195). Reflex effects are elicited by perfusion of the frog heart with 5-HT (340).

3. Gastric and intestinal muscle. Cyclostomes: The intestinal muscles are poorly developed in both the hagfish and lamprey. In the hagfish (*Myxine*), the intestine is contracted by ACh (10^{-8} to 10^{-5}) and relaxed by adrenaline (10^{-7} to 10^{-5}) and atropine (103, 106). The rectal part of the intestine of the lamprey (*Lampetra*) shows strong rhythmic contractions after 5-HT (10^{-4}) (180).

Elasmobranchs: All parts of the stomach and intestine are stimulated by ACh, pilocarpine, and adrenaline (82, 232, 270), but high doses of the latter may cause inhibition.

Teleosts: Muscles of the stomach (*Salmo*) are contracted by both ACh and adrenaline (55). The intestines of many fishes are contracted by ACh but relaxed by adrenaline (55, 82, 103, 386). In the tench (*Tinca*), the intestinal wall contains both smooth and striated muscle, and therefore gives a double response to ACh (10^{-5}). In addition to the slow smooth muscle contraction, there is an initial fast contraction of the intestinal striated muscle. The fast response is blocked by curare (251).

In their sensitivity to histamine and 5-HT, fish intestines show remarkable variations among species. Especially high sensitivity to 5-HT (10^{-9} to 5×10^{-9}) is found in the flounder (*Pleuronectes*), cod (*Gadus*), trout (*Salmo*), and pike (*Esox*) (55, 103, 351). Histamine is active on the intestine of some species, but not of others. In all teleost species investigated the intestine was contracted by substance P (103, 136a).

Amphibians: The intestine of a frog (*Rana pipiens*) is contracted by ACh and by 5-HT (10^{-8}), and relaxed by adrenaline (113, 323). Adrenaline also relaxes the intestine of *Xenopus* (African clawed toad) and the rectum of the toad (*Bufo regularis*) (93). The toad rectum (*B. arenarum*) is said to be 30 to 200 times more sensitive to adrenaline than noradrenaline (300). The intestine of the frog (*Rana esculenta*) is relaxed by adenine (302).

Innervation of the alimentary canal: The autonomic innervation of the intestine has been studied in the hagfish (106), in fish (55, 56, 57, 273, 386), and in amphibians (131, 174, 281, 387).

In the hagfish (*Myxine*) and in teleosts the intestine has a single nerve supply, in the hagfish from the vagus nerve (106) and in teleosts from the sympathetic system (55, 271) (Fig. 1). Nevertheless, ACh and adrenaline act as antagonists in a similar way as on the doubly innervated mammalian intestine. In cyclostomes

and fishes, it is impossible to distinguish functionally between "parasympathetic" and "sympathetic" nervous systems, since in these lower vertebrates the autonomic nerves probably are mixed nerves containing different kinds of fibres. The presence of adrenergic elements in the vagus trunk of the hagfish (*Myxine*) and the cod (*Gadus*) is indicated by their content of catecholamines (102). Also, the postganglionic vagus nerve fibres of the trout (*Salmo*) stomach probably are adrenergic, while splanchnic nerve fibres to the stomach and intestine may be cholinergic (*cf.* Fig. 1). Experiments on the trout gut have shown a good correlation between nervous action and pharmacodynamic activity (55).

Phylogenetically, an antagonistic double autonomic innervation seems first to occur in amphibians, for in a salamander (*Necturus*) and in the frog (*Rana*) the stomach muscles are inhibited by the vagus nerve and stimulated by the sympathetic nerves (281, 323).

4. Other smooth muscle. The frog lung is contracted by ACh and relaxed by adrenaline, noradrenaline and 5-HT (43, 80). The sensitivity to ACh is extremely high, but the preparation reacts also to slight mechanical stretch.

The muscularis mucosae of the swimbladder of fish (cod, eel, *etc.*) consists of two kinds of smooth muscle (Fig. 1). One type is contracted by adrenaline (10^{-8} to 10^{-6}) and noradrenaline but unaffected by ACh; the other is relaxed by the catecholamines and contracted by ACh (5×10^{-6}). Both types of muscle are supplied by fibres from the vagus nerve (107).

The gallbladder of the hagfish (*Myxine*) and the gallbladder, urinary bladder and the ovaries of a teleostean fish (*Uranoscopus*) are contracted by ACh and relaxed by adrenaline (110, 386).

In the mesentery of elasmobranchs (dogfish, skate) a sheet of smooth muscle is found. No extrinsic innervation of this structure is known. Contractions are elicited by ACh, adrenaline, and pituitrin (296).

The muscles of the urinary bladder of the frog (*Rana*) are stimulated by pilocarpine, choline, nicotine, and phenethylamine. The effects of adrenaline are variable, whereas histamine has no effect (5).

Iris. Teleosts: In the angler fish (*Lophius*) and in *Uranoscopus* perfusion of the excised eye with dilute solutions of ACh, pilocarpine, or adrenaline constricts the pupil. The effects of ACh and adrenaline are blocked by atropine and ergotoxine, respectively (385).

Amphibians: Adrenaline causes dilation of the pupil in the frog (89, 235). Acetylcholine has an uncertain effect on the frog eye, but constriction of the excised iris has been observed (153).

In the axolotl (*Ambystoma*) ACh causes constriction of the pupil; sensitivity to ACh is increased by removal of the cornea, which has ChE activity (13). After removal of the cornea the iris reacts to ACh in concentrations of 10^{-10} to 10^{-9}.

Reptiles: Like birds, reptiles possess striated muscle in the iris. Acetylcholine (10^{-6} to 10^{-3}) causes constriction, which is increased by eserine. Nicotine (10^{-5} to 10^{-4}) gives the same response as ACh (turtle). The responses to ACh and to electrical stimulation are reduced by curare (10^{-6}) and by atropine in high doses (14, 256).

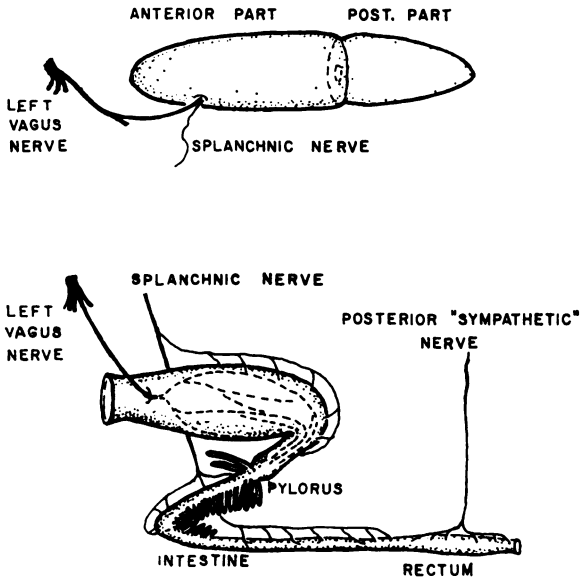

FIG. 1. Examples of visceral innervation in teleosts.

Upper figure: Swimbladder (*Labrus*) with its smooth muscle layer divided into two parts, both innervated from the vagus nerve. The anterior part is contracted by adrenaline and noradrenaline and contains considerable amounts of the latter substance (102). The posterior part is relaxed by catecholamines (107). See p. 285.

Lower figure: Gut of the trout (*Salmo trutta*), after Burnstock (55). The stomach, which receives vagal as well as sympathetic fibres, is contracted by adrenaline. The intestine, innervated solely from the sympathetic system, is relaxed by adrenaline. See p. 285.

Innervation of the iris muscles: In fishes the pupil is constricted by sympathetic impulses and dilated by the oculomotor nerve (385). In amphibians, mydriasis is brought about by both the sympathetic system and by oculomotor nerve (209, 334). Sympathetic denervation causes supersensitivity to adrenaline (153). The striated muscles of the iris in the reptilian eye are innervated by the oculomotor nerve (14).

Photoexcitation of the iris muscles: The pupils of the isolated eyes of the eel (*Anguilla*) and of the frog (*Rana*) constrict in response to light. It is uncertain whether the response represents direct photoexcitation of the iris muscles, or is due to an intraocular reflex. The fact that isolated frog iris preparations respond to light seemingly excludes the possibility of a nervous reflex action (31). However, Magnus more than 60 years ago showed that the response to light is blocked by atropine, and from this he concluded that nervous elements situated in the iris muscle are responsible for the reaction to light (235). This explanation may still hold.

5. Somatic muscles and their derivatives. a) Skeletal muscle. It has long been known that in amphibians and reptiles the striated muscles show two different kinds of response to ACh: muscles composed of slow or tonic fibres produce a prolonged contraction, while fast or phasic muscles respond with a twitch-like

contraction (355). The fast and the slow fibres have a different type of motor innervation. The two neuromuscular systems have been investigated mainly by electrophysiological methods.

Cyclostomes: The body wall muscles of the hagfish (*Myxine*) give a contraction with ACh which is counteracted by tubocurarine (113). Morphological and physiological studies have revealed that in *Myxine* there are two distinct kinds of muscle fibre. Only the "fast" fibre system responds to ACh, while the "slow" fibres (in similarity with the heart muscle of the same animal) seem to be ACh-insensitive. The fast fibres have an endplate region at each pole. The slow fibres have a more diffuse kind of motor innervation; they are also richer in mitochondria and respiratory enzymes than the fast fibres (11). In the lamprey two kinds of striated muscle fibres have been demonstrated histologically (282).

Elasmobranchs: The frequency of the myogenic movements of early dogfish embryos is increased by ACh (10^{-6}), while eserine is without effect. Cholinesterase cannot be demonstrated in the early stages (156).

Teleosts: Morphologists long have distinguished between white and red muscles in fish. The red muscles are rich in respiratory enzymes and contain myoglobin. Investigations with intracellular electrodes have shown that in many respects the white and red muscles in fish (*Ophiocephalus*) resemble fast and slow neuromuscular systems of amphibians (336). Electrophysiological observations of the effects of ACh, eserine, and curare on fish skeletal muscle indicate the presence of cholinergic transmission (214).

Amphibians: The "fast" and "slow" neuromuscular systems have been extensively studied in amphibians. Muscles like the sartorius and the adductor longus belong to the fast system, while the rectus abdominis, on which pharmacological assays of ACh are generally performed, consists predominantly of slow fibres (216, 217). Fast fibres are stimulated by ACh at the endplate region only, where application of as little as 10^{-15} mole of ACh produces transient depolarization and a muscle spike (64). Slow fibres probably contain numerous and diffusely distributed neuromuscular junctions (217). The spontaneous discharge of miniature endplate potentials in fast muscles is thought to be due to a quantal release of ACh from the motor nerve endings. The miniature potentials, which have an amplitude of 0.25 to 0.5 mV, increase in size and duration after neostigmine (10^{-6}), and are reduced in amplitude or blocked by tubocurarine. After degeneration of the motor nerves the sensitivity of the frog fast muscle to ACh greatly increases, probably due to an increase in the ACh-sensitive area of the muscle fibres (39).

Reptiles: The retractors of the head and the limbs of the turtle (*Emys*) are slow fibre muscles which give prolonged contraction with ACh (355). The special sensitivity of the endplate region to ACh was demonstrated years ago on what were probably fast muscles of a lizard (*Lacerta*) (46).

b) Lymph hearts. Lymph hearts occur in cyclostomes, teleosts, amphibians and reptiles. The coccygeal lymph hearts in amphibians (*Rana*, *Bufo*) consist of specialized striated muscle. They are particularly sensitive to ACh, muscarine, nicotine and *d*-tubocurarine; *d*-tubocurarine (10^{-5} to 10^{-4}) causes diastolic stand-

still; block of lymph heart contractions is also produced by atropine in high concentration. The rhythmicity of the lymph hearts is neurogenic and disappears after denervation, but automaticity has been claimed to reappear in transplanted lymph hearts. The electrical activity of the amphibian lymph hearts resembles closely that of the amphibian slow muscle system (65, 77, 168, 303).

c) *Electric organs.* Although electric organs are supposed to have evolved independently in different groups of fishes, they probably all consist of modified skeletal muscle (18).

Elasmobranchs: The plate-like structures called electroplaques in skates (*Torpedo*, *Raja*) are homologous with motor endplates. The electroplaques are excitable by stimulation of the nerve or by ACh, but do not respond to direct electrical stimulation. Discharge of the electric organ after injection of ACh has been demonstrated in the electric ray (*Torpedo*) (115).

Teleosts: In the electric eel (*Electrophorus*) the electroplaques consist of two morphologically indistinct components, corresponding, respectively, to a motor endplate and to a spike-conducting electrically excitable muscle membrane. After treatment with curare the electroplaques are still directly excitable. Eserine, *d*-tubocurarine, DFP, and procaine inactivate, but do not depolarize, the synaptically excitable membrane, whereas it is depolarized and inactivated by ACh, carbamylcholine, and decamethonium (8, 9).

A review (149) and a symposium (65a) have recently been published on the physiology of electric organs.

6. Pigment effectors (Fig. 2). The melanophores (melanocytes) are effector cells containing dark pigment granules. The granules may be "dispersed" or "concentrated" in the cells. Theories have been proposed to explain the mechanism of these movements (128); it has been assumed that they are associated with ionic exchange between the intracellular and extracellular media (381). Pharmacological studies of melanophore responses have been made on intact animals and on isolated pieces of skin, in some cases with photoelectric recording methods (338, 382).

Teleosts: Catecholamines are potent pigment-concentrating agents. In the cod (*Gadus*) and the guppy (*Lebistes*), noradrenaline is highly active; the melanophores in isolated scales of the guppy react to noradrenaline in concentrations of 10^{-11} to 10^{-10} (113). Other concentrating agents are ephedrine and thyroid extracts, whereas ACh, eserine, pilocarpine, atropine, and sympathicolytic agents cause dispersion (280, 308). Intraperitoneal injection of hexamethonium or decamethonium causes distinct melanophore responses, but the mechanisms involved are obscure (378).

Darkening effects of reserpine (341) and LSD 25 (36) have been observed on fish melanophores. The teleostean melanophores are very sensitive to the ion ratios Na^+/K^+, potassium ions causing concentration and sodium ions dispersion. For rainbow trout (*Salmo irideus*), a balanced saline solution should contain 0.8 % NaCl and 0.1 % KCl (308). Rhythmic pulsation of melanophores is induced by barium ions (280).

Amphibians: Frog melanophores are dispersed by pituitary hormones (*alpha* and *beta* MSH, ACTH) and by caffeine. Concentrating agents include adrenaline,

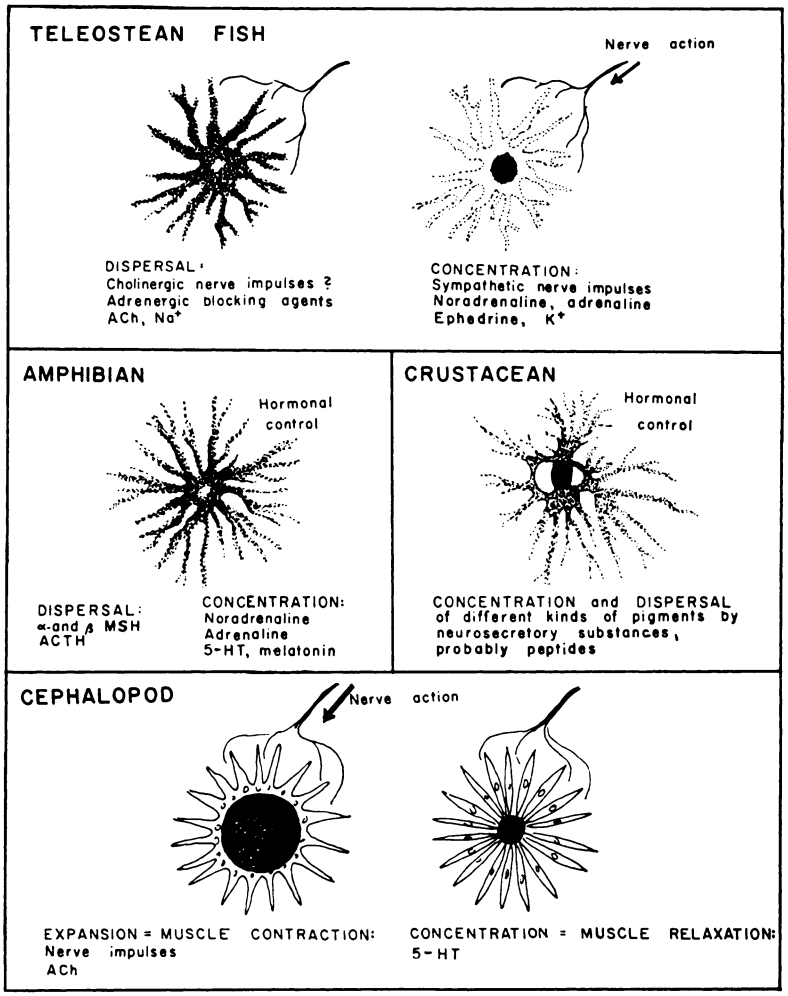

FIG. 2. Pigment effectors.

In teleosts (p. 288), amphibians (p. 288), and crustaceans (p. 297) the chromatophores have movable pigment granules. Cephalopods (p. 297) have chromatophores with muscles. [Diagram modified from Bozler (42a)].

In teleosts and cephalopods the chromatophores are controlled nervously, but also to some extent influenced by hormones.

noradrenaline, 5-HT, melatonin (N-acetyl-5-methoxytryptamine), cortisol (hydrocortisone), and triiodothyronine (222, 223). The actions of adrenaline, noradrenaline, and 5-HT are reversed by ergotamine (221, 381, 382). In the toad (*Bufo*), and on melanophores of the axolotl (*Ambystoma*) in tissue culture, adrenaline is more active than noradrenaline (332, 333, 390), but in the frog noradrenaline is more potent (224). In *Xenopus*, catecholamines cause dispersal of pigment instead of concentration. On specimens kept for a long time on a white

background, isoprenaline has a stronger darkening effect than noradrenaline or adrenaline. The receptors of the *Xenopus* melanophores for catecholamines are thought to be of the *beta* type. Pigment concentration obtained with LSD 25 is seen only in whole animals, not in isolated skin, and is therefore supposed to be due to a central action (54, 146, 276).

Reptiles: Isolated pieces of the skin of the lizard *Anolis* show the following responses: Dispersal of pigment is caused by pituitary hormone (MSH), whereas adrenaline, tyramine, and 5-HT cause concentration. Experiments with substances thought to interfere with the cellular utilization of energy, such as uncoupling agents (*e.g.*, 2,4-dinitrophenol), have given conflicting results (165).

Biological control of melanophores: In teleosts the melanophores are controlled by tonically active sympathetic nerves, sectioning of which causes darkening. The existence of dispersing nerve fibres has been much debated but is uncertain (158, 280). In amphibians melanophores are regulated by hormones from the hypophysis. The paling effect observed in the toad (*Bufo*) after stimulation of peripheral nerves is probably due to liberation of adrenaline-like compounds in the skin (333). In the lizard (*Anolis*) the colour change is humorally controlled, but the lively play of colours in the chameleon is regulated by nerves (128, 280).

7. Glands. a) Digestive glands. Gastric and pancreatic secretion has been studied in elasmobranch fishes (rays, dogfish), but no agent which stimulates gastric secretion has been found (32). Inhibitory effects observed may be explained as due to vasomotor responses.

In the frog (*Rana*), flow of gastric juice is produced by adrenaline or histamine. While the secretion produced by adrenaline contains both pepsin and acid, histamine causes acid secretion only (132). Submicroscopic changes in the acid cells in the stomach of the toad (*Bufo*) have been observed after stimulation with histamine (354). In the salamander (*Necturus*), no influence of injections of ACh, pilocarpine, atropine, or histamine on gastric secretion could be observed (131, 133).

b) Gas gland of the swimbladder. Yohimbine given intramuscularly increases the oxygen content in the swimbladder of the cod (*Gadus*), whereas adrenaline has the opposite effect. The effects are probably mediated by smooth muscle and vasomotor responses (107, 320).

c) Skin glands. In the African clawed toad (*Xenopus*) injection of adrenaline causes secretion of the skin glands (54). Similar secretion is obtained in the toad (*Bufo*) after stimulation of peripheral nerves (333).

The light-emitting organs (photophores) in the skin of certain marine fishes are stimulated by adrenaline (273).

8. Ciliary epithelium. The ciliary activity of the frog esophageal mucosa is stimulated by ACh, which is supposed to act as a local hormone (206).

9. Ion transport systems. Results with anticholinesterase agents suggest that the active transport of sodium ions in a variety of tissues is in some way dependent on cholinesterases. Effects of atropine and curare on the active transport of sodium in the frog skin have been described (197, 200, 202). Substances like adrenaline, pitressin, strophanthin, and metabolic inhibitors also influence ion

transport in the frog skin (163, 171, 375). Vasopressin has been shown to increase the short-circuit current across the wall of the toad urinary bladder (44).

10. Nervous system. The Siamese fighting fish (*Betta splendens*) and other fishes show characteristic behavioural disturbances when treated with psychotropic drugs (1, 266, 275, 298). Tryptophan prolongs LSD-induced behaviour, whereas reserpine has a restorative effect (189). In *Xenopus*, LSD causes concentration of pigment in the skin by a central action.

Several studies have been made on the effects of certain drugs on sense organs, for instance muscle spindles (327) and touch receptors in the frog skin (176). Effects of amino acids on the perfused spinal cord of the toad have been investigated (73).

Isolated frog spinal ganglia have been used in experiments where the effects of quaternary ammonium ions on ganglion cells were investigated. When sodium in the medium was replaced by choline, TEA, or TMA, the excitability of the cell membrane was maintained but the action potential was prolonged. It is surprising that electrical activity can be maintained in the sodium-free medium, since the quaternary ammonium ions do not seem to act as charge carriers (205).

B. Invertebrates

General remarks. The vertebrates are a fairly homogeneous group of animals, but in contrast, the invertebrates are separated into widely different phyla (230). Schemes have been put forward in order to show possible phylogenetic relationships (331). Some of the invertebrate groups consist of small or rare animals, which have never been studied physiologically.

In general the invertebrates possess the same kind of effectors as the vertebrates, but, for example, smooth and striated muscles in invertebrates do not necessarily resemble the tissues bearing those designations in vertebrates. Many invertebrates are very small animals and in order to study pharmacological effects it is necessary to adopt special methods, such as immersing whole animals in drug solutions. Electrophysiological methods offer great possibilities for the study of small animals and single cells. By means of intracellular electrodes, it has been possible to investigate the effects of GABA and other substances on protozoans (208), and to study pharmacological responses of single neurons (142). The use of specially composed physiological saline solutions has been of utmost importance in pharmacological work on crustaceans and other invertebrates (277).

1. Heart and pulsatile vessels. Hearts are found in arthropods, molluscs, and a few other groups. Reviews on the cardiac physiology of the hearts of invertebrates have been published recently (184, 210, 211, 212, 244, 292). Cardiac muscle fibres in arthropods are striated, while those in molluscs resemble smooth muscle.

Arthropods. a) Large crustaceans (crab, lobster, crayfish). Acetylcholine: Low concentrations of ACh accelerate the heart. The action resembles that obtained by stimulation of excitatory nerve fibres, but while the action of applied ACh is blocked by atropine, the effect of nerve stimulation is not. Eserine potentiates

the effect of ACh. After eserinization the heart of lobster (*Homarus*) reacts to ACh in a concentration of 10^{-9} (75, 330, 357).

Nicotine and pilocarpine excite the heart in the same way as ACh. The heart is also stimulated by anticholinesterase agents. This possibly indicates that ACh functions as a neurohumor in the pacemaker mechanism (210). In high concentrations ACh may cause inhibition (301).

5-Hydroxytryptamine: The heart of a crab (*Cancer borealis*) is stimulated by concentrations of 10^{-9} to 10^{-8} M (125, 362). The stimulatory effect of 5-HT is about 100 times greater than that of adrenaline, which is also excitatory (210, 356).

Other substances: Factor I (inhibitory factor from mammalian brain) and GABA cause diastolic arrest in the lobster, whereas glutamate and aspartate are stimulatory in doses down to a few micrograms (92, 119). Extracts of the pericardial organs have a strong stimulatory effect, probably due to the combined action of an unidentified peptide and small amounts of 5-HT (245).

b) *Small-sized crustaceans.* In *Daphnia* (water flea) and *Artemia*, common laboratory animals, the heart is accelerated by adrenaline and inhibited by ACh (127, 210).

c) *Xiphosura (king crabs and scorpions).* The heart of *Limulus polyphemus* is a classical object in cardiac physiology, but there are few modern studies on its pharmacological responses.

Acetylcholine in high doses (10^{-4}) has a stimulatory effect which is potentiated by eserine. No effect was observed on ganglion-free portions of the heart muscle, or on non-innervated embryonic hearts (63). Probably ACh affects only the ganglia, which are rich in cholinesterase (326). Adrenaline is supposed to stimulate both the ganglia and the muscle (63, 137, 326). 5-Hydroxytryptamine appears to have an inhibitory effect (53). The heart of a scorpion (*Palamneus*) reacts as a myogenic vertebrate heart, in that it is stimulated by adrenaline and inhibited by ACh (188).

d) *Insects.* Most insect hearts are difficult to record from owing to their small dimensions. The hearts of large aquatic species have been studied; in these, adrenaline stimulated and ACh had no effect (254). In other investigations on the heart of the cockroach (*Periplaneta*), stimulation was obtained with ACh, nicotine, lobeline, and adrenaline (210). Neurohormonal factors affecting insect hearts have been demonstrated. Apparently the biological control of heart movements in insects is different in different species (76, 183, 318, 347).

Innervation of the arthropod heart: It is generally accepted that in large crustaceans (crabs, lobsters, crayfish) and in the king crab (*Limulus*) the heart beats are initiated by rhythmic activity in cells of a cardiac ganglion. The ganglion in its turn is influenced by extrinsic acceleratory and inhibitory nerves. A detailed study of the effects of stimulation of the cardiac nerves has led to the conclusion that an inhibitory transmitter substance is inactivated at a faster rate than the accelerating transmitter (120). Nothing is known of the nature of the hypothetical transmitters, but there is a possibility that the presynaptic acceleratory nerve fibres are cholinergic (210) while the inhibitory nerves might act by liberat-

ing "Factor I." γ-Aminobutyric acid simulates inhibitory nerve action, but this amino acid has not been found in crustaceans (243).

Annelids (segmented worms): Annelids have a closed blood circulation in which tiny contractile vessels function as hearts. Hearts of the earthworm (*Lumbricus*) and the lugworm (*Arenicola*) are stimulated by ACh (10^{-9} to 10^{-7}) and nicotine. The effect of ACh is potentiated by eserine and blocked by atropine. The hearts of the earthworm are also accelerated by adrenaline (194, 292). In the leech (*Hirudo*), pulsatory vessels are accelerated by adrenaline but inhibited by muscarine (138). The leech seems to possess accelerating and inhibitory nerve control of its pulsatile vessels. A neurogenic origin of heart beats has been suggested for the earthworm.

Molluscs (snails, mussels, squids, and octopodes): The heart consists of a ventricle and one or two auricles. Somewhat more complicated pulsatile structures are found in cephalopods (squid, octopus). The usual technique for study of the hearts has been to perfuse the isolated ventricle or to suspend the ventricle in a bath of saline solution (360, 370). Pharmacological effects on the circulatory system of intact animals (*Octopus*) have also been studied. Some typical drug effects on molluscan hearts are listed below.

ACh: The effect is inhibitory (decrease in amplitude and frequency and, with large doses, diastolic arrest), but there is a wide range of sensitivity (287). The heart of the clam *Venus mercenaria* is probably the most sensitive of all invertebrate preparations known to react to ACh (287, 359) as it responds to concentrations as low as 10^{-12}, but the hearts of several other species are also very sensitive (287, 360, 361). At the other end of the sensitivity scale is the heart of the fresh-water mussel, *Anodonta*, which either does not respond to ACh or reacts only to high doses (108, 339).

The effect of ACh is only slightly potentiated by eserine, possibly because the cholinesterase activity of the heart is low (388).

In contrast to conditions in vertebrates, atropine usually does not block the effect of ACh (75, 215, 304), but Mytolon (benzoquinonium) is a very effective ACh-blocking agent on the heart of *Venus mercenaria* (344, 358).

5-HT: This and other indolealkylamines have a remarkably strong excitatory effect on all molluscan hearts tested (95, 98, 238, 360, 361). Methylated derivatives of 5-HT, such as bufotenine (N,N-dimethyl-5-hydroxytryptamine), are more active than 5-HT on the heart of the clam, *Venus mercenaria* (52, 344).

The action of 5-HT on the molluscan heart is blocked by ergot alkaloids, *e.g.*, LSD 25 and ergotamine, which cause irreversible increase in rate and amplitudes, presumably by occupying the receptor sites for 5-HT (19, 215, 362, 365, 370). Bromo-LSD antagonizes the action of both 5-HT and LSD 25 (365). High concentrations of 5-HT or other tryptamine derivatives render the *Venus* heart tachyphylactic to low doses (147).

Catecholamines stimulate the heart of some species (212, 292), but have little or no inhibitory action on other species (98, 101, 108, 212). High sensitivity is found in cephalopods (19, 112, 215). Thus the heart of a squid (*Eledone cirrosa*) reacts to adrenaline and noradrenaline at about 10^{-10} (112). In high doses adren-

aline may produce increase of tone and diminished amplitude (147, 215), effects which are also seen after osmotic changes in the medium (286) or high doses of 5-HT (147). In intact non-anaesthetized cephalopods, *e.g.*, *Octopus*, large doses of adrenaline and noradrenaline depress blood pressure and heart activity, effects probably elicited *via* nervous pathways (178).

Histamine has unspecific and variable effects on the heart of mussels (288).

Unidentified cardioactive substances have been demonstrated in tissue extracts of the snails *Helix aspersa* and *Aplysia* (141, 190).

Innervation of the molluscan heart: Accelerating and inhibitory cardiac nerves have been found in many species. Nervous inhibition is blocked by curare (212) or Mytolon (benzoquinonium) (362), indicating a cholinergic transmitter. The transmitter of the accelerating nerves might be 5-HT (365). Both ACh and 5-HT are present in molluscan nerve tissue (255, 366).

Echinoderms: Blood vessels in a sea cucumber (holothurian) pulsate with a slow frequency, 4 to 5.5 beats/min. The pulsations are accelerated by adrenaline (10^{-5}) and inhibited by low concentrations of ACh (10^{-14}) and nicotine (10^{-11}) (294, 384). The responses seem to be somewhat irregular.

Tunicates (ascidians, primitive chordates): The ascidian heart shows a periodic reversal of the heart beat, a phenomenon which has intrigued biologists for a long time. No specific effects from ACh or eserine have been observed on the heart of *Ciona intestinalis*. The ascidian heart is probably devoid of nerve elements (211, 213), but there are conflicting views on this point.

2. Locomotor and body wall muscles. As a rule the effects of drugs have been studied not on isolated pure muscle tissue, but on what may be called neuromuscular preparations. Sometimes these preparations, like the "dorsal muscle of the leech," represent half the bulk of the animal.

Coelenterates (sea anemones, jellyfish, corals): Muscles of sea anemones are capable of quick or slow contractions (169). Stimulatory effects have been observed after tryptamine, 5-methoxytryptamine, tyramine, and adrenaline in high concentrations, but there is no indication that these substances are of functional importance (312, 313). Acetylcholine has no effects whatsoever. The coelenterate muscles are innervated by a nerve-net.

Worms and worm-like animals. a) Trematodes, nematodes (primitive worms). In the liver fluke (*Fasciola*), ACh, carbaminoylcholine, and eserine cause either contraction or paralysis, and adrenaline-like amines stimulate rhythmical activity (66). Biochemical effects of 5-HT on phosphorylating systems have been observed (236). The stimulatory effects of ACh (10^{-6} to 10^{-5}) and nicotine on nematode muscle (*Ascaris*) are abolished by tubocurarine and cocaine, but not by atropine (30).

b) Annelids (segmented worms). The body wall muscles of these animals are extremely sensitive to ACh, which produces a prolonged contraction potentiated by eserine. The eserinized dorsal muscle of the leech (*Hirudo*) reacts to doses of ACh down to concentrations of 0.5 to 2×10^{-9} (335). Almost the same sensitivity is found in the earthworm (*Lumbricus*) and in other species (21, 22, 27, 272, 329, 383). The action of ACh is blocked by curare in some species but not in others (27, 194, 272). Eserine potentiates the effect of indirect stimulation, and an ACh-like

substance is liberated during stimulation (25, 27). Thus, the innervation of the body wall muscles is probably cholinergic.

5-Hydroxytryptamine has a relaxing effect on annelid muscles (290, 316). Adrenaline is without effect (22, 260, 272) unless given in very high doses.

c) Sipunculids (annelid-like marine worms). The proboscis retractors of *Phascolosoma* (293, 295, 296, 297) are short-fibred non-striated muscles innervated by parallel nerve fibres. The action potential has a fast and slow component. Acetylcholine (5×10^{-5}) causes contraction. The muscle potential is enhanced by eserine at 10^{-7} to 10^{-6} and reduced or blocked by atropine and tubocurarine (10^{-5} to 10^{-4}).

Arthropods. a) Crustaceans (crab, lobster, crayfish). The unique mode of innervation of the striated muscles of crustaceans by motor and inhibitory nerve fibres allows study of both motor and inhibitory phenomena at neuromuscular junctions in easily accessible preparations (376). The use of adequate physiological saline solutions has made possible electrophysiological work on such delicate structures as single axons. A quantal release of an excitatory transmitter from motor nerve endings is indicated by the appearance of spontaneous miniature potentials (84). Other observations indicate the release of an inhibitory transmitter from inhibitory nerve endings.

Acetylcholine, nicotine, eserine, atropine, and curare are ineffective at neuromuscular junctions (91, 376), but certain amino acids and tissue extracts are able to excite or inhibit crustacean muscles. L-Glutamic acid depolarizes at neuromuscular junctions, causing contraction. The action has been compared with that of ACh on vertebrate muscle, but the concentrations required are unphysiologically high (155). Contraction is also obtained with an extract of crustacean tissue, and the active substance has been suggested as a possible transmitter (201).

γ-Aminobutyric acid and a few other related amino acids inhibit contraction of the opener muscle of the claw of the crayfish (*Orconectes*). Probably GABA combines with an inhibitory receptor in the muscle membrane.

Factor I (extract from mammalian brain) also produces inhibition. The effects of GABA, Factor I, and inhibitory nerve stimulation are abolished by picrotoxin (306).

Experiments involving replacement of the external sodium with certain quaternary ammonium ions, such as choline and TEA, show an increase and prolongation of the action potential of crustacean muscles (114).

b) Insects. Insect muscles show physiological and histological resemblances to crustacean muscles. Electronmicroscopic demonstration of synaptic vesicles and electrophysiological observations of spontaneous miniature potentials indicate the existence of an excitatory transmitter substance (328, 348). Curare, ACh and some other drugs do not affect neuromuscular transmission (309). Nicotine and venom of the wasp, *Habrobracon*, inhibit somatic muscular activity presumably by blocking at neuromuscular junctions (35). A blocking effect by large doses of tryptamine and 5-HT has been observed on the jumping leg of a grasshopper (162).

The blood of insects contains trehalose instead of glucose as the main carbo-

hydrate. The sugar concentration influences the frequency of the wing beats in blowflies (69). The peculiar ionic composition of insect blood, with its high content of potassium, magnesium and amino acids, may be of significance in interpreting the results of electrophysiological work (169).

c) Xiphosura (king crabs). The striated muscles of *Limulus* have a double motor innervation, but just as in insects no inhibitory innervation seems to have been demonstrated (170).

d) Onychophora. These slug-like animals have been considered as "missing links" between annelids and arthropods. The muscles of *Peripatopsis* are contracted by ACh (3×10^{-6}), the effect being enhanced by eserine. Atropine, curare and adrenaline have no effects (104).

Echinoderms (sea urchins, sea cucumbers, sea stars): The muscles of echinoderms are extremely sensitive to ACh. The effect of ACh (10, 22, 23) and electric stimulation (83, 291) is potentiated by eserine and abolished by atropine or curare. An ACh-like substance is liberated during electric stimulation (24).

A relaxing effect of adrenaline (10^{-4}) (83) and a contracting effect of histamine (22) have been seen. Examples of preparations studied are longitudinal body wall muscles, pharyngeal retractors of sea cucumbers (holothurians) and the retractor muscle of Aristotle's lantern of the sea urchin (*Echinus*).

Tunicates (ascidians, primitive chordates): In spite of their relationship to vertebrates the neuromuscular system of ascidians is little developed. The sensitivity to ACh is extremely low, and eserine has no effect (20, 24). The muscles in some respects present a parallel to those of coelenterates. Cholinesterase is present in early neural stages, but disappears during metamorphosis (86).

Molluscs (snails, mussels, squids, octopodes): The muscles, which usually are of a smooth type, can make fast (phasic) or slow (tonic) contractions. Whether tonic contraction is due to a "catch" mechanism or to continuous nerve activity has been much debated (61, 181, 182, 342, 343).

The anterior byssus retractor muscle (ABRM) of the blue mussel (*Mytilus*) shows the following reactions: *ACh* produces a prolonged contraction which is potentiated by eserine; the effect is depressed by propantheline and methantheline and to a lesser degree by atropine, benzoquinonium, tubocurarine and hexamethonium (61, 343). *5-HT* causes relaxation. It also alters the response of the byssus retractor muscle to ACh. In the presence of 5-HT, ACh produces spike-like potentials and rhythmic contractions.

Electrical stimulation of the ABRM may produce tonic or phasic contraction, or inhibition. The electrically induced phasic contraction is blocked by ACh antagonists, which indicates the presence of a cholinergic innervation (342, 343).

In the ABRM, ChE activity has been found in nerve fibres (61).

In marine snails (*Buccinum, Busycon*) a group of red myoglobin muscles in the buccal region is characterized by a high tendency to rhythmical activity after treatment with ACh and 5-HT simultaneously. Acetylcholine alone provokes prolonged contraction (111, 161). Atropine (0.5×10^{-5}) blocks the effect of nerve stimulation. Eserine prolongs the relaxation period after a tetanus. A cholinergic system is therefore probably involved in the neuromuscular excitation of the buccal muscles of *Buccinum* (109a).

3. Visceral muscles. In a few cases mechanical recordings have been made from the intestine and other visceral organs of invertebrates.

Echinoderms: The cloacal muscles of sea cucumbers (holothurians) show rhythmical, heart-like activity initiated by the nervous system (48, 384). The inhibitory effect of adrenaline (10^{-5}) is reversed by cocaine, atropine, and ergotoxine. The oesophagus of the sea urchin is contracted by ACh and inhibited by "Factor I," but not by GABA (126, 164).

Arthropods (crustaceans, insects): Arthropod visceral muscles are striated. Contractions of the crayfish intestine caused by ACh (10^{-8}), adrenaline, or noradrenaline (10^{-6}) are prevented by GABA and related amino acids, and by "Factor I." The action of ACh is furthermore blocked by atropine. It is thought that ACh acts by stimulating nerve cells (121). Striated muscles other than the intestinal muscles in the crayfish do not respond to ACh.

The intestine of a mosquito larva (*Corethra*) is stimulated by ACh, eserine, and histamine, and inhibited by adrenaline (139). Methacholine (Mecholyl) seems to be more active on insect intestine than ACh (204). Peristalsis of the foregut of *Galleria* larvae is stimulated by 5-HT and adrenaline (35). Rhythmically contracting Malpighian tubules of insects react with increase of amplitude and decrease of frequency to ACh (10^{-4}) and veratrine (2×10^{-5}), but are not affected by adrenaline (279).

Molluscs: In molluscs, smooth muscles seem to have more or less taken the place of connective tissue, and most internal organs contain smooth muscles. In a cephalopod (*Loligo*) many visceral organs are contracted by both adrenaline and ACh, the former more active (19).

Annelids (segmented worms): The intestine of the earthworm (*Lumbricus*) might not seem a promising object for investigation, but this small organ nevertheless offers a most striking parallel to the mammalian intestine in its responses to drugs and in its double, antagonistic type of nerve supply (258).

4. Pigment effectors (chromatophores) (Fig. 2, p. 289). *Arthropods* (crustaceans, insects): Physiologically regulated chromatophores are found in crustaceans (crabs, shrimps) and in rare cases in insects (140, 280). The pigments of crustacean chromatophores are red, yellow, black, or white. Very probably each kind of pigment is controlled by a specific set of dispersing and concentrating hormones produced by neurosecretory cells in the nervous system. The exact number of hormones is not known. Different pigment activating substances, probably peptides, can be separated by chromatography and electrophoresis (62, 87, 116, 364). The hormones are extremely active in very dilute solutions, but no cells other than chromatophores are affected.

Molluscs: A peculiar pigment effector system which allows extremely rapid changes of colour is found in cephalopods (squid, octopus). The skin contains pigment bladders surrounded by radially oriented smooth muscles. At contraction of the muscles the animals darken, because the bladders are flattened and expanded. The muscles are controlled by nerves. Effects of drugs on colour change in cephalopods have been described but, due to the abundance of nerve elements in the skin, the results are difficult to interpret (185, 280, 311). On isolated pieces of skin of *Octopus*, 5-HT produces paling and ACh darkening (185).

5. Glands. Molluscs: Perfusion studies have been performed on the large posterior salivary glands of *Octopus*. Addition of 5-HT to the perfusion fluid resulted in secretion of a saliva free from 5-HT and protein. During electrical stimulation, on the other hand, a fluid with a high concentration of 5-HT was produced (28). The gland contains much muscle tissue, which might have complicated the experiments.

Annelids (segmented worms): Acetylcholine and nicotine evoke luminescence in gland-like organs of some species (273).

6. Ciliary epithelium. Molluscs (mussels, clams, snails): Ciliary activity in gill plates of mussels (*Mytilus*) is stimulated by ACh at low concentrations. Other stimulatory agents are 5-HT, adrenaline, and the alkaloid veratrine. Slowing of cilia is caused by atropine, tubocurarine, and high concentrations of ACh and eserine (7, 58, 262a). According to one theory, ciliary rhythmicity is maintained by locally produced ACh (58, 206). It has also been suggested that ciliary movements are initiated by the release of a 5-HT-like substance from the branchial nerves (7). The gill plates of *Mytilus* are claimed to contain both ACh and 5-HT as well as enzymes metabolizing these substances (41, 259, 262a, 366). 5-Hydroxytryptamine intensifies ciliary activity in embryos of nudibranch molluscs at extremely low concentrations (207).

7. Nervous system. Molluscs (snails, mussels, squids, octopodes): By the use of intracellular electrodes and electrophoretic administration of drugs, it has been possible to demonstrate in the nervous system of a mollusc (*Aplysia*) two types of neurones with different and specific pharmacological properties. One type is hyperpolarized (inhibited) by ACh in a concentration of 10^{-12} and the other type is depolarized. The neurones also differ in their responses to 5-HT, GABA and noradrenaline (142). On giant cells in the marine pulmonate mollusc, *Onchidium*, GABA has no effect, but ACh in high concentration (10^{-3}) produces inhibition (151).

Synaptic transmission has been investigated in an isolated ganglion (squid, *Loligo*) kept in cool sea water saturated with oxygen. Effects were obtained with only a few blocking agents in high concentrations (45). Isolated giant fibres from which the axoplasm has been squeezed out and which are perfused by isotonic solutions still conduct action potentials (29). Initiation of spontaneous activity in the cerebral ganglion of a mussel (*Mya*) is produced by 5-HT (166). Acetylcholine and 5-HT are excitatory, dopamine inhibitory, on the brain activity of a snail (*Helix*) (190a).

Arthropods. a) Crustaceans (lobster, crab, *etc.*). The central nervous system of crustaceans consists of a few neurones and has no tracts. The structure makes it suitable for certain types of analyses which are difficult to make on vertebrate nervous systems. Surveys on the central nervous system and its reflexes (377), and of neurohumors and neurosecretion (364) in crustaceans have recently been published.

Acetylcholine increases the spontaneous activity in the abdominal ganglion of a crayfish (*Orconectes*) and in the isolated ganglionic trunk of the lobster heart (*Palinurus japonicus*) (159, 242). Dimethylaminoethanol (DMAE) has greater effect than ACh, probably due to quicker penetration (159, 160). Quaternary

amines (ACh) permeate cells with difficulty in contrast to tertiary amines (like DMAE). Quaternary amines which are fat-soluble and biologically active, such as the dodecyltrimethylammonium ion, block conduction in crab and lobster nerve (319). Besides ACh, 5-HT and LSD-25 also excite the cardiac ganglion (lobster), while GABA and Factor I activate inhibitory nerves (243). The insecticide pyrethrum (10^{-7}) blocks synaptic transmission in the central nervous system of the crayfish (317).

Stretch receptor neurons in crayfish are stimulated by ACh and inhibited by Factor I and GABA (248, 289). The effect of GABA seems to be due to an increase in conductance of the stretch receptor membrane (152).

b) *Xiphosura* (king crab). The rhythmic activity of isolated cardiac ganglia of *Limulus* is inhibited by 5-HT (5×10^{-8}) and GABA (53).

c) *Insects and spiders.* A correlation between spontaneous electrical activity and content of ACh in insect ganglia has been observed in the silkworm (*Cecropia*) and cockroach (*Periplaneta*) (71, 199). The effects of ACh, urocanylcholine, and catecholamines on synaptic transmission have been investigated in the abdominal ganglion of the cockroach (345). The insecticide DDT [1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane] causes an increase and prolongation of the negative after-potential in cockroach nerve (267), and this is considered to be an important part of the toxic action of DDT. This compound has also been studied on a ganglion-muscle preparation of the large beetle *Dytiscus* and proved to have both central and peripheral effects (134). γ-Aminobutyric acid and *beta*-alanine inhibit the bioelectrical activity of ganglia of the pine moth caterpillar (353). Extracts of the corpus cardiacum, an endocrine organ, cause excitation of the central nervous system in insects, probably by block of an inhibitory system (257).

Various effects of psychotropic drugs, like reserpine and chlorpromazine, have been observed on the shape of the cocoons in silkworms (337), behaviour of meal beetles (*Tribolium*) (173) and net-building of spiders (380).

8. General features of pharmacological effects in invertebrates. High sensitivity to ACh, eserine and ACh-blocking substances is shown by the neuromuscular systems in annelids, molluscs, holothurians, and several other groups. In coelenterates, ascidians, and arthropods the muscles do not respond to ACh, but interneuronal synapses are affected in arthropods.

A very strong stimulatory effect of 5-HT is observed on the heart of molluscs; 5-HT also stimulates the heart of certain crustaceans and relaxes molluscan and annelid muscles.

Effects of catecholamines are relatively rare in invertebrates, but are obtained in annelids, cephalopods, and a few other cases. Histamine as a rule shows no effects. Certain amino acids have remarkable actions on neurons and muscles in crustaceans.

There are indications that ACh and 5-HT have transmitter functions in many invertebrates. Arthropod muscles seem to be brought into contraction not by ACh, but by some other transmitter substance acting in a similar way as does ACh at cholinergic myoneural synapses of vertebrates. Dopamine may be an inhibitory transmitter in the nervous system of molluscs (190a). Neurohumoral

transmission in invertebrates has been dealt with in recent reviews (122, 135, 364).

IV. PREPARATIONS USED FOR BIOASSAY

A. Poikilothermic vertebrates

The cannulated perfused heart, the perfused hindlegs (299) and the isolated rectus abdominis muscle from frogs and toads (6, 78, 234, 263) have been extensively used for studies on adrenaline, ACh, and curare-like substances. Eserinization (5×10^{-6}) and treatment with acetone (0.5 to 1%) are said to increase the sensitivity to ACh of the rectus abdominis muscle of the frog (263). For demonstration of adrenaline (and noradrenaline) the isolated frog eye and the toad rectum have occasionally been used (89, 300).

Similar preparations as the above mentioned may be obtained from reptiles (*e.g.*, turtle heart) and from fishes, although it seems that use has seldom been made of the latter animals. The goldfish intestine has been suggested for the assay of substance P (136a). Assays of psychotropic drugs have been tried on aquarium fishes (275). Salamanders have been used for investigation on testing anticonvulsant properties of drugs (283), and tadpoles for studying thyroxin analogs (130). The isolated amphibian skin provides possibilities for evaluating pituitary hormones and other substances active on melanophores (129, 198, 219, 225, 228). Thus, dispersal of pigment in hypophysectomized frogs after injection of urine has been utilized for pregnancy diagnosis (276). The relatively high sensitivity to noradrenaline of the melanophores in fish scales (105) could possibly be of value for bioassay purpose.

B. Invertebrates

The wide register of pharmacological responses in invertebrates gives numerous possibilities for biological assays. In Table 1 are listed some preparations which have been recommended for biological tests. Many of the species used occur in special localities, but usually related species may be used instead. Suitable techniques for measuring muscle activity in invertebrate preparations have been described (368, 370).

The eserinized dorsal leech muscle preparation has been much used for estimation of ACh and is also useful for assay of 5-HT in concentrations down to 10^{-12} (290).

The use of the earthworm for *in vitro* test of anthelmintics has been criticized on the basis of the different muscle physiology of annelids and intestinal worms (30).

One of the best known and most studied invertebrate preparations is the heart of the clam, *Venus mercenaria*. The isolated ventricle of this animal, suspended in aerated sea water, is used for assays of ACh as well as 5-HT. Pretreatment with ergot alkaloids (LSD 25) produces increase of amplitudes and makes the *Venus* heart insensitive to 5-HT without influencing the response to ACh, while benzoquinonium (Mytolon) makes the heart insensitive to ACh (362, 370).

In work with the *Venus* heart and other organs of marine animals sea water

TABLE 1

Invertebrate preparations used for assay

Substance	Animal	Organ	Effects	References
ACh and related compounds	Molluscs:			
	Clam (*Venus mercenaria*)	Heart	Inhibition	369, 370
	Other species			123, 172, 218, 250
	Sea cucumbers (holothurians)	Body wall muscle	Contraction	10, 23, 360
	Worms:			
	Leech (*Hirudo medicinalis*)	Body wall muscle	Contraction	234, 260, 335
	Other worms			324, 329
5-HT and related compounds	Molluscs:			
	Clam (*Venus mercenaria*)	Heart	Stimulation	362, 370
	Other molluscs			108, 125, 136, 237, 238, 360, 361, 389
	Larvae of marine molluscs (nudibranchiates)	Cilia	Stimulation	207
	Leech (*Hirudo medicinalis*)	Body wall muscle	Relaxation	290
	Mussel (*Mytilus*)	Byssus retractor muscle	Relaxation	60
GABA and Factor I	Crayfish (different species)	Stretch receptor	Inhibition	90, 118
	Crayfish	Intestine	Inhibition	121
	Sea urchin	Oesophagus	Inhibition	126, 164
Glutamic acid, Factor S	Crayfish	Claw muscle	Contraction	154, 155, 201
Adenosine-triphosphate (ATP)	Firefly (*Lucilia*)	Tail (dried)	Luminescence	67

may be used as physiological saline. For organs of fresh water invertebrates, frog Ringer solution (for leech muscle) or other special solutions are recommended (108, 227, 237, 238).

A fluorimetric method, not a bioassay, is the estimation of adenosinetriphosphate (ATP) which employs an extract prepared from firefly tails (67).

V. DISTRIBUTION OF BIOLOGICALLY ACTIVE SUBSTANCES

A. Poikilothermic vertebrates

Catecholamines: The lancelet (*Amphioxus*) contains a distinct amount of noradrenaline, but probably no adrenaline (100). In *cyclostomes* both these amines occur. The remarkably high amounts of adrenaline and noradrenaline in the hearts of the hagfish (*Myxine*) and the lamprey (*Lampetra*) are due to special kinds of chromaffin cells (17, 42, 179, 277, 278). Similar cells have been observed in the heart of the Californian hagfish (177). The auricle and also the so-called portal vein heart of the hagfish contain mostly noradrenaline, while in the ventricle adrenaline predominates. Noradrenaline is found also in the kidney and the pronephros (102).

Organs of cartilaginous and bony fish (elasmobranchs, teleosts) contain varying amounts of catecholamines. Very high amounts are found in chromaffin tissue associated with the sympathetic trunks in dogfish (*Squalus*) (102, 371). In a bony fish (cod, *Gadus*) adrenaline is found in the cranial kidney, the kidney and the vagus, while noradrenaline is found in the muscularis of the swimbladder (102). Reserpine causes disappearance of noradrenaline and nearly total disappearance of adrenaline from adrenals of the dogfish (*Scyliorhinus*) (285).

In amphibians catecholamines are found in the adrenals, in the heart, and in the skin of many species (117, 148, 220, 371). In the frog (*Rana temporaria*) heart, adrenaline accounts for about 90% of the total catecholamines (277).

It was shown by Otto Loewi in his classical experiments that during stimulation of the sympathetic nerves to the frog heart there occurs a release of a substance which is probably identical with adrenaline. It is not known whether the adrenaline of the frog heart is present in nerve endings, or if it is enclosed within chromaffin or muscle cells. Inasmuch as about 10 to 15% of the catecholamine content of the frog heart consists of noradrenaline, there is the possibility that noradrenaline is a sympathetic transmitter in amphibians as it is supposed to be in homoiothermic vertebrates. In poikilothermic vertebrates, probably much of the catecholamines found in different organs occurs in chromaffin cells rather than in sympathetic nerve endings. The suggestion has been made that the development of adrenergic fibres represents a late step in the evolution of the autonomic nervous system (102).

ACh: The distribution of ACh probably corresponds to its function as a transmitter substance. In electric fishes, very high amounts of ACh (up to 100 μg/g tissue) are present in electrical tissue (18, 115, 292). In the frog, ACh has been demonstrated in the heart, and ACh or an analogue is liberated during vagal or direct stimulation (229, 268). The ACh occurs predominantly in the richly

innervated auricles (1 to 2 μg/g tissue) (246, 314). γ-Butyrobetaine, with effects similar to those of ACh, has been identified in fish, reptiles, and mammals (167). Leptodactyline, *m*-hydroxyphenethyltrimethylammonium, a substance with nicotine-like effect, is found in the skin of some South American amphibians (99).

Substance P: High values for substance P activity are found in the central nervous system of amphibians and reptiles. This or a similar peptide has also been demonstrated in brain and intestine of cod (*Gadus*) and dogfish (*Squalus*) (72, 74, 145).

Indole alkylamines: 5-Hydroxytryptamine (5-HT) has been found in the male reproductive tract of the dogfish (233), and 5-HT and related compounds (bufotenine, bufoviridine, bufotenidine, *etc.*) are present in the skin of many amphibians (95, 96, 97, 346, 352). 5-Hydroxytryptamine is also found in the amphibian intestine, from which it is partly liberated by reserpine, which has no effect on the indole alkylamines in the skin (285). Increased release of 5-HT from the stomach of the frog (*Rana tigrina*) has been observed during nervous stimulation (325). Enterochromaffin cells, which are supposed to contain 5-HT, have been demonstrated in *Amphioxus* and in all vertebrate groups except bony fishes (teleosts) and cyclostomes (94, 143).

GABA and Factor I: Factor I is a substance in mammalian brain which inhibits spontaneous impulse generation by stretch receptor neurons of crayfish (118). *Gamma*-aminobutyric acid seems to account for a part of the Factor I activity (34, 226). By paper chromatography GABA has been demonstrated in brain, but not other tissues of poikilothermic vertebrates (Pacific mackerel, alligator, and both adult and tadpole bullfrogs) (307).

Various other substances: Toxic substances, often of protein nature, are found in many fishes, amphibians and reptiles. Snake venoms contain toxic albumins and hydrolyzing enzymes. The amphibian skin glands contain digitalis-like substances besides indole alkylamines and catecholamines (47, 186, 269). From injured skin of fresh-water fishes a "Schreckstoff" is emitted (284). Steroid-like alkaloids constitute part of the venom secreted by skin glands of the salamander (*Salamandra maculosa*) (321). The buccal glands of the blood-sucking lampreys (*Cyclostomata*) secrete an anticoagulant (32a).

Cholinesterases (ChE's): High activity of ChE is found in the electric organs (292) and skeletal muscle (70, 231) of fish. Specific ChE has been demonstrated in the optic nerve of the skate (*Raja*), in the splanchnic nerve of the bullfrog (50). Cholinesterase, choline acetylase, and various choline esters occur in frog skin (202, 203). The digestive tube of fresh-water fish has a considerable activity of specific ChE, but no activity is found in the blood cells (70).

B. Invertebrates

An extremely large number of substances with strong biological effects are found among invertebrates. Cantharidin from the blister beetle may be mentioned as an example well known from materia medica.

Below is given a very incomplete record of the distribution of certain biogenic amines and other compounds.

ACh and related compounds: Acetylcholine occurs in protozoans, where it is supposed to be of importance for their movements (59). In multicellular organisms, ACh has a very wide distribution (22). It has been found in nerve tissue of molluscs (255) and crustaceans (123). Acetylcholine has been demonstrated in many insects (71, 252, 253), in wasp venom (186), and in the silk glands of the caterpillar *Arctia caja* (garden tiger moth). In the adult moth another choline ester, probably β-dimethylacrylylcholine, occurs in the prothoracic glands (263).

In addition to acetylcholine, many other choline esters such as urocanylcholine (murexine), acrylylcholine, dimethylacrylylcholine, and senecioylcholine have been demonstrated in molluscs (97, 191, 192, 373, 374). The hypobranchial gland of marine snails (*Murex* and others) is an especially rich source of choline esters (373).

Tetramethylammonium occurs in coelenterates (jellyfish, sea anemones), possibly in the stinging capsules (241, 367), and in large amounts in the salivary glands of certain marine snails (*Neptunea*) (15, 109).

Catecholamines: Adrenaline or a related substance has been found in a protozoan (33). An adrenaline-like substance (catechol 4) has been found in sea anemones (277). Small amounts of adrenaline and noradrenaline are present in annelids, and noradrenaline occurs in high amount in insects (100, 277). Noradrenaline has been demonstrated in the posterior salivary glands of *Octopus*. These glands also contain tyramine, octopamine (*p*-hydroxyphenylethanolamine), and several other amines (68).

Indole alkylamines: The posterior salivary glands of *Octopus* are very rich in 5-HT, probably stored in special cells (68, 95). Injections of reserpine cause decrease of 5-HT in optic ganglia, but not in the salivary glands of *Octopus*. A spectrofluorimetric examination of a series of different animals has shown a wide distribution of serotonin among invertebrates. Especially large amounts were found in molluscan nervous tissue and in pericardial organs of crustaceans (366). In molluscan ganglia, 5-HT is bound in granules (363); it occurs also in wasp venom (37) and in scorpion venom (4).

GABA and Factor I: Research on inhibitory substances extracted from nerve tissue is in a somewhat confused state due to a premature acceptance of GABA as the substance responsible for the so-called Factor I activity. Some authors consider that GABA accounts for the main part of the Factor I effects from extracts of mammalian brain; yet in crustaceans GABA has not been demonstrated (34, 226). Dopamine is a possible inhibitory substance in the molluscan nervous system (190a).

An unidentified inhibitory substance of crustacean nerves has been given the name Substance I in order to distinguish it from the Factor I and associated GABA. Substance I occurs only in inhibitory axons, not in motor or sensory nerves (123, 124).

Various substances: Recent papers on the pharmacology of marine organisms (68, 274) and on animal venoms (47, 186) show that a great development is taking place in these fields. Proteins and peptides are probably more important

constituents of invertebrate venoms than are amines (88, 144, 249, 261, 262). Smooth muscle-contracting peptides have been found in wasp venom (315). Histamine-liberating, mast-cell disrupting principles are contained in extracts of jellyfish and parasitic roundworms (*Ascaris suis*) (349, 350).

Histamine is found in insects (47, 186) and in marine invertebrates (2). A nicotine-like base is the toxic principle of a nemertean (*Amphiporus*) (196). Ergot-like alkaloids occur in the king crab (*Limulus*) (3). Powerful toxins causing block of bioelectrical activity are formed by certain marine micro-organisms (79).

Blood sucking invertebrates are provided with glands secreting substances which block coagulation of vertebrate blood. Mouth glands of the medical leech form a peptide called hirudin, which was much used instead of heparin in physiological experiments in the beginning of the century (240). Tabanids (insects) also produce anticoagulating substances (239).

Endocrine organs of insects and crustaceans produce a large number of hormones which, although extraordinarily active on their specific target tissue as a rule, do not affect organs of vertebrates. The crustacean neurosecretory hormones are probably peptides (62, 87).

Enzymes: Different kinds of ChE have a wide distribution among invertebrates (16). In many cases the presence of ChE is probably associated with cholinergic transmitter mechanisms. Cholinesterase activity has been demonstrated in trematodes and other primitive worms (25), but not in nematodes (30). Extremely high enzyme activity was found in a nemertean (187). Although certain coelenterates have been reported not to possess any ChE activity (22, 310), a highly specific ChE has been found in the hydrozoan *Tubularia* (51). In ascidians, ChE occurs only in early embryonic stages (86).

Insects are a rich source of ACh-synthesizing enzymes (253) as well as of ChE. The relation between the effectiveness of certain insecticides and their blocking effect on ChE has been much debated (38, 305).

Enzymes converting 5-hydroxytryptophan to 5-HT are present in nerve tissue of molluscs (366). Enzymes responsible for the oxidation of 5-HT and related compounds are also found in invertebrate organs.

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