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## AUTONOMIC NEURO-EFFECTOR SYSTEMS

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# AUTONOMIC NEURO-EFFECTOR SYSTEMS

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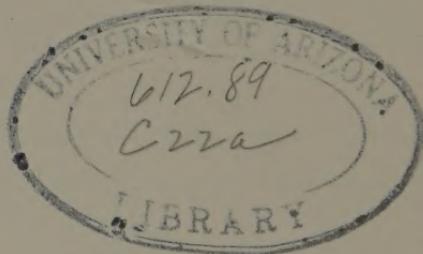
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*Gratefully dedicated  
to the memory of  
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## PREFACE

Our central interest in writing this monograph has been to render an organized account of recently acquired evidence regarding the chemical step which intervenes between the nerve impulse and the effector in the functioning of the autonomic nervous system. A wider range of interest, however, is implied in the title, *Autonomic Neuro-Effector Systems*. This title was chosen because we have considered not only the processes in the junctional region but also those occurring in the effectors themselves.

Attention to these aspects of physiology has been a natural outgrowth of antecedent studies in the Laboratory of Physiology in the Harvard Medical School. The completely sympathectomized animal, first prepared about eight years ago, raised questions regarding the natural functions of the sympathetic system, questions which, when answered, brought forth the concept that this system serves to maintain steady states in the internal environment. With removal of the sympathetic system, however, there was a disappearance of a mysterious cardiac acceleration attendant on emotional excitement. When that phenomenon was carefully examined, at once the realm of chemical mediation of sympathetic impulses was entered. During the past six years many of the researches in the Harvard Physiological Laboratory have been concerned with problems in that realm.

The preparation of a summary of progress when progress is rapidly continuing involves hazards. In the quick movement of events the maintenance of a proper perspective is an almost impossible achievement. Discrimination between what is important and what unimportant, which always requires perspicacious judgment, becomes especially difficult, and if attempted, is liable to wide error. Also there

appears acutely the problem of deciding where to stop the record; even while we are writing and seeing the book through the press, new and, it may be, highly significant discoveries are being reported. Such are some of the hard conditions which are necessarily encountered in an attempt, at this time, to report the advances of knowledge of chemical mediation of nerve impulses.

We have not striven to present an encyclopedic review of all previous researches on autonomic neuro-effectors and the occurrences at their synapses. The valuable recent surveys of the literature by Bacq (1935) and Gaddum (1936) eliminate the need for such treatment of the subject. Furthermore, we have not touched on the controversial features of the publications in the 20's, when Loewi was defending the reliability of his experiments. The interested reader will find that part of past history in Bacq's treatise. Also we have not considered the evidence for chemical transmission of nervous influences to melanophores—the excellent monograph by Parker (1932) has summarized investigations in that field. After these various eliminations we trust that we have not directed undue attention to researches in which we personally have been concerned; they are perhaps more emphasized than in other summaries of the literature, but, if so, merely because, with regard to them, we are in a position to offer direct, first-hand testimony.

Some readers may think that our theoretical treatment of certain problems of autonomic neuro-effector functions is too elaborate and even redundant. We wish to be explicit in declaring that we look upon the theories which we have projected as quite tentative modes of conceiving the facts now available. New facts may appear which may effectively destroy these theoretical concepts. If fresh evidence should require us to change our views, we shall part from the old theories with no more regret than we should feel in relinquishing a once helpful tool for a newer and more efficient instrument.

We wish to express our thanks to the following persons for

permission to reproduce figures which have appeared in publications which they control: Mr. W. W. Norton, Figure 1; the Editor of the *Anatomischer Anzeiger*, Figure 3; the Trustees of the Sir Edward Sharpey Schafer Trust, Figure 5; the Editors of the *Journal of Physiology*, Figures 8, 11 and 36; the Publisher of Pflüger's *Archiv für die gesammte Physiologie*, Figure 9; the Editor of the *British Medical Journal*, Figure 12; and the Editor of the *American Journal of Physiology*, Figures 6 and 7, 10, 13-35 inclusive, 37-40 inclusive, and 42.

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## AUTONOMIC NEURO-EFFECTOR SYSTEMS



# AUTONOMIC NEURO-EFFECTOR SYSTEMS

## CHAPTER I

### THE ORGANIZATION OF THE AUTONOMIC SYSTEM

The writing of a survey of autonomic neuro-effector systems raises immediately the question as to how much it is desirable or necessary to consider at the outset details of the organization and function of the autonomic system and the finer structure of its effectors in order to assure understanding. Obviously judgment concerning the readers' previous acquaintance with the subject must determine to an important degree the extent of the exposition. The writers of this monograph are disposed to assume that interested readers will not look into the following pages with the hope of finding an elaborate account of the gross morphology or the minute histological characteristics of either the autonomic nerves or their end-organs. A brief outline of the organization of autonomic neurones and related functions should be sufficient. In presenting this outline emphasis will be laid especially on such aspects of the subject as are pertinent to later discussion.

For purposes of description the nervous organization of vertebrates can be separated into two grand divisions, the exteroffective and the interoffective. The exteroffective division is comprised of the brain and spinal cord with the subservient afferent and efferent nerves; the afferent, bearing impulses inward from peripheral receptors from body surfaces and moving parts, and the efferent delivering impulses to the exteroeffectors, the skeletal muscles. By action

of the exteroffective division, commonly called the "voluntary" nervous system, we change the outer world and our relations to it.

The interofective division of the vertebrate nervous organization is known also as the autonomic, "vegetative," or "involuntary" nervous system. In its interofective functions it is concerned with adjustments which, in the most highly evolved animals, maintain the stable states of their internal environment. Thus the autonomic system is concerned when, because of high or low external temperature, the temperature of the body tends to rise or fall. And in muscular exercise it is concerned in preventing disturbances due to excessive production of heat and acid. The most general descriptive statement which can be made regarding the service of the autonomic system is that its prime function is the maintenance of stability or homeostasis in the fluid matrix of the organism (78).<sup>1</sup> There are some functions, e.g., the emptying of pelvic organs, the protective action of the lachrymal glands and the nictitating membranes, that lie outside this broad generalization.

*The Main Features in the Organization of the Autonomic System.* These will be presented in a series of brief statements, based mainly on the studies by Langley (1903)<sup>1</sup> and his students. Figure 1 illustrates the important points, and frequent reference to it will help to fix them in memory.

First, the autonomic system in relation to the central nervous axis is an arrangement of *efferent* nerve paths. The afferent fibers from the viscera that accompany the efferent do not belong to the autonomic system proper and should be designated sensory visceral fibers.

The autonomic is peculiar in the nature of the agents which it employs as effectors. It provides the nerve supply for glands, smooth muscle, the special muscle of the heart, and the contractile elements in capillaries. The *glands* which receive autonomic fibers are mostly those having an

<sup>1</sup> Articles are referred to either by years, after the names of authors, or by italicized numbers, corresponding to those in the list of references at the back of the book.

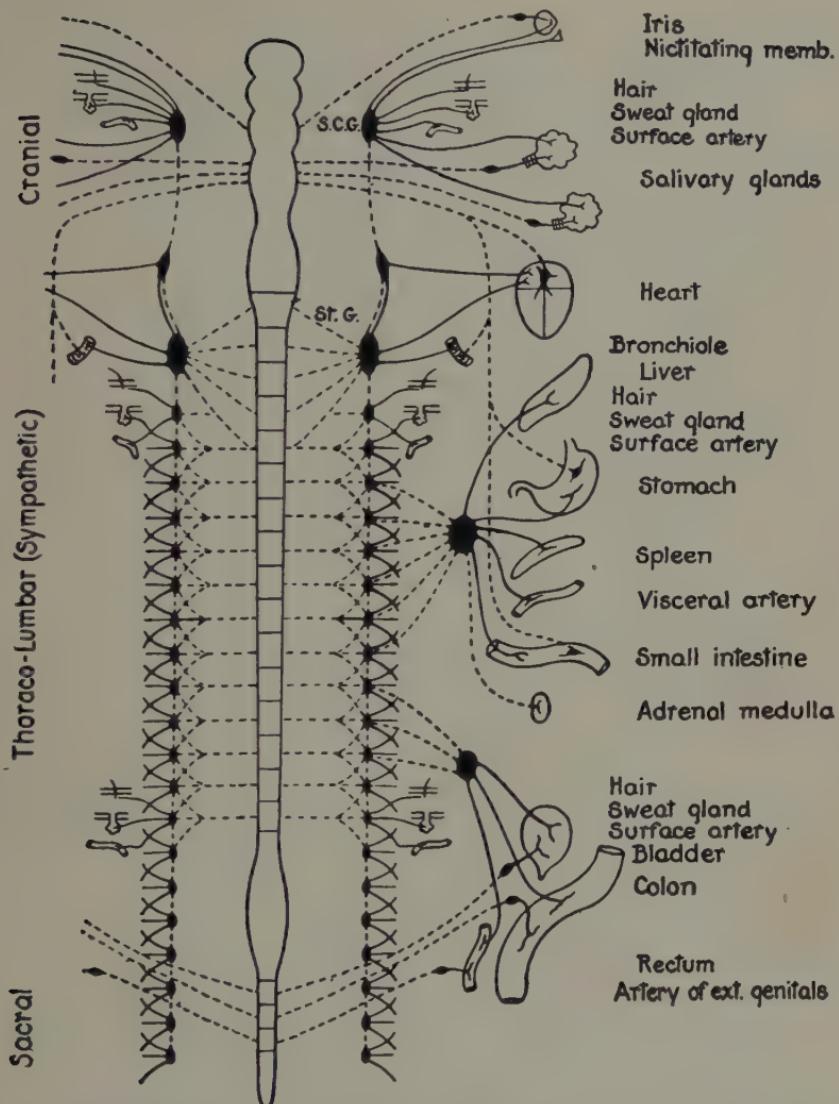


FIG. 1. General arrangement of the autonomic nervous system. The brain stem and spinal cord are represented between the two chains of sympathetic ganglia. The cranial, sacral and sympathetic preganglionic fibers (dash lines) and the postganglionic fibers (solid lines), symmetrically distributed, are represented more completely on the right than on the left of the diagram. The large *unpaired* prevertebral ganglia are shown on the right.

external secretion, *i.e.*, the lachrymal glands and those secreting into the digestive tract, notably the salivary and gastric glands, the pancreas and possibly the liver. The unique innervation of the sweat glands will be considered later. Of the endocrine glands there is evidence that the adrenal medulla, the anterior lobe of the pituitary body and the islet cells of the pancreas are under control of one or another part of the autonomic system. The *smooth muscle* effectors include those in the vascular system, the bronchioles, the alimentary canal, spleen, ureters, bladder and genitalia, iris, nictitating membrane, Müller's muscle and the pilomotor apparatus. Finally, as reviewed by Hinsey (1934) the evidence that ordinary striate or *skeletal muscles* are directly innervated by any autonomic fibers is highly questionable.

The autonomic innervation of smooth and cardiac muscle is not direct from the brain and spinal cord, and, with one exception, the same statement is true for glands. Between the outreaching neurones, whose cell bodies are in the central nervous axis, and the interefective muscular and glandular structures there are *interposed peripheral neurones*. The exception just mentioned is found in the nerve supply to the adrenal medulla. It appears to be well established that the cells of that endocrine organ receive nerve impulses directly and immediately from the central nervous system (133).

The collections of nerve fibers having cell bodies in the central nervous axis and reaching out to the peripheral neurones of the autonomic system, are separable into *three divisions*: the thoracolumbar or sympathetic, passing outward between the brachial and sacral enlargements of the spinal cord, and the parasympathetic which is commonly described as two divisions, the cranial and the sacral. The connections of the *cranial division* are by way of four of the twelve pairs of cranial nerves: the third pair related peripherally to the iris and ciliary muscle of the eyes; the seventh to the lachrymal and to the sublingual and submaxillary salivary glands; the ninth to the parotid glands; and the tenth pair to the heart, bronchioles, lungs, esophagus and

gastro-intestinal tract. The connections of the *sacral* division are in neurones issuing from the spinal cord below the sacral enlargement. Peripherally the effectors are the smooth muscles of the lower colon and rectum, the bladder, genital glands and the blood vessels of the external genitalia.

The cell bodies of the peripheral neurones of the autonomic system are arranged in ways characteristically different for the two main divisions. In the *sympathetic* division these cell bodies are grouped in a *series of ganglia* (called "vertebral ganglia") lying on either side of the midline at the back of the body cavities and reaching into the neck. The chains are represented diagrammatically in Figure 1. They extend from the superior cervical ganglion (S.C.G.) near the base of the skull to the closely joined pairs of ganglia deep in the pelvis. Connecting the successive ganglia with one another are the fibers from the spinal cord. The bridging of the ganglia results from the mode of distribution of these fibers. They arise as branches of the segmentally arranged spinal nerves. The branches, whitish in appearance because composed almost entirely of myelinated fibers, are the "white rami." The fibers of a white ramus coming from any segment not only join the ganglion nearest that segment, but extend upwards or downwards through perhaps two or three of the nearest ganglia, giving off branches in each. Obviously there must result an overlapping of these fibers as they bind together the ganglia in the two chains. It is characteristic of the *vertebral ganglia* that they send fibers outward—mostly non-medullated (the "gray rami")—to be distributed to sweat glands and the smooth muscle of peripheral blood vessels and hair follicles, as they are found in the range of distribution of cerebral and spinal nerves. The fibers emerging from the spinal cord and reaching and uniting the ganglia are "preganglionic" (represented by dash lines in Figure 1); those distributed to peripheral glands and smooth muscle are "postganglionic" (solid lines in Figure 1). In addition to the vertebral ganglia there are the *prevertebral* found at the origin of the main branches

of the abdominal aorta—the solar (commonly separated into the semilunar or celiac and the superior mesenteric) and the inferior mesenteric. The preganglionic fibers which enter these masses pass through vertebral ganglia on the way. Typically the prevertebral ganglia send postganglionic fibers only to glands and smooth muscle of the abdominal viscera (see Figure 1). The large stellate ganglion (St.G.), high in either side of the thorax, belongs to the vertebral series, but is peculiar in supplying postganglionic fibers not only to spinal nerves but also to the heart (the "cardio-accelerators"). Traversing this ganglion on either side are preganglionic fibers which end in the inferior or superior cervical ganglia. In the two parasympathetic divisions (*cranial and sacral*) the cell bodies are characteristically found in a more or less scattered distribution *in or near the organ to which they directly deliver impulses*. They lie inside the heart and the wall of the alimentary canal, and in the pelvic plexus of nerves near the bladder and rectum. The preganglionic fibers of the parasympathetic divisions, therefore, like the nerves supplied to skeletal muscles, are distributed to or within the organs which they influence. The ciliary ganglion, in the course of the third cranial nerve, is a noteworthy place in the organism where parasympathetic fibers have a relay well separated from the innervated structures (iris and ciliary muscle).

There are many more postganglionic fibers passing out from a ganglion than preganglionic fibers coming to it. Billingsley and Ranson (1918) found that the ratio between the preganglionic fibers in the cervical sympathetic strand and the cell bodies in the superior cervical ganglion is as 1 to 32. Although the same ratio may not prevail in other parts of the system, it is clear from the evidence of branching of preganglionic fibers, as they pass through ganglia, that an important function of the outlying neurones centered in autonomic ganglia is that of spreading widely the area of distribution of nerve impulses.

Since the preganglionic fibers in the sympathetic division

may traverse two or three vertebral ganglia before ending and may give off side branches in each one, and since these fibers may grow either upwards or downwards so as to produce in their combinations an interlacing of nerve tendrils, the *sympathetic division* is clearly *organized for diffuse distribution of the nerve impulses*. Related to this arrangement for diffuse distribution is the innervation of the adrenal medulla by sympathetic preganglionic fibers. Since medulli-adrenal secretion (adrenine<sup>1</sup>) mimics generally throughout the organism the action of sympathetic nerve impulses (130), the diffuse distribution of this secretion by the blood stream, simultaneously with the diffusely distributed sympathetic impulses, brings about a coöperation whose intimate nature has only recently been revealed. The sympathetic division may, indeed, be considered as a sympatho-adrenal system. Although adrenine generally affects smooth muscle and glands as do sympathetic impulses, there are discrepancies. For example, in some animals sympathetic impulses cause secretion from sweat glands, whereas these glands are quite unaffected by an injection of adrenine. Also, smooth muscles of hairs, normally indifferent to adrenine, contract readily in response to sympathetic stimulation; this discrepancy, however, is quantitative, for, when sensitized, they respond to the hormone. Amending the evidence of organization of the sympathetic division for widespread and general influence is the evidence that it is not always active in all its parts. Impulses are being discharged continuously to the smooth muscle of blood vessels and of the nictitating membrane, for example, as proved by the prompt relaxation of these structures when the sympathetic nerves are cut; on the other hand, the pilomotors are not obviously influenced by denervation; they normally lie quiescent until stimulated. The arrangement of the sympatho-adrenal system for diffuse effects allows, however, bringing into

<sup>1</sup> The term, adrenine, is used to designate the secretion from the adrenal gland and, generically, preparations containing extracts of the adrenal medulla. Adrenalin is used when, specifically, the commercial product bearing that name is employed.

action at one time the whole congeries of organs under control of that system—a function important for emergencies and for preservation of steady states in the organism (79).

Many structures in the body are innervated from both the sympathetic division and one of the parasympathetic divisions. Examples of organs with *double innervation* are the salivary glands, the heart, the stomach and intestines, the bladder and the blood vessels in some parts, *e.g.*, in digestive glands and in the external genitalia. As a broad rule when representatives of either of the parasympathetic divisions meet representatives of the sympathetic division in any organ, the two are opposed in their effects. Thus the sympathetic contracts blood vessels of the salivary glands, accelerates the heart beat, has a relaxing influence on gastric and intestinal muscle and shuts down the blood flow to the genitals; nerves belonging to the cranial or the sacral division that supply these structures act conversely. This general rule has exceptions. There is evidence of accelerator fibers in the vagal supply to the heart (206); and there is evidence also of inhibitory fibers for the cardiac end of the stomach which are distributed to that organ by way of the vagus nerves. The examples just cited show clearly that a given nerve may carry impulses which have opposite actions in different effectors; the vagus, *e.g.*, has predominantly an inhibitory action on the heart and a stimulatory action on the stomach.

Not all smooth muscle is supplied with nerves from two divisions of the autonomic system. *Only sympathetic fibers* innervate the nictitating membrane of the cat, for instance, and this is true also for the uterus, the pilomotor muscles, the spleen and the sweat glands. Probably the blood vessels over a large part of the body are governed solely by the sympathetic division.

*The General Functions of the Autonomic System.* The diffuse influence of sympatho-adrenal activity allows, in structures where that activity is continuous and moderate, a widespread increase or decrease of function. Thus aug-

mented activity of the sympatho-adrenal system may evoke extensive changes, as in dilating the pupils, accelerating the pulse, and contracting the blood vessels of the splanchnic area. Reduction of activity, on the other hand, permits these widely scattered organs to resume their former state or to become less affected. On the other hand, the particular and individual innervation of organs by way of parasympathetic fibers deals with these organs, not in a great group, but separately. For example, there may be specific constriction of the pupil (in bright light), or discharge of saliva, or slowing of the heart rate, or contraction of the stomach or the bladder or the rectum, without any one of these acts affecting any other. Obviously in this realm, also, in so far as activity is continuous and moderate, it can be varied upwards or downwards. The vagal impulses delivered to the heart illustrate such tonic nervous functioning—increase or decrease of vagal tone makes the heart contract, respectively, less frequently or more frequently. Because of their double innervation the organs under tonic control of the autonomic system can be made to alter the degree of their functional use either as an *integrated group*, through sympatho-adrenal influence, or as *separate structures* through the direct effects of parasympathetic impulses.

The *sympathetic division* of the autonomic system, despite its extensive range of action, is *not essential for existence*. The two ganglionic chains, as well as the prevertebral ganglia, have been removed from animals and yet, within the confines of the laboratory, such altered animals seem not to differ markedly from the normal (86). When they are exposed to heat or cold, however, or when subjected to lowering of blood sugar, or tested by loss of blood through hemorrhage, they show definite deficiencies (304). These deficiencies more clearly define the statement, previously made, that the autonomic system is concerned with preserving steady states in the fluid matrix of the organism; it is especially the sympathetic division which has the general function of *maintaining homeostasis*.

The *cranial division* of the autonomic system may be regarded as protective, conservative and upbuilding in its services to the organism. Examples of these modes of functioning are seen in the constriction of the iris, brought about by the third cranial nerve, which shields the retina when illumination is intense; in the check on the heart rate by tonic vagal impulses when the body is at rest and the energy of cardiac muscle may be saved; and in the augmented tone of gastric and intestinal musculature established when that state is favorable for the peristaltic and segmenting motions which are essential for the digestive process. The parasympathetic control of the digestive glands, which is exercised at the start of digestion, is another illustration of the value of this division for constructive use, for thereby is assured the first of the linked series of events in the alimentary tract which render food available to the organism.

The functions of the *sacral division* are mainly the reflex emptying of hollow organs, *e.g.*, the urinary bladder and the rectum. These organs are stimulated by distension as their natural contents accumulate and thus reflex contraction is excited. The bladder has a double innervation, with sympathetic and sacral nerves playing variable rôles in different species of animals.

## CHAPTER II

### THE EFFECTORS AND THEIR FINER INNERVATION

The three types of effectors innervated by the autonomic system will be considered in the order, smooth muscle, cardiac muscle and glands.

*Smooth Muscle.* The cells which compose a sheet or mass of smooth muscle differ from the cells of skeletal muscle not only in an absence of cross-striations, but in many other respects. The non-striate cells are short, varying in length between 0.02 mm. (in the arterial wall) and 0.5 mm. (in the pregnant uterus), as compared with striate muscle fibers, 20–30 mm. long. The diameter of the cells ranges from a fifth to a tenth the diameter of skeletal muscle cells. The non-striate units are spindle shaped, not cylindrical like the striate; and again, unlike the striate with its many nuclei, each non-striate cell has a single elongated nucleus, situated near the middle and occupying there a large proportion of the cross-area (see Figure 2). Masses of smooth muscle are difficult to tease apart; the elements do not separate readily as do those of the striate variety.

There has been considerable discussion as to whether there are, inside the cell, specialized mechanisms for contraction. Fibrils running longitudinally have been described by a number of histologists after examination of fixed tissues, and the suggestion has been offered that these are contractile agents. Bozler (1927), in examining isolated smooth muscle cells of the ctenophore (*Beroë*), was able to see extreme contraction which was characterized by irregular adjustment of the sarcoplasm, with *straight* fibrils which thickened as they shortened. Arguing against the idea that the fibrils in vertebrate smooth muscle are artifacts, the results of coagulation produced by the fixing agent, Tiegs

(1924) has reported two significant observations. First, the fibrils are larger in hypertrophied cells (those of the pregnant uterus, for example) than in the resting cells, and there appears to be no reason for larger coagula in these circumstances. Second, when regions of contraction are examined the fibrils there are seen to be considerably thicker

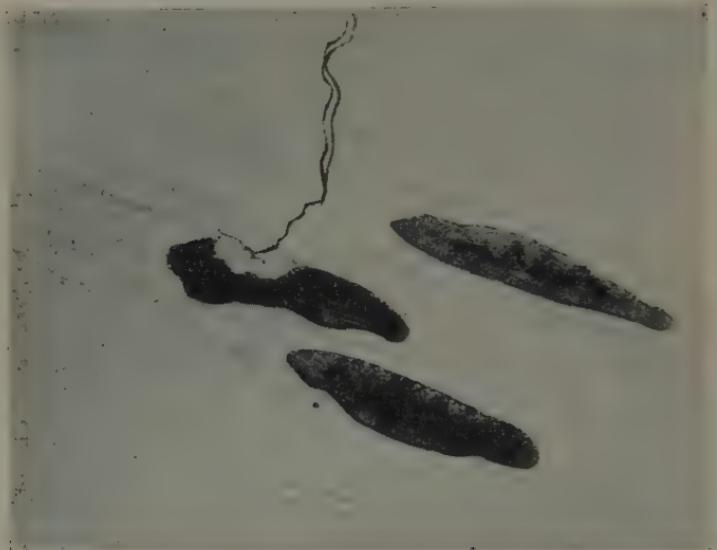


FIG. 2. Nerve ending in the nuclear region of smooth muscle.  
(Lawrentjew, 1926.)

than elsewhere. He explains the absence of an apparent fibrillar structure in the living tissue by assuming that the fibrils have a refractive index too nearly that of the surrounding fluid to permit them to be distinguished, just as the nucleus of the living infusorian is often difficult to detect, for the same reason. The longitudinal fibrils are described as being arranged in the circumference and periphery of the muscle cell, enclosing therefore the undifferentiated cytoplasm in which the nucleus lies. Mallory (1918) states that they more or less fuse as they come together in a bundle at the end of the cell. Although Boeke (1932) admits the presence of very fine internal fibrillae lying immersed in the myoplasm, he declares that with proper

staining the so-called "boundary fibrils" can be seen to be composed of longitudinal rows of dots. These dots, because they are arranged close together in rows and also because they seem connected by delicate protoplasmic threads, give the appearance of a fibrillar structure. It is of interest that Tiegs (1924) also has described in the myofibrils of smooth muscle serially arranged darkly staining portions.

Whether the myofibrils are artifacts or not, it is clear that the cellular protoplasm is not wholly undifferentiated, for it is doubly refractive from one end to the other (309). Here again it differs from cross-striate muscle in which only the dark bands display double refraction. This property appears to be invariably associated with contractility. It has been ascribed to the presence of extremely minute parallel rods, small even when compared with the wave length of light, which themselves have some power of double refraction. The suggestion has been offered that these are elongated molecular groups or chains or loops which are arranged lengthwise of the spindle-shaped muscle cell and which by change of relations of their parts bring about the shortening or lengthening of their extent (329).

It seems probable that the presence of the large nucleus in the middle of the cells of smooth muscle precludes the existence of contractile elements along the cellular axis. Just as the longitudinal fibrils in fixed and stained tissues have a peripheral location, the molecular chains (which may be related to the fibrils) are probably arranged in a similar manner. As previously noted, the nucleus would then be left imbedded in the "undifferentiated" cytoplasm, surrounded by a grill of minute contractile threads. Tiegs (1924) has suggested that the relatively simple sarcoplasm around the nucleus would provide a region where the chemical changes could occur which induce contraction or relaxation.

The difficulty of pulling apart masses of non-striate muscle and isolating the individual units has been attributed to the presence of intercellular protoplasmic bridges holding the

units together. According to McGill (1909) the smooth muscle cells of the alimentary canal are developed from a mesenchymatous syncytium surrounding the embryonic entoderm. It has been suggested that the connecting bridges are remnants of the embryonic state, and that even in the adult, therefore, a mass of smooth muscle retains a syncytial structure. As we shall see later (p. 134), the physiological evidence lends no support to the idea that a syncytium exists—as it exists in the heart, for example—at least in the sheet of smooth muscle present in the nictitating membrane.

*The Nerve Endings in Smooth Muscle.* In relation to later discussion a question of considerable importance is whether autonomic nerves end on or inside smooth muscle cells. Older histologists represented the terminations as button-like enlargements in contact with the surface, *i.e.*, not entering (197). There were, to be sure, observations as early as 1867 that fine nerve filaments penetrate the sarcoplasm of cells of the ureter (see Stöhr, 1928). This and other suggestive evidence to the same effect was not accepted, however, because of defective technique. The brilliant technical skill of Boeke (1915), according to Stöhr (1928, p. 104), first brought reliable evidence that the nerve twig enters the muscle fiber and terminates there in an end ring or in a minute net, often close to the nucleus. Boeke's studies were made on the ciliary muscle of the human eye. He has argued that the evidence is definite because smooth muscle cells are frequently isolated at the inner parts of the ciliary body so that one can see the end rings of the twigs in the cell without confusion from neighboring cells; and also because the end rings or nets lie so close to the nucleus that they occupy an indentation at the side of it at one pole. "Such cases are not rare . . . indeed, in nearly every section through the *m. ciliaris* were found one or two of them." Boeke's observations were confirmed by Stöhr (1926) in an examination of the neuromuscular elements of the human urinary bladder. Lawrentjew (1926) and Hill (1927) likewise have described intracytoplasmic nerve

endings in the contractile cells of the stomach and intestines (see Figure 2). Hill's observations might reconcile the earlier and the later evidence regarding the character of the innervation, for she found on the surface of the cells varicose swellings of the fine nerve filaments, and in addition extremely delicate fibrillae, originating from the swellings, that penetrated into the cytoplasm.

Since there are areas of smooth muscle—for example, those of the intestinal wall—which are under both sympathetic and parasympathetic control, and since these two influences may have opposite effects, the question arises as to the nature of the innervation in such areas. Are some smooth-muscle cells innervated from the sympathetic division only and others from the parasympathetic, or do single cells receive, for example, both the stimulatory impulses from the parasympathetic and also the inhibitory impulses from the sympathetic? Boeke (1921) has figured a single cell of the ciliary muscle of the human eye, in which two nerve filaments are present, one ending in a little net situated in a dent in the nucleus, the other lying free in the cytoplasm. In the musculature of the frog's stomach Tiegs (1924) likewise has found two nerve filaments terminating in single muscle cells. Here again one of them was close to the nucleus, spreading over it and sometimes winding around it; the other, smaller and slightly branched, ended in the granular cytoplasm close beside the nucleus. Unfortunately these two observers leave the question of the source of the endings quite undetermined. No histological evidence, of which we are aware, has yet been adduced to prove that representatives of two divisions of the autonomic system meet in a single cell. The alternative mode of organization would be the distribution of sympathetic fibers to some cells and of parasympathetic fibers to others. Observations by Frank (1907) have been interpreted as supporting that condition in the heart. He found that when the body temperature of dog or rabbit is gradually reduced there is a gradual lessening of the accelerator effect of sympathetic stimulation

on the heart rate; at the same time the inhibitory influence of the vagus remains unchanged. As we shall see later (p. 184), however, there is a reasonable alternative to his explanation. The specializing of autonomic effectors in accordance with opposed nervous influences has not yet been proved.

A fact of considerable significance is the inability of competent histologists to find nerve filaments distributed to all smooth-muscle cells. Since 1850 an impressive series of reliable investigators have reported that not every cell receives a nerve ending. Among these experts may be mentioned Kölliker (1863), Engelmann (1869) and Huber (1897). This negative testimony might be attributed to defects of technique. Although admitting that possibility, Stöhr (1928, p. 107), after examining large numbers of preparations most carefully and finding a lower ratio than one cell in a hundred with a nerve supply, has declared that he will not believe in the dependence of each muscular unit on direct nervous control until there is clear proof to the contrary. As shown by the blood vessels of the placenta, which are quite lacking in a nerve supply, smooth muscle can serve its proper function without any nervous control (308). On the other hand, the testimony of Agababow (1912) and of Boeke (1932) indicates that in one region—the ciliary muscle of the eye—the nerve terminals are so numerous that Boeke inferred that every muscle fiber could have its own. In the gut, however, Boeke found only the rare innervation of the smooth-muscle cells that Stöhr had reported. Boeke suggests that the meagerness of innervation there may be due to difficulties of staining. That is only a suggestion, however, and the actual evidence—negative, but repeatedly confirmed—does not support it. The favored region in the eye may be peculiar, and associated with the requirement of rapid changes in quick adjustments of vision to shifting external conditions. To what degree the peculiar relations of nerve endings and smooth-muscle fibers in the ciliary muscle may be extended to other parts must remain, for the present, an open question.

If there is not a detailed one-to-one relation between nerve ending and smooth-muscle cell, the nerve impulses, delivered to the select, innervated cells—which we may call “key cells”—might be effective on neighboring, common cells by some special agency of transmission. The inference from embryological evidence that smooth muscle is a syncytium, like cardiac muscle, is not confirmed, as previously remarked, by physiological observations. Connective protoplasmic bridges between cells are not needed, however, to explain the transfer of action from one part to another. We now have proof that a chemical agency, set free by the nervous influence over the muscle cells in one region, can affect other, remotely situated muscle cells which have been completely deprived of their autonomic innervation. The requirement of an individual nerve supply for each contractile element is thereby rendered unnecessary.

*Cardiac Muscle.* The muscle of the heart belongs to the cross-striated variety, but the nerve fibers distributed to its elements do not terminate in an end plate as they do in ordinary skeletal muscle. Instead, the finer innervation is remarkably similar to that of smooth-muscle cells. To be sure, almost all of the older histologists described the endings in the heart as knob-like thickenings of the nerve filaments lying on the surface of the muscle fibers, but Gerlach (1876) and more recent investigators (Boeke [1925], Woppard [1926], Jones [1927]) have all found fine terminals reaching within the sarcoplasm. Gerlach described in the frog heart an intramuscular nervous net from which twigs penetrated into the substance of the muscle cells. Boeke's studies on the tortoise heart led him to the conclusion that very fine varicose branches of a pericellular nerve network pass into the muscle cells and terminate in minute nets or loops or knobs, imbedded in the protoplasm. According to Woppard, who stained with methylene blue, the innervation of cardiac fibers in the rabbit and cat is like that in the lower forms. The nerve filaments, after entering the muscle cells, run in the protoplasm and can be traced across the bridges between

the fibers. Their intraprotoplasmic position can be seen in cross-sections of the muscular elements. The endings are little swellings or loops, similar to those noted by Boeke, and are often located near the nuclei. In his report on conditions in the cat's heart Jones has described two types of nerve endings—one a complicated net which "occupies one pole of the undifferentiated perinuclear protoplasm," the other a single varicose fibril extending along the length of the muscle fiber. The resemblance between the mode of innervation of cardiac muscle and that of smooth muscle is obvious.

Nerve filaments are found diffusely distributed in the myocardium. Stöhr (1928, p. 86) has declared that his preparations do not permit him to assume that every cardiac muscle nucleus in a given region has a nerve ending; indeed, he regards the provision of every muscle fiber with a nerve fiber as being improbable. The syncytial character of the muscular organization of the heart would render that elaborate arrangement unnecessary. Furthermore, the diffusion of chemical agents from innervated key cells to other cells in the myocardium or in Purkinje tissue might be expected to be efficacious in spreading the effects of nerve impulses, much as in structures composed of smooth muscle. Certainly such an agent, carried in the blood stream from other organs in the body, can have an influence on the performance of the heart typical of the sort of nerve that is stimulated (see p. 28).

*Glands.* The operations of muscle cells and gland cells are markedly different. Contraction involves a rearrangement of multitudes of minute units in the cell without much change of place for any one of them; secretion, on the other hand, involves an inflow of substance from blood plasma or lymph, an elaboration of this substance into new chemical forms, and a continual or an occasional discharge of the elaborated substance on a body surface or into the circulation. In short, gland cells are microscopic shops in which material is altered on its way through from entrance to

exit, and in which there may be considerable shifts of internal parts in the alternations of rest and activity. Because of the different modes of functioning, gland cells and muscle cells might have different sorts of intimate innervation.

Unfortunately the testimony of various observers as to the finer relations of nerve terminals to secreting units is either defective in detail or is not concordant. There ap-

pears to be general agreement that a tangle of winding nerve fibers lies about the single parts of a compound gland, whether salivary, hepatic or pancreatic. From this mesh of fibers the directly innervating filament reaches out and ends in numerous knob-like enlargements or varicosities which, as a rule, are described as lying on the surface of the gland cell.<sup>1</sup>



FIG. 3. Nerve endings on isolated cells of the parotid gland of the rabbit. (Arnstein, 1894.)

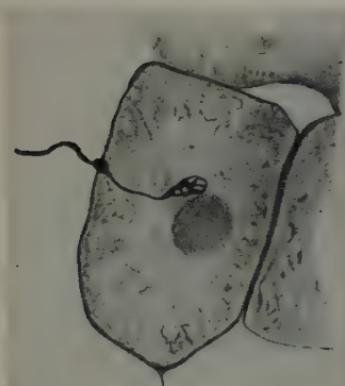


FIG. 4. A reticular nerve ending inside a liver cell of the rabbit. (Riegele, 1928.)

the cell membrane. In the gastric glands the terminal knobs or discs are said to be applied directly to the cell

<sup>1</sup> There is an apparent resemblance between these minute knobs or varicosities and the "boutons terminaux" in which nerve filaments end on the body of a nerve cell. Is it possible that they have similar functions?

membrane. In the liver, however, conditions are different. The network between the cell masses is formed of very fine nerve filaments which have numerous varicose enlargements. From this net extremely delicate nerve threads enter the liver cell, and, as in smooth muscle, they end near the nucleus, according to Riegele (1928), in a tiny fibrillar reticulum (see Figure 4).

For only few of the endocrine glands is there physiological evidence of nervous control; at present these are the adrenal medulla, the islets of Langerhans and the anterior and posterior lobes of the pituitary. The ultimate connections of nerve fibers with the cells of these organs appear to be undetermined, except in the adrenal medulla where Fusari (1891) found the nerve filaments ending on the cell surface in a delicate net. The medullary portion of the adrenal is peculiar in receiving a direct supply of preganglionic fibers. Elliott (1913) who first called attention to this condition, associated it with the fact that the medullary cells are modified neuroblasts which elsewhere develop into the outlying neurones of the sympathetic system, the implication being that preganglionic fibers would thus in a natural way innervate the secreting cells directly. In support of this inference Elliott reported finding no ganglion cells in the adrenal medulla of the cat, and he cited other observers who found only few in the mouse and rat. In man Renner (1924) has declared that the medulla has many multipolar nerve-cell bodies. This discrepancy of testimony may be due to specific differences. When ganglion cells are present they, like similar cells elsewhere, may serve as the immediate transmitters of nerve impulses from preganglionic fibers to the secretory elements.

As we shall see later, the histological testimony that nerves end inside of cells has significance for interpretation of certain facts observed in studies of chemical mediation of nerve impulses.

## CHAPTER III

### STEPS IN THE DEVELOPMENT OF EVIDENCE FOR CHEMICAL MEDIATION OF NERVE IMPULSES

We have now surveyed the organization of the autonomic system and the finer structure of its effectors. What is the relation between the nerve impulses which course along autonomic nerve fibers and the muscles and glands which they influence? Since the chief manifestation of a nerve impulse is electrical, it was natural to infer that the terminal organs are acted upon physically. We now have evidence, however, that a chemical step intervenes between the phenomena of the nerve and those of the effector.

*The Theoretical Basis.* The first suggestion of a chemical mediation of nerve impulses was offered by Elliott in 1904. Struck by the fact that after sympathetic fibers had been cut and had degenerated the structures previously innervated by them respond in a characteristic manner to adrenine, he raised the question whether the sympathetic impulse might not normally produce its effects by liberating adrenine "on each occasion when the impulse arrives at the periphery." The sympathomimetic effect of adrenine would thus be explained, for it would be, according to Elliott, the same substance which serves locally as a mediator between the events in the nerve and the reacting mechanism in the cell—secretion, contraction or inhibition.

An idea essentially analogous to that expressed by Elliott was put forth by Dixon and Hamill in 1909. "There is no inherent difference," they wrote, "between the action of muscarine on the heart on the one hand, and electrical excitation of the vagus nerve on the other. So similar are the two effects that it is not unwarrantable, in the absence of any evidence to the contrary, to assume that they are brought

about in the same way. If it is permissible to argue from analogy, there is reason in the suggestion that excitation of a nerve induces the local liberation of a hormone which causes specific activity by combination with some constituent of the end organ, muscle or gland." In support of this idea Dixon (1907) had earlier made an extract of the heart after vagal stimulation in animals freshly killed and had found that it had "the power of inhibiting the frog's heart, and like muscarine the effect was completely antagonized by atropine." Whatever the active agent Dixon saw in action, it was not the highly unstable substance we now recognize as the chemical intermediary of vagal impulses, for it would have disappeared in the process of extraction which he employed.

The next advance, made by Dale (1914*b*) and Dale and Ewins (1914), was related to a research reported in 1906 by Hunt and Taveau. Noting that a blood-pressure reducing extract of the adrenal gland lost its potency as its choline content increased, Hunt had inferred as early as 1901 that in the extract there might be an ester-like precursor of choline. With Taveau's coöperation a number of esters were made, among them acetylcholine. This remarkable substance was shown, in favorable circumstances, to be 100,000 times more active than choline in causing a fall of blood pressure, and 100 times more active in that effect than is adrenine in causing a rise. Dale's studies in 1914 disclosed that certain ergot extracts produced changes in the body closely resembling those produced by muscarine, and that the agent responsible for them was acetylcholine. On investigating the results of injecting acetylcholine Dale found that it produced a "pronounced vagus-like inhibition of the heart, and various other effects of stimulating nerves of the cranial and sacral divisions of the autonomic system—secretion of saliva, contraction of the oesophagus, stomach and intestine, and of the urinary bladder." These effects, which were remarkably evanescent, were wholly abolished by small doses of atropine. Here was definite intimation

that acetylcholine mimics the action of parasympathetic nerve impulses, much as adrenaline mimics the action of most sympathetic impulses.

*Experimental Support.* The Great War delayed further progress of knowledge of chemical transmission of nerve

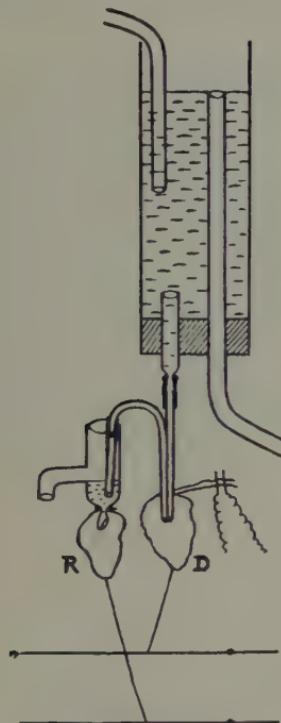
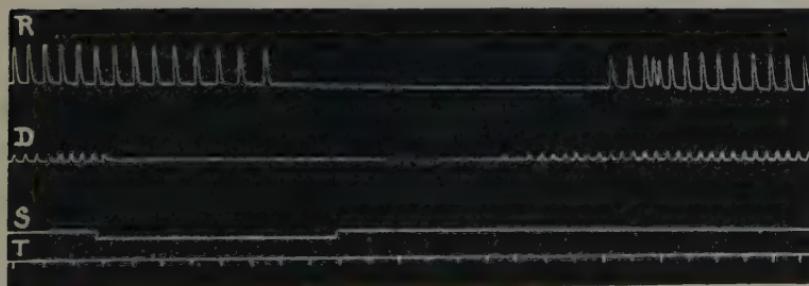


FIG. 5. Bain's modification of the original experiment performed by Loewi in 1921. At the left is represented a reservoir of salt solution from which there is a passage to the donor heart (D); pressure from the reservoir assures a continuous flow of the solution through that heart to the recipient heart (R). The donor heart still has its proper nerves. Each heart is attached to a writing lever.

Below are the records of the two hearts, donor and recipient. When the vagal fibers of the donor were stimulated (S), there was a prompt arrest of that heart (D), and later a slowing and arrest of the recipient heart (R), with gradual recovery. Time (T) is recorded in 5-second intervals. (Bain, 1932b.)



impulses until 1921. In that year Loewi published an investigation, simple in method, ingenious and decisive. Using Straub's technique he set up a frog heart filled with

Ringer's fluid, and found that after stimulating the vagus nerve the fluid acquired a new property—that of being able to induce in another frog heart typical inhibitory vagal effects (see Figure 5). Furthermore, he demonstrated that when the sympathetic fibers were stimulated, to make the heart beat more rapidly, the Ringer solution in contact with it became endowed with cardio-accelerator influence,

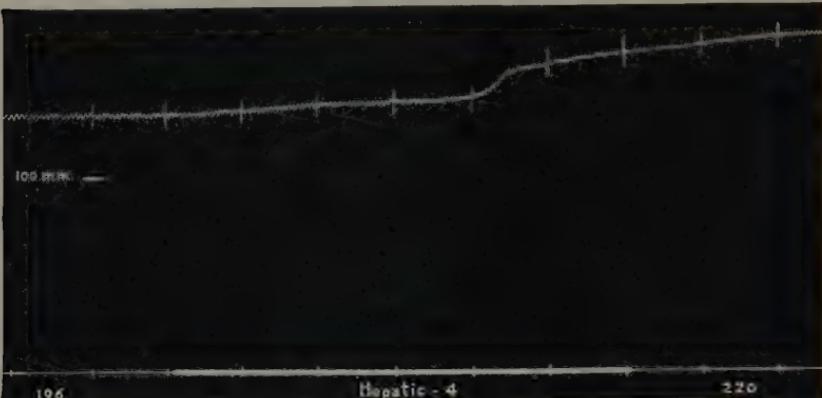


FIG. 6. Sharp rise of blood pressure and increase of heart rate (from 196 to 220 beats per minute) about 20 seconds after the start of stimulation of the hepatic nerves. Time, 5 seconds. (Cannon and Uridil, 1921.)

i.e., an agent was added to it, which, like adrenine, had sympathomimetic influence.

The same year that Loewi published his classic experiment Cannon and Uridil (1921) reported that stimulation of the splanchnic nerves after the adrenal glands had been removed, or direct stimulation of the hepatic nerves, caused, especially in animals digesting meat, the discharge of a substance from the liver, which, like adrenine, accelerated the heart and raised blood pressure (see Figure 6). Unlike adrenine, however, it did not cause a dilation of the pupil. Not until years later did it become clear that the cardio-accelerator substance from the liver has properties fitting into Elliott's suggestion of an adrenine-like mediator of sympathetic nerve impulses. And not until still later was the curiously anomalous effect on the iris explained. In due time these matters will be considered.

Further progress in experimentation was made possible by learning that the vagus substance is probably acetylcholine. Witanowski (1925), working in Loewi's laboratory, found that it had the physical and chemical characteristics of acetylcholine. Related to this evidence was the fact that watery extracts of the heart inactivate both the vagus substance and acetylcholine, as Loewi and Navratil (1926) showed—an effect explicable as resulting from the destructive action of a choline-esterase.

It is interesting to note that Dale (1914a) had assumed the presence of an esterase as the reason why injected acetylcholine was ephemeral in its effects. That the esterase in blood rapidly destroys the vagus substance of the frog heart was demonstrated by Plattner (1926).

*The Carriage of Chemical Mediators in Circulating Blood.* In all the earlier experiments, except those of Cannon and Uridil, the transfer of the mimetic chemical agent from the activated to the recipient organ was by way of salt solutions. In 1929 Demoer pointed out that these artificial fluids "may create new conditions of existence for the tissues, conditions accompanied by permeabilities which do not exist in physiological states, and that the escape of vagal and sympathetic substances, though proved experimentally, may not occur normally." Demoer's skepticism has been met by evidence that the substance representing parasympathetic impulses can be effective *via* vascular channels under nearly normal conditions, and that the substance representing sympathetic impulses can act typically after traveling in the blood stream under conditions which are wholly normal.

The early attempts to prove the presence of vagus substance in blood were not convincing. Duschl and Windholz's (1923) testimony that vagal stimulation of one parabiotic rat caused not only direct slowing of the heart but also indirect slowing in the other member of the pair was not confirmed by Enderlen and Bohnenkamp (1924) when tried on dogs. By taking carotid blood from a rabbit during

vagal stimulation and injecting it into the ear vein of a second rabbit Brinkman and van de Velde (1925) claimed to have shown that the heart rate of the latter was inhibited and that therefore a "Vagusstoff" was transmitted. Tournade, Chabrol and Malméjac (1926), using the heart as an indicator in crossed circulation experiments on dogs, were unable to confirm their results; and Plattner (1926) likewise failed, even though he drew blood from the coronary sinus during vagal stimulation and injected it directly into the left auricle of the second animal. In these experiments eserine, which inhibits the esterase (p. 85), was not used. Even when that drug is injected into the circulation to protect the vagus substance from destruction the effort to find signs of it in the blood may fail. Freeman, Phillips and Cannon (1931), employing a variety of anesthetics or decerebration, stimulating the entire vagal distribution and also shunting the portal blood flow past the liver, were unable (in cats) to register any effects on a variety of test organs, including the iris and the submaxillary gland, even when these organs had been previously denervated. It seems that too little eserine (physostigmine) was used in these experiments, for when Babkin, Alley and Stavracky (1932) gave repeated doses, and also limited the circulation to the fore part of the body, they found that stimulation of the left chorda tympani nerve (causing secretion from the left submaxillary gland) was followed shortly by secretion from the previously denervated right submaxillary. If the vein conveying blood from the stimulated gland was closed, reapplication of the stimulus did not produce its regular effect on the other side. Not only secretion, but also dilation of blood vessels on the denervated side was recorded, and attendant thereon was a fall of blood pressure. Henderson and Roepke's (1933b) observation that vagal stimulation produces an increased activity of the denervated submaxillary gland in the eserized dog, and von Saalfeld's (1934) confirmation of this result, together with his evidence that the "vagus substance" from the heart can contract bronchial muscles in the dog,

under physostigmine, are further proof that if the mediating substance, produced when a typical parasympathetic nerve is stimulated, is properly protected from destruction, it can be carried in the blood and can mimic elsewhere in the organism the effects of parasympathetic impulses.

That the adrenaline-like substance, liberated when sympathetic impulses are discharged, is a quite natural product, commonly transported in the blood stream, was first proved in surviving unanesthetized cats with the heart surgically denervated and the adrenal glands rendered inactive. In such animals Cannon and Britton (1927) had noted that a brief excited struggle, which brought sharply into action the sympathetic system, no longer provoked the large, sudden increase of heart rate (possibly 80 to 100 beats per minute within 60 seconds) that had occurred before the adrenals were inactivated and that was typical of medulli-adrenal secretion, but, instead, a moderate, gradual increase, which might amount to 25 to 30 beats per minute, reached at the end of three minutes. This strange phenomenon must have been due to some change in the circulating blood, for only by that medium was the denervated heart connected with the rest of the organism. Previous experiments had proved that it did not result from increased blood pressure, from increased temperature of the blood, or from metabolites thrown off by active muscles. In efforts to solve the problem, Newton, Zwemer and Cannon (1931) removed successively the medulla of the adrenals, the accessory chromaffin tissue between the kidneys, the adrenal cortex and the pituitary body, and then excluded from participation the agent from the liver (by severing the hepatic nerves), the alimentary canal and its associated glands (by extirpating the abdominal sympathetic chains), and the thyroids and parathyroids (isolated by excision of the stellate ganglia). The mysterious acceleration persisted; whenever there was excitement and struggle the heart would be slowly accelerated. At this stage the only remnants of the sympathetic system left in the animals were short strands in the lower thorax. When

these were removed the strange emotional increase of the cardiac rate disappeared. Vigorous and intense activity would send the beat up perhaps 4 beats per minute, but not 25 or 30.

The explanation of the part played by lower thoracic sympathetic chains was undertaken by Cannon and Bacq (1931). Acting on the probability that the sympathetic impulses released into the flowing blood a substance which, carried to the heart, made it beat faster, they prepared animals with denervated hearts, and then stimulated the lower abdominal sympathetics, isolated from the spinal cord. The only functional connection between the denervated heart and the tail region was the vascular system. When the sympathetic strands were stimulated for 30 or 60 seconds and the smooth muscles of the hairs and blood vessels contracted, there followed in about 2 minutes a marked elevation of arterial blood pressure, and in about 3 minutes an increase of heart rate which might be as great as 30 beats per minute. By blocking the blood flow into and out from the tail region it was possible to show that the same stimulation had no noteworthy effect until after the block was removed (see Figure 7).

The conclusions drawn from these experiments were that in consequence of parasympathetic or sympathetic stimulation a substance is liberated into the blood stream and that this substance, carried elsewhere in the organism, may have effects similar to parasympathetic or sympathetic nerve impulses. It is noteworthy that the substance produced by sympathetic stimulation acts under natural conditions and that no drug—analogous to physostigmine—is required to protect it from being broken down. Previous investigators, who had found a sympathomimetic substance in salt solution coming from organs stimulated through sympathetic nerves, called it "sympathetic substance." In place of this expression Cannon and Bacq suggested the name *sympathin*. Although sympathin produces many effects which are typical of the action of adrenine, evidence will be offered

-A-



-B-

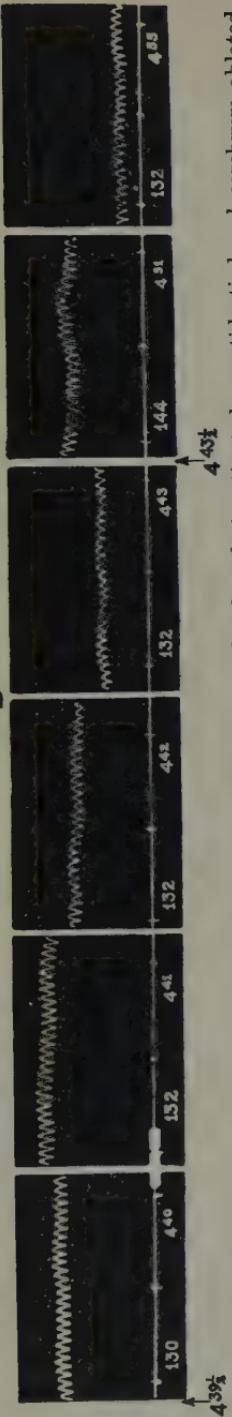


FIG. 7. Excerpts from a record made on a cat with heart denervated, adrenals inactivated, carotids tied and cerebrum ablated. Electrodes placed on the lower abdominal sympathetic strands which were isolated from the spinal cord. A loop of thread was passed around the aorta and the inferior cava above the iliac branches. A cannula for the blood-pressure record was inserted into the right carotid artery. A, stimulation for 1 minute, between 4:21 and 4:22, was followed by a slow rise of blood pressure (24 mm.) and a slow increase of heart rate (134 to 152 = 18 beats) at the times indicated. B, stimulation as in A, between 4:40 and 4:41, while the blood flow into the hind part of the animal was checked by closure of the aorta and inferior vena cava, between 4:39 1/2 and 4:43 1/2 (see arrows). Note differences at corresponding intervals after stimulation. At 4:48, the heart rate had risen 12 beats, at 4:51 it was 14 beats above the initial level. (Cannon and Baeg, 1931.)

later that it differs from adrenine in several important features.

*The Explanation of Chemical Blocking of Autonomic Impulses.* In 1870 Schmiedeberg showed that atropine prevents the inhibitory action of vagal impulses on the heart, an effect which was proved to be peripheral, for it occurred when the heart was isolated. In 1906 Dale demonstrated that ergotoxine produces an analogous block of the excitatory impulses of the sympathetic. Evidence as to the nature of the interferences yields important insight into the process of chemical mediation of nerve impulses.

Although atropine fully checks cardiac inhibition by the vagi, it does not interfere with the production of the mimetic vagus substance. Using two frog hearts Loewi and Navratil (1924) demonstrated that although vagal stimulation of heart A, lightly atropinized, did not inhibit its contractions, the Ringer solution, taken from this heart during the stimulation period and applied to heart B, produced typical inhibition of heart B.

In a similar manner Navratil (1927) showed that ergotamin which prevents the acceleratory action of sympathetic impulses on the frog heart does not prevent the formation of effective amounts of the chemical accelerating agent. And Cannon and Bacq (1931), by employing a dose of ergotoxine which blocked the excitatory action of sympathetic impulses on smooth muscles but did not affect the freshly denervated heart, found that stimulation of the lower abdominal sympathetic strands evokes, *via* the blood stream, typical cardiac acceleration.

From these observations it is clear that the paralyzing agents, atropine and ergotoxine, interpose a barrier, not between the nerve impulse and the production of the chemical mediator, but between the mediator and the responsive mechanism in the cell. This evidence is quite in accord with the fact that atropine and ergotoxine obstruct, respectively, the operations of acetylcholine and adrenine (in its excitatory influence).

*Adrenergic and Cholinergic Nerves.* To this point the association of sympathetic impulses with an adrenaline-like mediator and parasympathetic impulses with acetylcholine has been described without qualification. It has long been known, however, that certain sympathetic effects, *e.g.*, the contraction of the smooth muscle of some blood vessels and the secretion of sweat in some animals, have peculiarities which would class them with parasympathetic phenomena. Thus sweating can be induced in the cat by the parasympathomimetic drug, pilocarpine, but not adrenaline; and once started, it can be stopped by atropine. In 1921, Langley suggested that if two kinds of nerve fibers exist in a division of the autonomic system (*e.g.*, the sympathetic division) they might be distinguished by the "understandable—though inelegant—words, cholinophil and adrenophil." With the evidence before him that acetylcholine is set free when sympathetic impulses excite sweat glands to action, Dale (1933) proposed that fibers having such effects be designated *cholinergic* sympathetic fibers, to distinguish them from *adrenergic* sympathetic fibers—those having an adrenaline-like mediator.

Whether there are adrenergic parasympathetic fibers has not yet been determined. Luckhardt and Carlson (1921) reported that adrenaline contracts the pulmonary arteries of the frog and turtle, and that vagal, not sympathetic, impulses have the same effect. Furthermore, there is evidence that the vagi contain cardio-accelerator fibers which might be adrenergic (see p. 46), but a liberation of sympathin by them has not been demonstrated.

Evidence will be presented later that nerve impulses delivered to sympathetic ganglia by preganglionic fibers or to skeletal muscle by ordinary motor fibers discharge acetylcholine at their terminals. These fibers are obviously cholinergic. So also, typically, are the postganglionic parasympathetics. And typically the postganglionic sympathetics are adrenergic. The exceptions mentioned above, however, prove that the use of adjectives related to the

chemical mediator involved in any situation is more exact than the anatomical reference to divisions of the autonomic system.

*The Argument.* No one has seen the process whereby nerve impulses first produce a chemical substance and the chemical substance then provokes its peculiar effect in the reacting cell. The phenomenon is inferred. The inference is based, however, on the steps which have been reviewed in the foregoing pages. Substances found naturally in the body, adrenine and acetylcholine, are capable of mimicking in autonomic effectors the changes produced by sympathetic and parasympathetic impulses. When sympathetic or parasympathetic nerves are stimulated a substance is given off into fluid bathing the stimulated organ, such that when transferred to another organ the substance has an adrenine-like or an acetylcholine-like action. In fairly typical instances paralyzing agents are effective for both the nerve impulses and the mimetic chemical; atropine blocking, for example, vagal impulses, and also acetylcholine; and ergotoxine blocking, for example, sympathetic impulses on contractile vascular muscle, and also adrenine. But these paralyzants, acting peripherally, do not interfere with the local production of the mimetic substances by the autonomic impulses. The simplest conclusion from these facts is that when a sympathetic or parasympathetic nerve is stimulated, it liberates a chemical deputy and this deputy exerts the influence formerly attributed directly to the nerve impulse.

There are other facts which we must consider in later discussions. Thus a mediator has different effects in different organs— inhibitory in some and excitatory in others. To account for this phenomenon Langley (1905) assumed that there was present in the responsive cells a differentiating receptive substance which would determine whether a single agent, adrenine, for instance, would produce relaxation or contraction in a given set of smooth muscles. Again, the histological testimony favors the ending of autonomic nerve filaments inside smooth-muscle units, and that raises the

question as to whether the mediator has a strictly neural or perhaps a neuromuscular origin. And furthermore there is evidence that a mimetic substance produced in key cells, supplied with nerves, diffuses to neighboring, less favored, cells. These and other related matters will be taken up in later chapters.

The sequence of functionally related events which probably occur in the electrical excitation of an autonomic neuro-effector system is as follows: 1) electric shock → 2) local excitatory state in the nerve → 3) nerve impulse (conducted disturbance in nerve) → 4) liberation of a chemical mediator → 5) combination of the mediator with a receptive substance → 6) specific reaction of the effector (contraction or relaxation of muscle, acceleration or deceleration of the heart, or secretion). Our attention will be applied chiefly to events 4 to 6.

## CHAPTER IV

### SOURCES AND INDICATORS OF THE PARASYMPATHOMIMETIC SUBSTANCE

As previously noted (pp. 3, 10, 22), the various influences of parasympathetic impulses on autonomic effectors include inhibition of the heart, contraction of the stomach and intestines, constriction of the bronchioles, narrowing of the pupil and dilation of blood vessels in various parts of the body—notably in the external genitalia, the face and salivary glands. The fibers distributed to these structures are cholinergic. But, as already observed, the sympathetic supply to the sweat glands in some animals is also cholinergic. And there is evidence that acetylcholine is liberated on the arrival of nerve impulses in sympathetic ganglia and in skeletal muscle. Theoretically each of these arrival stations of cholinergic impulses might be a source of acetylcholine and each might also be made an indicator of the presence of that substance in blood or perfusate coming from another station. To a large extent that possibility has been realized.

*Tests for Acetylcholine.* In order to indicate reliably the presence of either of the chemical mediators an organ should preferably be freed from any other influence, *i.e.*, disconnected from a complicating nervous control or wholly isolated from the body. Even when the situation has been thus simplified the possibility of action of another agent than that being sought must be critically regarded. Without separation from the nervous system the reaction of arteries in blood-pressure tests has been employed, to be sure, but the intricacies of the vascular apparatus make it a rather complex method, to be used cautiously.

For demonstrating the presence of small concentrations of acetylcholine a highly sensitive test-object is, interestingly

enough, not an isolated organ from a vertebrate, but a strip of longitudinal muscle from the leech. After being carefully prepared and cleaned it must be placed in a weak solution of eserine for 15 minutes (Fühner, 1918) before it is sensitized. Since Minz (1932) pointed out that its contraction offers a delicate means of identifying acetylcholine it has been used in a number of important researches. Although it is said to be specific in its response, eserine renders it more responsive to barium, as Vartiainen (1933) has shown, and also to potassium ions.

According to Gaddum (1936) the relative sensitiveness, measured in  $\gamma$  (0.001 mgm.) per liter, of common indicators of acetylcholine, is as follows:

Leech muscle (isolated and treated with eserine) . . . . .	2
Rabbit auricle (isolated and treated with eserine) . . . . .	4
Frog heart (Straub method) . . . . .	10
Mouse intestine . . . . .	10
Rabbit intestine . . . . .	20
Frog's rectus abdominis (isolated and treated with eserine) . . . . .	20
Cat's denervated gastrocnemius . . . . .	100

Since none of these organs is strictly specific, the suggestions of Chang and Gaddum (1933) as to ways of distinguishing between their reaction to acetylcholine and to other substances become important.

1. If the action of a tissue extract or perfusate is increased by protective eserine the conclusion is justified that it is not due to choline nor to any other known bodily substance than acetylcholine.

2. The activity must disappear quickly if the extract or perfusate is mixed with blood; but if eserine has been added to the blood the rapid destruction is markedly checked or suppressed.<sup>1</sup>

3. The active substance must not be stable in an alkaline solution. If a part of the extract or perfusate is mixed with

<sup>1</sup> In connection with this action of an esterase and the protective effect of eserine, the observations of Galehr and Plattner (1928) and Plattner and Bauer (1928) are of interest. They found that animals differ in the ability of the blood to break down acetylcholine; from strongest to weakest, the animals range as follows: man, pig, bovine cattle, dog, horse, rabbit, frog and cat. According to Feldberg and Rempel (see Feldberg and Krayer, 1933) the blood of the guinea pig is weaker than that of the cat.

an equal part of 2 N NaOH and allowed to stand 10 minutes at room temperature and then neutralized, acetylcholine is destroyed, while choline is not changed.

4. The active substance is not destroyed by boiling for several minutes in a weakly acid solution.

5. Many effects of acetylcholine are blocked by atropine, others by curare or nicotine; the active substance must be affected in the same manner by these drugs.

6. If the activity of an extract or perfusate has been quantified in terms of acetylcholine by use of various pharmacological tests, the results from all the tests must be the same. The special value of this criterion is that it separates acetylcholine from most other choline esters.

In presenting evidence for the existence of acetylcholine as a chemical mediator of parasympathetic impulses special emphasis will be laid on experiments in which one or more of these corroborative tests have been employed.

*Chemical Mediation of Cranial Parasympathetic Nerve Impulses.* Loewi's use of the frog heart as a source and as an indicator of "vagus substance" has been mentioned (see p. 23). The fluid from the stimulated heart is able to lessen the extent of contraction and reduce the frequency of beat of the recipient organ. These observations have been confirmed in modifications of the method by various observers. The arrangement of the two hearts and the records of direct and indirect vagal action, as reported by Bain (1932b), are shown in Figure 5.

That the mammalian heart, subjected to vagal influence, may serve as a source of mediating substance was indicated by the transfer of blood or perfusate to another heart of the same species (Rylant, 1927) or to a frog heart (Popper and Russo, 1925). Recording simultaneously by electrical methods the heart beats of a pregnant guinea pig and a fetus, Hansen and Rech (1931) found that the bradycardia produced by stimulation of the mother's vagus was followed after about 10 seconds by a similar change in the fetal heart rate—an effect which they attributed to the passage of the

vagal mediator through the placenta. Although the guinea pig, because its blood is least destructive of acetylcholine (see p. 35), is naturally the animal most favorable to the success of this ingenious experiment, the positive effect, without use of eserine, seems strange in view of the extreme lability of the mediator. Possibly fetal organs are more sensitive than those of the adult, or fetal blood is less destructive, or perhaps the guinea pig's placenta is like the human in being especially rich in acetylcholine (Chang and Gaddum, 1933) and under experimental conditions is able to yield this substance to the circulation.

The absence of eserine or its ineffective use was a defect of the earlier experiments involving a blood transfer of acetylcholine from organ to organ. Using the heart as a source and salivary glands and other organs as indicators, Henderson and Roepke (1933b) and von Saalfeld (1934), as previously mentioned, were able to demonstrate definite effects in eserinized animals. Even more decisive were the procedures of Feldberg and Krayer (1933), who found that the blood which they collected from the coronary vein of eserinized cats and dogs during or shortly after vagal stimulation caused contraction of leech muscle and a lowering of blood pressure (in the cat). The hypotensive action on blood pressure was abolished by atropine. And the active substance, though destroyed by natural blood, was relatively stable in blood treated with eserine. By use of the same method Krayer and Verney (1934) showed that acetylcholine is set free from the heart when the vagus center is stimulated reflexly by increase of blood pressure.

It seems probable that vagus innervation endows cardiac muscle with an increased sensitiveness to acetylcholine. Armstrong (1935) found that whereas relatively large doses of that agent (up to  $0.135\gamma$ ), when injected into Fundulus embryos before nerves reached the auricle, did not inhibit the heart beat, a very small dose (down to  $0.000023\gamma$ ) lessened the auricular contraction, and about three times that small dose stopped both the auricle and the ventricle.

in diastole. (Like cardiac muscle, smooth muscle responds to acetylcholine when no nerves are present [Bauer, 1928]; whether it is rendered more sensitive after being innervated has not been determined.<sup>1</sup>)

The vagus is not only an inhibitor of the heart, but is a stimulator of the *gastro-intestinal tract* and its associated glands. In relation to atropine, however, the effects of the vagi and of acetylcholine on gastro-intestinal muscle present an anomaly; whereas atropine even in large doses does not completely block vagal action on the stomach and fails to paralyze at all vagal action on the intestine, it readily abolishes in small doses the mimetic influence of acetylcholine and related agents. This striking difference raised the question as to whether the mechanism of vagal impulses affecting the muscles of the alimentary tract could be the same as it is in the heart.

Evidence on this point was furnished by Feldberg and his associates (145, 150) and by Dale and Feldberg (1934c). The former found that the blood of the portal vein of an eserized dog or cat caused contraction of leech muscle and a fall of blood pressure in the cat; both effects were augmented by eserine, and the blood-pressure effect was abolished by atropine. Passage through the liver destroyed

<sup>1</sup> Elliott (1905) suggested that only after union with sympathetic fibers does smooth muscle acquire, at the myoneural junction, a mechanism which can receive the nerve impulse and turn it to contraction or relaxation, and which also is responsive in like manner to adrenine. In support of this suggestion Elliott relied on evidence that the muscles of the bronchioles lack sympathetic nerves and correspondingly are insensitive to adrenine. That evidence is now known to be incorrect. Furthermore, Langley (1905) found that spontaneous contractions of nerveless amnion of the chick are inhibited by adrenine. Unfortunately he did not report the relative strength of the solution which he used. It is noteworthy that Bauer (1928) produced only brief inhibition of the amniotic rhythm by direct application of adrenine, 1:150,000—a rather strong concentration. Indeed, Bauer specifically states that the amnion is relatively inert, *i.e.*, the concentrations of chemical agents, when effective, are relatively high. Thus, though nerveless smooth muscle may respond to adrenine, union with sympathetic fibers may sensitize it to that agent. In that respect Cattell and Wolff (1935) have generalized Elliott's suggestion so as to include cholinergic fibers, and have modified it by assuming that the nerve connections bestow on the muscle a specific sensitiveness which is retained after denervation. Smooth-muscle cells which do not receive a nerve supply may nevertheless be in the "sphere of influence" of the nerve endings, they suppose, much as in experimental embryology structural differentiation may be induced by the presence of neighboring cells.

the action, and only when eserine was given were any of the tests positive. The destructive influence of the liver is in accord with an observation by Hunt (1918), who noted that a dose of acetylcholine which would cause a marked fall of blood pressure when injected into a systemic vein, had no effect on blood pressure when it was injected into a mesenteric vein and required to traverse the hepatic capillaries. These phenomena support the inference that acetylcholine was present, although the vagi had not been

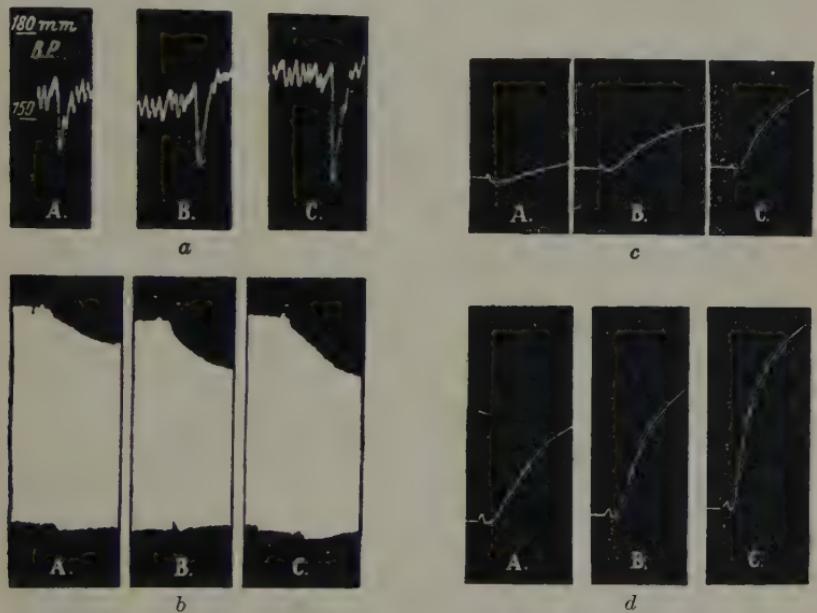


FIG. 8. Tests of a perfusate of eserinized Locke's solution passing through the vessels of the stomach of a dog during vagal stimulation. The perfusate before stimulation was little, if at all, active. Record *a*, effects on the blood pressure of an eserinized cat under chloralose; *b*, isolated frog heart (Straub method); *c*, eserinized rectus abdominis of the frog; and *d*, eserinized leech muscle. In each series *B* shows the effect of the concentrated perfusate, tested in a constant ratio to two strengths of acetylcholine, at *A* and *C*; that at *C* was double that at *A*. Cf. requirement 6, p. 36. (Dale and Feldberg, 1934c.)

stimulated. In the experiments of Dale and Feldberg an eserinized perfusate was collected from the stomach of a dog during the period of stimulation of both vagi in the lower thorax. Four different tests—the blood pressure of the cat, the frog heart, the rectus abdominis of the frog,

and leech muscle—all yielded quantitatively consistent results (see Figure 8). In every respect the signs were evident that vagal impulses are mediated by acetylcholine in the stomach as they are elsewhere.

Testimony confirmatory of observations on the stomach has been given by Bunting, Meek and Maaske (1935), who perfused the small intestine of the dog with a modified eserized Locke's solution. The perfusate was tested on the frog heart and on leech muscle. Although the heart was affected slightly by the control sample taken before vagal stimulation, it was brought to a prolonged standstill by the sample taken during the stimulation. Leech muscle likewise yielded positive results. These effects appeared only when the perfusate contained eserine, the inhibition of the cardiac beat was abolished by atropine, and the effective agent disappeared on standing. These typical features led to the conclusion that acetylcholine was the substance set free from the intestine when the vagi were excited.

That parasympathetic control of the *salivary glands* is mediated by acetylcholine is indicated by the experiments of Babkin, Alley and Stavracky on the submaxillary gland, already described (see p. 26). Confirmation of these experiments was brought by Babkin, Gibbs and Wolff (1932) in showing that when blood was allowed to flow through the submaxillary of an eserized cat during chorda stimulation a fall of blood pressure resulted, but not when the flow was prevented. Feldberg's (1933b) demonstration that this effect disappeared after atropine was given proved that it was due to an agent acting on the general circulation, for atropine does not prevent local vasodilation from chorda impulses.

In consonance with these results are those reported about the same time by other investigators. As well-controlled observations may be mentioned those of Henderson and Roepke (1932) and Gibbs and Szelöczey (1932). The former found in the perfusate from a dog's submaxillary during stimulation of the chorda tympani a substance which acted

like acetylcholine on the frog heart and was blocked by atropine. It was destroyed when warmed to 60° for 5 minutes in a weakly alkaline solution. As in the frog heart, atropine, though preventing the obvious effect (secretion, in this instance), did not prevent the liberation of the mediator. Gibbs and Szelöczey demonstrated that fluid perfused (interchangeably with blood) through the submaxillary gland of the cat acquired, when the chorda tympani was stimulated, an ability to cause a fall of blood pressure, a flow of saliva, inhibition of the isolated frog heart, and heightened tone of a strip of rabbit intestine—all effects characteristic of acetylcholine. Furthermore, the active agent, destroyed by standing in blood, could be preserved from this destruction by eserine. And again, doses of atropine which stopped secretion did not hinder the passage of the active agent into the perfusate when the secretory nerve was stimulated.

Although vagal stimulation of the *pancreas* in the dog evokes an external secretion (Pawlow, 1878), satisfactory experiments proving that acetylcholine is produced by the nerve impulses have not been reported. The procedure employed by Rasenkow and Ptschelina (1931) lacked adequate controls, and the treatment of the non-eserinized blood from the stimulated pancreas (collection and defibrillation before it was injected into the test pancreas, previously perfused with Ringer solution) was such as to permit total destruction of any acetylcholine which might have been present. The positive result was probably due to the change of the perfusate from salt solution to blood. The failure of Gayet and Guillaumie (1931) to evoke secretion when blood from the stimulated pancreas passed directly to the test pancreas may have been due to lack of eserine.

That the short ciliary nerves, acting on the smooth muscle of the *ciliary body* and the *iris sphincter* are cholinergic was shown by Engelhart (1931), who applied the aqueous humor to the toad heart. In the untreated humor of the rabbit and cat he found no sign of an active agent. From the

previously eserized eye of the rabbit, however, after it was stimulated reflexly by exposure to light, the watery fluid produced the typical effects of acetylcholine. And when strong electrical stimulation was applied to one oculomotor nerve of the cat the aqueous humor of that eye, as compared



FIG. 9. Effects of the aqueous humor of the cat's eye on the beat of the toad heart. *A*, acetylcholine (1: 100 millions). *N*, extract from the non-stimulated eye. *O*, extract from the eye stimulated through the oculomotor nerve. (Engelhart, 1931.)

with that of the other, strikingly reduced the cardiac contractions (see Figure 9). The "parasympathetic substance" was rendered ineffective by atropine.

In 1912 Dixon and Ransom reported that vagal stimulation contracted the *bronchioles* and that atropine blocked the action. Support for these indications of cholinergic activity of pulmonary vagal endings was brought by Thornton (1934) who perfused the lungs of the guinea pig with physiological salt solution containing eserine. The perfusate collected during

vagus stimulation acted like acetylcholine in contracting leech muscle and in producing a fall of blood pressure in the cat.

*Chemical Mediation of Sacral Parasympathetic Impulses.* The pelvic visceral nerves serve to dilate blood vessels of the external genitalia and contract smooth muscle of the distal colon and the body of the bladder. Relatively little work on chemical mediation has been done in this area. Pharmacological evidence is suggestive; Henderson and Roepke (1933a) and Bacq (1935) noted that eserine increases remarkably the influence of the *nervi erigentes* on blood vessels, and that atropine, though decreasing the efficacy of acetylcholine, has little influence on the efficacy of the nerve impulses. This phenomenon presents a problem similar to that encountered in examining vagal action on the

intestine (see p. 38), and probably will have the same explanation when illuminating facts are found.

By perfusing the vessels of the isolated *bladder* (*in situ*) Henderson and Roepke (1933a) were able to demonstrate that stimulation of pelvic autonomic fibers made the perfusate capable of reducing the contractions of the frog heart. In a later paper (1934) they pointed out that, whereas acetylcholine injections or nerve impulses produce a contraction which is sustained throughout the application of the stimulus and for a short period thereafter, the same stimulations, when applied after atropine, evoke only an initial, twitch-like response. The former they assume to be an exhibit of the "tonus mechanism"; the latter, a separate contractile mechanism. Atropine, they infer, depresses the tonus mechanism but not the contractile. When, after atropine, nicotine was used to paralyze the autonomic pathways at the ganglionic synapses, neither acetylcholine nor nervous influence had any noteworthy effect. It appears, therefore, that the twitch-like response to acetylcholine was not due to a direct stimulation of the muscle peripherally, but to stimulation of ganglion cells, *i.e.*, like the electric shocks it started nerve impulses which evoked the sharp contraction. The action of atropine on the bladder resembles in some respects its action on the intestine—it prevents the response to an injected mimetic substance, but has a slight or a modifying influence on the response to natural nerve impulses. The "contractile" reaction of the bladder, as contrasted with the "tonic," is like the behavior of the nictitating membrane after the drug, "933F" (piperidinomethylbenzodioxane), has been given. An analysis of that situation will be presented later (see p. 154). It will be obvious that Henderson and Roepke's view is not exclusive of another reasonable theory explanatory of the two types of contraction observed by them in bladder muscle.

*Chemical Mediation of Nerve Impulses Delivered to Blood Vessels.* As a rule cholinergic fibers relax the smooth muscle of blood vessels; the contraction of the coronary arteries by

vagal impulses (cf. Anrep, 1926) is an exception. Already dilation of the arteries of the submaxillary glands and the external genitalia by parasympathetic action has been mentioned; acetylcholine in both instances causes vascular relaxation, but definite proof that that substance is liberated by the nerve impulses is lacking. Atropine blocks the impulses only slightly if at all.

Besides these well-known parasympathetic vasodilators there are others which, when stimulated, have yielded evidence that acetylcholine is set free. Historically prominent among them is the lingual nerve whose function is that of dilating the vessels of the tongue. Philipeaux and Vulpian, in 1863, reported that after the motor nerve to the dog's tongue, the hypoglossal, had been cut and degenerated, stimulation of the lingual induced a slow, gradual contraction of that organ. A similar phenomenon was described by Rogowicz (1885) who, after denervating the facial muscles in the dog, found that stimulation of the cervical sympathetic would produce a sluggish response of muscles near the lips and the eye. Another instance of like character was reported by Sherrington (1894); he severed the anterior roots of the hind leg of the cat and after these motor fibers had degenerated he noted that stimuli applied to the peripheral nerve or to dorsal roots caused a slow contracture of the "paralyzed" muscles. This "pseudomotor" phenomenon Bremer and Rylant (1924) attributed to the liberation of a substance at the endings of vasodilator nerves that affected skeletal muscles which were sensitized by denervation. That acetylcholine is able to influence denervated skeletal muscle in like fashion was shown by Frank, Nothmann and Hirsch-Kauffmann (1922-23).

Further progress has supported the theory that the Vulpian-Rogowicz-Sherrington phenomenon results from a leak, onto denervated skeletal muscle, of acetylcholine which diffuses from blood vessels dilated by stimulation. Dale and Gaddum (1930) observed that the contracture of the tongue muscles was augmented by eserine—an effect

indicative of the presence of acetylcholine. By extending these observations to the muscles of the face in repeating the Rogowicz experiment, v. Euler and Gaddum (1931) proved that a true, anatomical sympathetic trunk (the cervical) may contain cholinergic fibers. And Hinsey and Cutting (1933) showed that the Sherrington phenomenon disappeared if the sympathetic supply to the blood vessels of the hind limb was abolished—*i.e.*, that it resulted from cholinergic fibers in the sympathetic distribution. Meanwhile Bain (1932a) had reported that salt solution which had flowed through the blood vessels of the dog's tongue, while impulses from the lingual nerve relaxed the vascular smooth muscle, became able to excite contractions of the rabbit intestine. And Feldberg (1933a) found that blood from the tongue of the eserized dog had effects on blood pressure and leech muscle and responded to other tests that indicated that acetylcholine was present. In addition, Büllbring and Burn (1935) have shown that stimulation of the sympathetic fibers of the hind limb of the dog causes to appear in a perfusate of eserized Locke's solution a substance which acts pharmacologically like acetylcholine. Although the foregoing observations clearly point to cholinergic vasodilators in sympathetic trunks, they do not exclude the existence of adrenergic vasodilators. Rosenblueth and Cannon (1935), using pharmacological methods, were led to conclude that the sympathetic contains some cholinergic and some adrenergic vasodilators in dogs and cats, but no vasodilators in rabbits.

*The Innervation of Sweat Glands by Cholinergic Fibers.* The anomalous responses of sweat glands—of the cat, for example—to parasympathomimetic drugs, which are blocked by atropine, although these glands are under sympathetic control, has long been a puzzle. The mystery has been solved by proof that the fibers governing the glands are cholinergic. By perfusing the cat's paw with eserized Locke's solution Dale and Feldberg (1934b) obtained, during sympathetic stimulation, a substance which by its action

on leech muscle and cat's blood pressure was identified as acetylcholine. It was destroyed in an alkaline medium, and its hypotensive effect on blood pressure was increased by eserine and abolished by atropine.

The existence of accelerator fibers in the cervical vagus trunk has been reported by a number of observers (see Tigerstedt, 1921). In 1934 Jourdan and Nowak showed that in the dog vagal cardio-accelerator impulses can be demonstrated to be effective after atropine. By direct stimulation and also by asphyxia of the isolated head the fibers which carry these impulses were shown to leave the medulla in the vagus root. Whether the impulses act by mediation of sympathin has not been learned. If they do there would be in the vagal supply to the dog's heart an instance of adrenergic fibers in the parasympathetic system.

## CHAPTER V

### ACETYLCHOLINE AS A MEDIATOR OF IMPULSES TO SKELETAL MUSCLES AND SYMPATHETIC GANGLIA

The evidence that autonomic impulses delivered to smooth muscle and glands exert their influence indirectly by setting free one or another effective chemical agent has naturally raised questions as to the manner in which nerve impulses elsewhere—at neuronal synapses and at the motor plates of skeletal muscles—exert their influence. Although the main interest of the present exposition is to report on autonomic neuro-effectors, the development of the concept of chemical mediation beyond the autonomic realm into other realms of nervous activity has brought forth facts pertinent to the general theme under consideration and significant for future growth of knowledge.

Since acetylcholine is indicated as the mediating agent in the speedy processes which occur at sympathetic ganglia and striated muscle, these phenomena will be considered now, before sympathetic mediation is discussed in the next chapter. As Dale pointed out in 1914(a), the mode of action of acetylcholine at synaptic junctions resembles the action of nicotine and should be distinguished from its action at parasympathetic effectors, where acetylcholine resembles muscarine (p. 22).

The main distinction between the two types of action of acetylcholine is pharmacological—*atropine* blocks the muscarine-like effects while leaving practically unimpaired the nicotine-like effects. But there is another difference of importance for this exposition; in its nicotine-like action acetylcholine is excitatory at relatively small doses, but becomes paralyzing when its concentration is high. This

paralyzing influence accounts for some of the apparent discrepancies in experimental results.

*Evidence that Motor Nerves of Skeletal Muscles Are Cholinergic.* Observations suggestive of a chemical step in the transfer of action from nerve to skeletal muscle were made by Zucker (1923), who noted that eserine lowered the threshold of frog muscle to electrical stimuli, and by Samojloff (1925), who found that temperature changes (which affect chemical processes more than they do physical) influence the excitation stage of muscle more than they influence the conductivity of nerve.

Direct evidence that motor nerves are cholinergic was obtained decisively by Dale and Feldberg (1934a; see also Dale, 1934). After degeneration of sympathetic fibers in the trunk of the hypoglossal nerve had left only motor fibers and some afferents from the muscle spindles, they stimulated the trunk and observed that the eserinized Locke solution flowing then from the tongue vessels contracted leech muscle and gave other signs of containing acetylcholine.

Even more demonstrative were the experiments of Dale, Feldberg and Vogt (1936), performed on the hind limbs of dogs, cats and frogs. Here they stimulated only motor fibers, those of the anterior roots, after removal of the abdominal sympathetic strands and severance of the dorsal roots. Again eserinized Locke solution, perfused through the limb vessels, revealed that stimulation had liberated from the contracting skeletal muscles a substance having acetylcholine characteristics. Direct stimulation of a normal muscle, or one deprived only of its autonomic nerve supply, had a similar result; but when the muscle was completely denervated no acetylcholine appeared in response to effective stimulation. Tetanizing the motor nerves after curarization still caused release of the active substance, although there was no contraction.

The observations just cited, suggestive of acetylcholine as a mediator in the excitation of skeletal muscle, receive

support from the evidence on the stimulant properties of that substance. Certain normal muscles of some cold-blooded animals exhibit a prolonged, low-tension contracture in response to acetylcholine (270). As illustrations the rectus abdominis of the frog may be mentioned, and the striated muscular coat of the intestine of the tench. Similar contractures have also been obtained in some muscles of birds and mammals (113, 123). Other muscles will only respond after denervation (157) or after injections of eserine (288). These contractures differ from the ordinary responses of skeletal muscle to nerve impulses in not having the characteristic quick development and subsidence of tension, and in lacking typical action potentials (cf. Gasser, 1930). It is possible, however, to demonstrate quick muscular twitches when acetylcholine is injected under favorable experimental conditions.

In 1933(a) Feldberg, studying the similarity between the action of acetylcholine on the muscles of the tongue and the normal effects of stimulating the lingual nerve, found that strong doses of acetylcholine injected into the carotid artery caused irregular contractions of the innervated muscles. Simonart and Simonart (1935) made the observation that ether anesthesia abolishes the influence of acetylcholine on contraction. When, in the early stages of barbital anesthesia, they tested the effects of intravenous injection of acetylcholine, they were able to provoke a rapid contraction of all the muscles of the body lasting from 2 to 5 seconds. The gastrocnemius, attached to a lever, registered a sharp shortening and relaxation. To prove that the twitch resulted from a peripheral stimulation they repeated the injection immediately after cutting the sciatic nerve and duplicated the previous response. The tension developed by the gastrocnemius after a single dose was as much as 400 gms. Like Feldberg, the Simonarts noted that during contraction the muscles manifested fibrillary activity.

The doses used by the Simonarts were large as compared with those which act on autonomic effectors; the least

amount used, in medium-sized cats, was 1 mgm.; and 5 mgm. were commonly injected. A. Simonart (1935) was able to obtain contractions of the gastrocnemius with a much smaller dose, 0.01 mgm., by introducing it directly into the artery supplying the muscle (cf. Brown, Dale and Feldberg, 1936). With doses varying between 0.5 and 5 mgm. he produced contractions which were equal to those produced by tetanizing the sciatic nerve. Moderate doses were shown to be adjuvant to motor impulses. And just as curare sup-

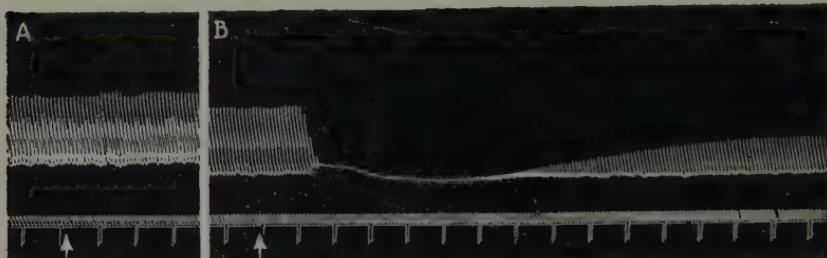


FIG. 10. Record of the tibialis anticus muscle of a cat; the muscle was left *in situ* and stimulated through its nerve. Adrenals ligated. Atropine (1 mgm. per kgm.) had been injected to prevent the fall of blood pressure caused by acetylcholine. A, before prostigmin; at arrow acetylcholine (1 mgm.) injected intravenously. B, after prostigmin (0.5 mgm. per kgm.); at arrow acetylcholine (1 mgm.) injected. (Rosenblueth, Lindsley and Morison, 1936.)

presses the response to such impulses, it suppresses the response to acetylcholine.

The quick and slow responses of skeletal muscle to acetylcholine are probably independent events denoting activation at different steps of the excitatory process. The quick contractions appear to correspond to twitches or short tetani set up at the neuromuscular junction. Brown (cf. 65) has found that repetitive muscle action potentials attend these responses. The slow contractures, which are not preceded by conducted action potentials, as was mentioned before, may be due to a direct effect of acetylcholine on the contractile system (279). It is possible to obtain both types of contraction in a given muscle when acetylcholine is injected after eserine.

The protective action of eserine on acetylcholine might

and, a poison, to increased muscular responses to motor nerve impulses. It may also lead, however, to decreased responses if the concentration of acetylcholine should reach sufficiently great. Experimentally the two opposite effects can be produced. Rosenblueth, Lindsley and Morison (1936), using prostigmin (a substance closely related to

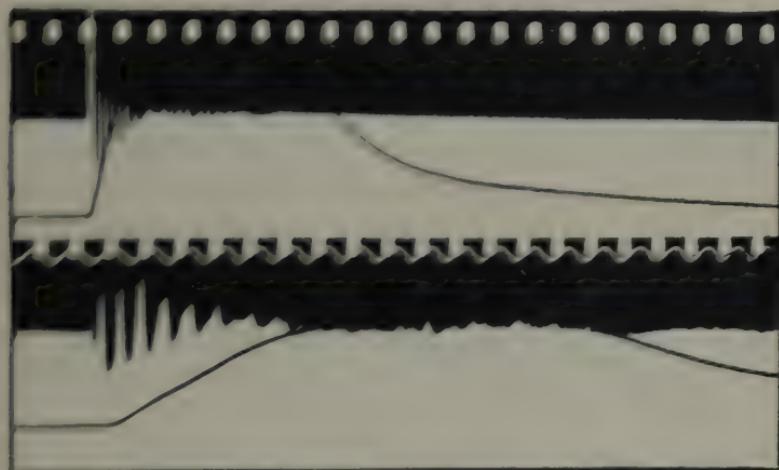


FIG. 10. Records of the spinal myogram and the action potentials of the quadriceps muscle stimulated through its nerve, in the spinal cat. Top record, 1.6 seconds after exercise. Lower record, two hours after exercise, at the same time. Normally a single nerve volley produces a single electrical wave (or spike) and a single twitch; after exercise, as here shown, it produces repetitive electrical responses and a broad tetanic contraction. (Brown, Dale and Feldberg, 1936.)

exercise), instead of eserine, found a paralysis of the responses of the lumbar muscles to stimulation of the peroneal nerve (Figure 10). On the other hand, Brown, Dale and Feldberg (1936), have demonstrated increased responses of the same muscles in spinal cats injected with eserine. This increase is due to the ability of each nerve volley to produce several propagated disturbances in the muscles—*i.e.*, single shocks induce tetani instead of twitches (Figure 11). Unpublished observations made by Rosenblueth and Morison show that the potentiation or depression induced by eserine depends on the frequency at which the motor nerves are stimulated. With slow frequencies—*e.g.*, 1 shock per 10 seconds—eserine

or prostigmin sensitizes the responses; with higher frequencies—e.g., 4 per second or more—a depression occurs. These results are in agreement with the explanation mentioned above, since high frequencies lead to the accumulation of acetylcholine, protected from destruction by eserine, until paralyzing concentrations are reached.

*Evidence that Preganglionic Fibers of the Sympathetic System Are Cholinergic.* In 1933 Kibjakow reported that Locke's solution, perfused through the superior cervical ganglion of the cat, acquired a new property when the pre-ganglionic fibers were stimulated. If reintroduced into the blood vessels of the ganglion it had the same effect as nerve stimulation—it caused contraction of the nictitating membrane. The perfusate taken before or after exciting the pre-ganglionic fibers was without effect. Using Kibjakow's technique Feldberg and Gaddum (1934), though unable to repeat his results, found that the perfusate, containing a small addition of eserine, bore away from the ganglion, while the cervical sympathetic trunk was being stimulated, a substance indistinguishable from acetylcholine. It was relatively stable in a slightly acid solution, but was destroyed by alkali; and it acted typically and with quantitative consistency on eserinized leech muscle, frog's heart and rectus abdominis, cat's blood pressure and rabbit's auricle. Similar results were obtained by Barsoum, Gaddum and Khayyal (1934) in perfusing the inferior mesenteric ganglion of the dog.

Further advances relative to the events in transmission of nerve impulses in sympathetic ganglia have been made by Feldberg and Vartiainen (1934). They found that antidromic impulses, when excited in postganglionic fibers, did not set free any acetylcholine from the ganglion. They found also that eserine in very slight concentration (1 in  $10^6$ ), when perfused through the ganglion, markedly augmented the responses of the nictitating membrane to submaximal preganglionic stimuli. Furthermore, stronger concentrations of eserine (1 in  $2 \times 10^4 - 10^5$ ) depressed the re-

sponses to preganglionic stimulation, just as they depress the action of small doses of acetylcholine. These results have been interpreted as sustaining the opinion that acetylcholine is a necessary agent in the passage of nerve impulses from neurone to neurone in sympathetic ganglia.<sup>1</sup>

Potassium ions, likewise, may stimulate normal and denervated sympathetic ganglia to discharge, as was demonstrated by Brown and Feldberg (1936a). Potassium liberates acetylcholine when applied to innervated ganglia, but activates denervated ganglia without demonstrable release of acetylcholine. There is thus an intimate relation between the two substances which is probably of significance in the transmission of the nerve impulses. Eccles (1935a) has suggested that the transmitter of the nerve impulse is the potassium mobilized by the passage of the impulse and that acetylcholine plays only an adjuvant rôle in the process. This view, however, is not tenable, for Brown and Feldberg (1936c) have shown that curare paralyzes the ganglia both to acetylcholine and to the preganglionic nerve impulses, whereas it does not prevent the action of potassium. The rôle of potassium in the transmission process remains to be elucidated by further investigation.

Granted that the transmission of nerve impulses at ganglionic synapses involves the release of acetylcholine, that agent must act in such locations in a remarkable manner. Bishop and Heinbecker (1932) have shown that each single impulse in preganglionic fibers activates a corresponding

<sup>1</sup> While this book was in press Cannon and Rosenblueth found that tetanic stimulation of cervical preganglionic fibers, after a dose of prostigmin, starts a contraction of the nictitating membrane but cuts it short and induces relaxation. This result is explained by an initial excess of acetylcholine because it is protected from prompt destruction, for a small amount of acetylcholine (without prostigmin), if injected when stimulation begins, has the same effect. Either prostigmin or acetylcholine raises the height of contraction of the membrane when, during continuous stimulation, it fails to maintain the original height. The failure, therefore, cannot be due to excess of acetylcholine. It is attributed to an insufficient production to keep all ganglion cells active; when more acetylcholine is injected or when it is preserved from prompt destruction by prostigmin the inactive cells become active and cause a higher contraction. These observations, made on a ganglion normally supplied with blood, bring further support to the concept that acetylcholine is an intermediary agent in the transmission of impulses at ganglionic synapses.

single impulse in the postganglionic fibers. At the synapse the delay is not longer than 2 milliseconds (64). These facts would require every preganglionic impulse to liberate a minute quantity of acetylcholine which, on starting a postganglionic impulse, would instantaneously disappear. At the normal rate of discharge from sympathetic centers—probably not more than 20 impulses per second—there would be no accumulation or exhaustion. When preganglionic fibers are excited more rapidly (*e.g.*, at the rate of 50 per second), conduction through the ganglion may be impaired (cf. Orías, 1932).

The cells of the adrenal medulla are homologous with the cells of sympathetic ganglia, and they are innervated by preganglionic fibers (see pp. 3, 20). In harmony with the evidence regarding conduction in sympathetic ganglia are the results obtained by Feldberg and his collaborators (148, 149), when, on stimulating splanchnic nerves, they found in eserized blood passing through the adrenal gland not only adrenaline but acetylcholine. Earlier, Feldberg and Minz (1931) had shown that acetylcholine is a powerful direct stimulus to secretion by the adrenal medulla.

*An Interpretation of the Compatibility of Cross-Sutured Nerves.* In a series of experiments on cross-suturing of nerves Langley and Anderson (1904) found some remarkable compatibilities and incompatibilities. Thus after various connections of motor fibers with the cervical sympathetic strand had shown, after regeneration, effects on the area of distribution from the superior cervical ganglion, and after similar fusions with the vagal supply to the heart had shown capacity for inhibiting that organ, the conclusion was drawn that any motor fibers can replace any preganglionic fibers. Also preganglionic fibers (vagal) can replace other preganglionic fibers (cervical sympathetic). Similarly when the cervical sympathetic was joined to nerves supplying skeletal muscles (diaphragm, sterno-mastoid), it could cause, after regeneration, a contraction of the muscles. Therefore preganglionic fibers can make functional union with motor endings. At-

tempts to produce an effective union of motor fibers with postganglionic sympathetic fibers, however, or *vice versa*, have been quite fruitless.

The general rule governing success and failure in these experiments, as pointed out by Dale (1935), is based on division of nerves into the cholinergic and the adrenergic classes. Any cholinergic fibers—preganglionic sympathetic or parasympathetic, or motor fibers of skeletal muscle—are interchangeably functional. On the other hand, adrenergic fibers—in general, the postganglionic of the sympathetic system—are interchangeable only with members of their own class, since they produce only sympathin.

The rule stated above is confirmed by a chance observation made by Anderson (1905). The parasympathetic supply to the circular muscle of the iris is cholinergic, and when eserine is administered the pupil becomes very small. After removal of the ciliary ganglion, however, and consequent degeneration of its postganglionic filaments, the efficacy of eserine disappears. Anderson performed this experiment and when several months had passed he noted that the activity of eserine was restored. Also the light reflex returned. He found that the recovery was due to the growth of preganglionic fibers and some injured motor fibers of the eye muscles into the postganglionic pathways and thus to the iris sphincter. The result was puzzling, because it did not accord with the evidence then accepted, that preganglionic and motor neurones could not replace postganglionic neurones. The explanation advanced by Dale is that Anderson was concerned altogether with cholinergic fibers, and for that reason regeneration to the parasympathetic effector could come from any other nerves which belong to the cholinergic class.

*Possible Significance of Chemical Mediation for Phenomena of the Central Nervous System.* The evidence of chemical mediation in the transfer of impulses from axons to effectors and from neurone to neurone is suggestive of similar phenomena in the central nervous system (cf. Forbes, 1934).

One of the most striking features of the simple reflex is "*forward conduction*"—from the afferent to the efferent path, but not in the opposite direction. As already mentioned, there is similar forward conduction in the superior cervical ganglion—from pre- to postganglionic fibers with liberation of acetylcholine. Impulses backfired from post-ganglionic axons into a ganglion do not produce the mediator and are not transferred through the ganglion (p. 52). Forward conduction in the spinal cord might be explained, if it should be shown that a chemical agent appears when afferent impulses come to the ends of axons and that dendrites are affected by the agent but do not produce it. Also the phenomenon of *summation*—the inefficiency of a single stimulus and the efficiency of multiple stimuli—could be explained on the assumption that the amount of the chemical substance produced by one stimulation is not enough to excite the motor cells of the reflex arc, and only after a series of stimulations is there an effective concentration. The phenomenon of *recruitment* could likewise be explained, for with repetition of the stimuli there would be an increasing concentration of the chemical agent and therefore an increasing number of neurones involved in the response. Furthermore, *reinforcement*, as a consequence of bringing to bear on a group of motor neurones impulses from different sources, would be readily explicable. Perhaps most striking of all is the possibility of accounting for *after-discharge*. The persistence of the state of excitation, or the state of inhibition, for some time after the stimulus which induced the state has ceased to act, might naturally result from a relatively slow disappearance of the accumulated chemical agent which is acting on the motor cells, much as in skeletal muscle, after eserine, a single nerve impulse can elicit repetitive responses.

Factual support for the foregoing suggestions is meager. Rosenblueth (1934) found that reflexes which involve autonomic effectors manifest an after-discharge which may last for minutes after the end of the stimulus has ceased acting—

a duration which would be too long to be explained by reverberations in the nervous system, but too long also, it would appear, to be consistent with the ephemeral existence of acetylcholine. On the other hand Dikshit (1934) has noted

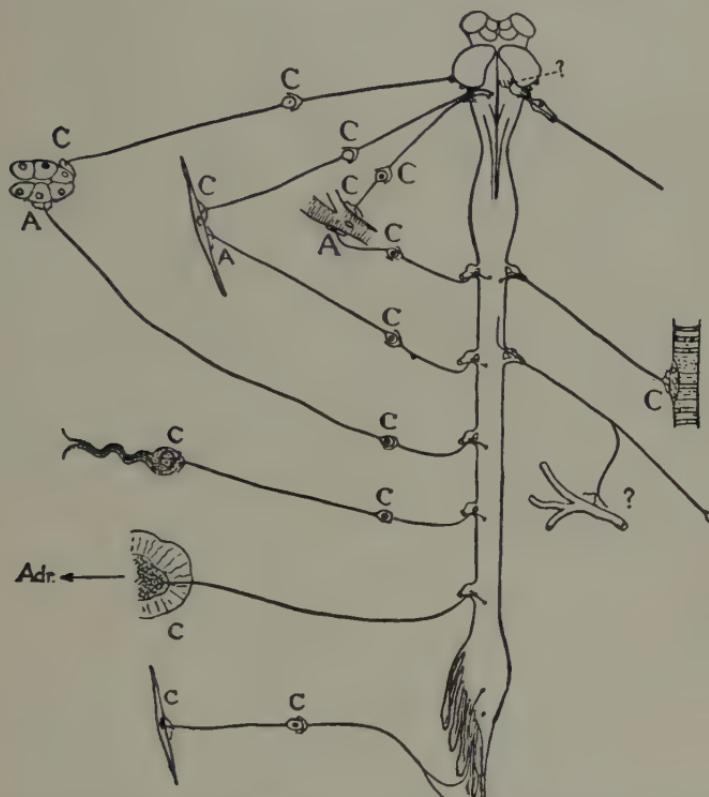


FIG. 12. Diagram of nerve fibers reaching out from the central nervous axis leftwards to a salivary gland, smooth muscle, peripheral blood vessel, sweat gland and adrenal medulla; and rightwards to skeletal muscle and blood vessel. At points marked C, there is evidence of a cholinergic transmission, and at those marked A, of an adrenergic transmission. Doubtful cases are marked ?. (Dale, 1934.)

that acetylcholine, injected in minute quantities into the cerebral ventricles, produces effects like those of central vagal stimulation. And Suh, Wang and Lim (1936) have reported that acetylcholine applied on a cotton pledge to the floor of the fourth ventricle raises blood pressure and increases respiration. In the presence of this discrepancy it is well to

consider that perhaps more mediators exist in the body than the two found in autonomic effector systems. In his admirable studies on the nervous control of chromatophores Parker (1932) has had to postulate a variety of transmitting agents for nerve impulses, among them a lipoid-soluble mediator which might explain purely local effects. Possibly such an agent is active in the central nervous system.

As a final feature of this survey Figure 12 is presented—a diagram in which Dale (1934) has marked (C) the various stations where evidence at the time indicated that nerve impulses are mediated by acetylcholine, *i.e.*, that the fibers are cholinergic. The rapid advances since the opening of the field of chemical mediation of nervous activity has transformed ideas of the ways in which efferent nerves perform their functions and in which the effects of those nerves are modified by drugs or otherwise. The possibility that these ideas will be extended by further researches renders Dale's diagram a measuring point for past and future progress.

## CHAPTER VI

### SOURCES AND INDICATORS OF THE SYMPATHOMIMETIC SUBSTANCE

The general rule, illustrated by Elliott in 1905, that adrenine mimics the action of sympathetic impulses, was impaired by the anomalous fact that sweat glands, innervated by sympathetic fibers, do not respond to adrenine but respond to parasympathomimetic drugs. The difficulty was reduced when Dale and Feldberg (1934b) brought proof that acetylcholine appears in the perfusate of the cat's paw if sweat glands are stimulated *via* their nerve supply. The existence of cholinergic fibers in the sympathetic system was thus demonstrated. In making the distinction between these fibers and adrenergic fibers Dale (1933) was careful to state that he did not thereby imply that the substance set free on sympathetic stimulation is definitely adrenine. No doubt the substance reacts to chemical and physical tests as if it and adrenine are closely related structurally (see p. 87), but evidence will be presented later that they are not the same. Because the term "adrenergic" is likely to intimate, however, that the sympathomimetic substance is adrenine, it is well to hold in question the idea of identity until the indications of differences between the two are discussed.

*Tests for Sympathin.* Theoretically, just as every source of acetylcholine might be also an indicator of its presence in perfusates and blood, so likewise every source of sympathin might serve also as an indicator of the presence of sympathin. Bearing on the methods of testing is an important contrast between the two mimetic substances—their relative stability. Whereas acetylcholine so readily disintegrates that even in saline perfusates it must be protected by eserine in order to have remote effects, sympathin

requires no protection, although conveyed in blood, and continues active for minutes. The test organs for sympathin, therefore, have been found, as a rule, in the animal from which that substance has been experimentally derived. And the means of transport from source to indicator has been the animal's own circulation.

Two precautions must be taken when the source and the indicator of sympathin are in the same organism. First, both the organ stimulated to produce sympathin and also the test organ whenever possible must be disconnected from the central nervous system; thus the opportunity for reflex effects on the indicator is assuredly excluded. And second, the adrenal glands must be rendered completely inactive, for otherwise secreted adrenine may confuse results. In chronic experiments one adrenal may be removed and the other demedullated (by sucking out the medulla) or denervated (by cutting the ipsilateral splanchnic nerves and removing the ipsilateral abdominal sympathetic strand from diaphragm to kidney). In acute experiments the two glands may be lifted and tied off or they may be removed. If these simplifying measures are employed, the demonstration of sympathin in the circulating blood, by its effect on a proper organ, is quite reliable.

In the course of experimental testing, the following organs have been employed as indicators of the presence of the sympathomimetic substance: heart, blood vessels and pilomotor muscles, leg volume, nictitating membrane, intestine, retractor penis, uterus, salivary gland, spleen and iris. Of these the cat's nictitating membrane is undoubtedly the most convenient. As shown by Rosenblueth and Bard (1932) the fan of smooth muscle reaching out from the inner canthus to the cartilaginous border of the membrane has as its only nerve supply fibers from the superior cervical sympathetic ganglion. Denervation, therefore, is simple.<sup>1</sup> Attachment of a recording lever to the cartilage at the free

<sup>1</sup> The striated muscle fibers of the membrane are innervated by a branch from the sixth cranial nerve. We shall use the expression "denervated nictitating membrane" as meaning abolition of the sympathetic supply to the smooth muscle.

edge is also simple—no operation is required. And the muscular contraction exerts a relatively simple, direct pull. Still another important advantage of the membrane is the constancy of its response to the same stimulus; as shown by

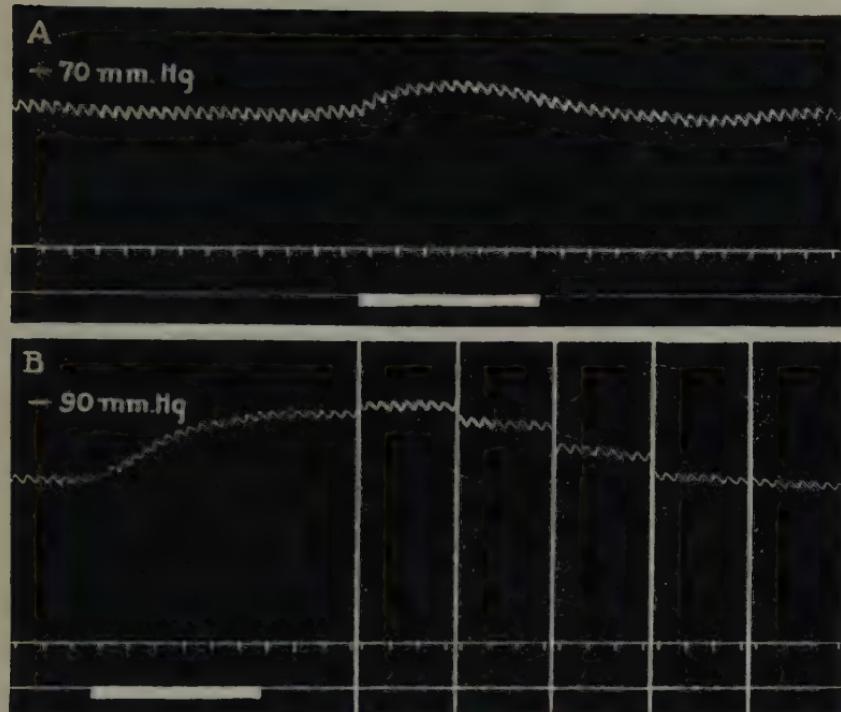


FIG. 13. Blood-pressure records showing the effect of cocaine on the action of sympathin. The lower abdominal sympathetic chains, severed at the third lumbar ganglia, were stimulated for 30 seconds. A, before, and B, after injecting intravenously 25 mgm. cocaine hydrochloride into a cat weighing 3.2 kgm. Brain pithed, both sciatics cut. The first minute of record B is reproduced, and thereafter 15 seconds of the record of each minute until the original level is recovered. The lower line indicates 0 blood pressure. (Rosenblueth and Schlossberg, 1931.)

Rosenblueth and Cannon (1932), it serves as a satisfactory quantitative indicator of the presence of a sympathomimetic agent, *e.g.*, adrenine.

Although the presence of sympathin can be demonstrated in circulating blood by use of a test organ acutely denervated, the response to it is more striking if the organ has been sensitized. Sensitization may be produced by dener-

vating the indicator and allowing a week or ten days to pass before using it (see p. 189), or by injecting cocaine hydrochloride, or by both procedures. In 1910 Frölich and Loewi found that cocaine increases the effectiveness of adrenaline, and in 1931 Rosenblueth and Schlossberg proved that it also increases the effectiveness of sympathin (Figure 13). If injected intravenously, in doses of 8 mgm. per kgm., and at a slow rate, to avoid injury to the heart, it greatly increases both the degree and the duration of the effect of the circulating sympathetic mediator.

*Sources of Sympathin.* It is well to recognize at the outset that when any particular organ is stimulated by way of its sympathetic nerves not only are the proper cells of that organ affected but also the smooth-muscle cells of the blood vessels distributed to the organ. To what degree each source contributes to the result is not yet known. When the hepatic nerves are stimulated, for example, the sympathin which appears in the blood is probably derived from sympathetic impulses acting on the hepatic parenchymal cells and on the arterioles. Since we know that smooth muscle elsewhere yields sympathin we may be quite sure about the arterioles as a site of origin; we assume the participation of the parenchyma. In muscular organs, of course, and in glands with an external secretion, the assumption is supported by visible signs of participation.

The first evidence that the *liver* yields a sympathomimetic substance on sympathetic stimulation was reported by Cannon and Uridil in 1921. They found that when the hepatic nerves were excited there resulted an increased rate of the denervated heart, and a rise of blood pressure which was not due to check of blood flow through the hepatic vessels (see Figure 6). The effects appeared later and lasted longer than when adrenal secretion was induced, and occurred when the adrenal glands were not present. The increments of heart rate varied widely in different animals, being slight if the animal had been fasting or was in poor condition and much greater if it recently had been or was

digesting meat. Cannon and Griffith (1922) proved that the active agent was carried in the blood stream—indeed, that blood drawn from the hepatic veins during stimulation would, when reinjected into the inferior vena cava, accelerate the heart. The effectiveness of the agent was not influenced by feeding fat or carbohydrate, but was increased by a diet of meat or milk. Amino acids introduced into the intestine or injected intravenously did not render hepatic stimulation more efficacious. The conclusion was reached that “a substance of special and unknown nature, which increases the rate of the denervated heart and raises blood pressure, is discharged into the blood stream when the hepatic nerves are stimulated.” About a decade later Rosenblueth and Cannon (1932) showed that the “substance” is capable of causing contraction of the nictitating membrane of the cat; this observation has been confirmed in the dog by Rosenblueth and Phillips (1932) and also by Gayet (1932) in a crossed circulation experiment. It is also capable of contracting the spleen and the volume of the leg in spite of a concomitant rise of blood pressure (89). These sympathomimetic actions, and other characteristics which identify the substance with sympathin from other sources, will be considered later (p. 90).

That the *heart* may be made a source of the sympathetic mediator was first demonstrated by Loewi (1921) when he found that the fluid from an isolated frog heart, made to beat faster and stronger by stimulating the accelerator nerves, produced the same effects in another isolated frog heart to which it was transferred. This phenomenon has been repeatedly reported in later experiments by Loewi (see 1936b), Kahn (1926) and Lanz (1928). Using the frog stomach as an indicator Brinkman and van Dam (1922) found that the fluid from a frog’s heart, when the heart was accelerated by nerve stimulation, produced inhibition as adrenine does. Lanz confirmed this observation and showed also that the active agent constricted the frog’s blood vessels. Further confirmatory evidence has been adduced in studies

on mammalian hearts. By means of a saline perfusate Rylant (1927) duplicated in essential features on rabbit hearts Loewi's experiment on the hearts of the frog; and Rylant and Demoer (1927) were able to obtain a transfer of sympathetic effects from a stimulated heart to a recipient heart (of the cat) by using blood as a vehicle. Inhibition of the motions of the rabbit intestine was produced by Jendras-sik (1924) by means of a substance given off from the isolated heart when accelerated by sympathetic stimulation. In the intact animal the heart has been used in a number of investigations as a source of sympathin; as shown by Cannon and Rosenblueth (1933) and by Rosenblueth and Morison (1934) sympathin from that source causes a strong contraction of the nictitating membrane. Experiments by Bodo and Benaglia (1936) have brought forth data indicating that stimulation of the cardio-accelerators, in an adrenalec-tomized animal, produces an increase of blood sugar.

The first evidence that excitation of the nerves of the *intestine* can set free a sympathomimetic agent was described by Finkleman (1930). He found that Ringer solution, running over a pulsating piece of excised rabbit intestine (A, still supplied with its mesenteric nerves) and passing to a similar pulsating piece of intestine (B), acquired a new property when the nerves were stimulated; piece A was inhibited and thereupon piece B also was inhibited. The transmitted action was ephemeral; a second stimulation of the nerves inhibited A but did not alter B. Cannon and Bacq (1931) reported that when the peripheral ends of the cut splanchnic nerves were excited, after adrenal extirpation and severance of the hepatic nerves, the rate of the denervated heart was increased in a typical manner, as much as 30 beats per minute. A similar effect was produced by impulses delivered to the large intestine *via* the inferior mesenteric nerves, and it was increased by ergotamine (see Figure 14). Splanchnic stimulation also makes the non-pregnant uterus of the cat relax (89). Employing the nictitating membrane as a quantitative indicator (see p. 60) Rosenblueth and Cannon

(1932) found that the degree of response depended on the extent of the area of intestine affected when a large or small distribution of mesenteric nerves was stimulated. This conclusion was confirmed by Liu (1935) who noted that stimulation of both cardio-accelerators had greater effects than either one by A itself.

That sympathetic discharge into the *uterus* and *bladder* is able to cause acceleration of the denervated heart was shown by Cannon and Bacq (1931).

In their early study Cannon and Bacq used the activation of the *vascular* and *pilomotor* muscles of the tail region as a source of sympathin. Stimulation of the lower abdominal sympathetic strands influences these muscles in legs, rump and tail, and the chemical mediator conveyed away in the blood has been shown to speed up the heart rate, increase salivary secretion, raise blood pressure (294, 80), contract the nictitating membrane (281), and augment the glucose content of the blood (19). The cervical sympathetic innervates similar muscles in the head, and also other structures (*e.g.*, the salivary glands); it is of interest to note that Bacq (1933a) was able to produce in the cat a lessened tonus of the small intestine and also contraction of the denervated

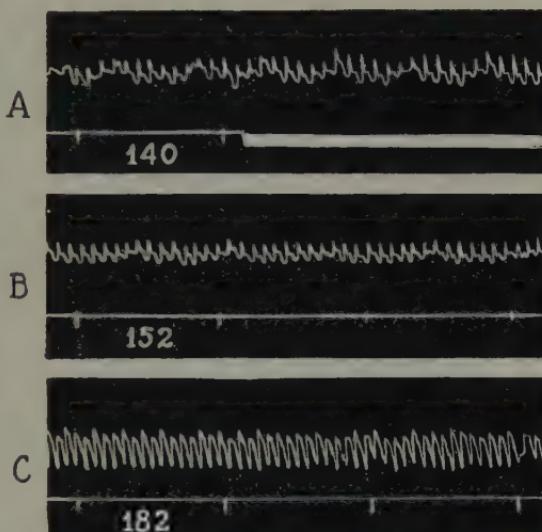


FIG. 14. Records of the rate of the denervated heart before and after stimulation of nerves on the inferior mesenteric artery. A, the start of stimulation. B, 2.5 minutes after stimulating for 30 seconds (increase of 12 beats per minute). C, the same stimulation as produced B, but applied 6 minutes after an intravenous injection of 1 mgm. of ergotamine (which had increased the rate to 150 beats per minute). This stimulation increased the rate by 32 beats. (Cannon and Bacq, 1931.)

retractor penis by applying a current of tetanizing frequency to both cervical sympathetic strands.

In experiments directed towards learning whether sympathin production might be different when nerve impulses



FIG. 15. A, contraction of a sensitized nictitating membrane when the sympathetic fibers to the pilomotors of a patch of skin on a cat's rump were stimulated 30 seconds. B, absence of the effect when the same stimulus was repeated, after removal of the patch of skin. (Rosenblueth and Cannon, 1932.)

are sent to muscle held in tonic contraction (vascular) and to muscle which is only occasionally active (pilomotor), Rosenblueth and Cannon (1932) attempted to separate the two. They cut the caudal connections of the lower abdominal sympathetic chains in the cat, severed the sciatic nerves and excised the inferior mesenteric ganglion. When the chains were stimulated the only obvious effect was an erection of the hairs over a small region on the rump where blood vessels are few. Such stimulation induced a contraction of a long-denervated nictitating membrane, further-

sensitized by cocaine. After removal of the patch of skin a repetition of the stimulus was without effect (Figure 15). Clearly, when smooth muscle which contracts phasically is brought into action sympathin is put forth. The other test was made after severing all connections of the lower abdominal chains except those leading to the legs and rump; then the patch of skin of the rump that had been responding with erect hairs was removed, and the chains were again stimulated. A characteristic response occurred in the sensitized nictitating membrane. When, now, the sciatic nerves were cut and the stimulus was reapplied there was no response. Similar results were obtained by Bacq and Brouha (1932). Wishing to exclude any possibility that chromaffine tissue in sympathetic ganglia might be involved in the distant, secondary influence of sympathetic stimulation, they excited the peripheral end of the cut sciatic and thereby evoked a faster rate of the denervated heart. That the distant action was due to sympathin was shown by obtaining it when the procedure was repeated after degeneration of the motor fibers to the skeletal muscles, and also by finding that it did not occur if the sciatic was stimulated after the postganglionic elements in the nerve had been severed and allowed to disappear. From these experiments it is obvious that when nerves which deliver tonic impulses to smooth muscle are specially excited extra sympathin is given off.

In their experiments Bacq and Brouha confirmed in a more precise manner the conclusion reached by Cannon and Bacq (see p. 28) that the sympathomimetic substance is carried in the blood stream. They observed the typical increase of heart rate when sciatic stimulation affected a leg which was connected with the rest of the body only through the crural artery and vein; if the flow through the vessels was blocked while the stimulus was applied the cardiac acceleration was delayed until the block was removed.

In connection with evidence of the output of sympathin from the blood vessels of the leg the phenomenon of Orbeli

(see Brücke, 1927) may be mentioned. He and others have shown that sympathetic impulses, delivered to a muscle which is being repeatedly stimulated through its motor nerve fibers, delay the onset of fatigue, or increase the performance of the muscle if it is already fatigued. In experiments confirmatory of the Orbely school Baetjer (1930) found that the improved action of the cat's tibialis anticus occurred after ergotoxine had "paralyzed" the vasoconstrictor nerves. Although ergotoxine, indeed, prevents vasoconstriction, it does not check the production of sympathin (see pp. 30, 88, 89). Baetjer, furthermore, was able to obtain improvement of the muscular contractions by exciting the *contralateral* sympathetic chain. These results, which led to the conclusion that the sympathetic impulses affect the muscle in some unknown manner, are all consistent with the suggestion made by Bremer (1932) that sympathin set free from the smooth muscle of the blood vessels exerts on fatigued muscle the favorable action which has been observed. That adrenine has a similar influence brings support to this hypothesis.

The clear aqueous humor of the eye, close to the smooth muscle of the *iris* and *ciliary body*, offers a unique opportunity to test for sympathin by both chemical and biological methods. Bacq (1931, 1933d) has found in the aqueous humor from the dog and rabbit (not from the cat), after excitation of the cervical sympathetic, a substance which has sympathomimetic action on the isolated toad heart and also on the cat's pilomotors when it is injected intradermally. Unfortunately the chemical test (Viale's color change, as modified by Bacq [1932]), though positive after cervical sympathetic stimulation, is not sufficiently specific to bring convincing proof of the presence of adrenine; the reaction occurs to various polyphenols and may merely indicate, therefore, that sympathin belongs in that group.

Stimulation of the *salivary* glands may be used to bring forth sympathin. After destroying all branches of the cervical sympathetic except those supplied to the submaxil-

lary on one side, Cattell, Wolff and Clark (1934) applied a stimulus to the trunk and were able to record contraction of the denervated nictitating membrane of the opposite side. The response, which was enhanced by cocaine, was absent if the venous blood flow from the gland was prevented. The sympathomimetic substance thus demonstrated had other consistent effects—causing a rise of general arterial pressure and a decreased blood flow through the opposite, denervated submaxillary gland. All these actions were shown to be in sharp contrast to those induced when, in the eserized animal, the chorda tympani was excited and acetylcholine was liberated.

The foregoing instances prove that sympathetic stimulation of a wide range of organs, including the three varieties of tissue—cardiac, smooth-muscular and glandular—that are under autonomic control, produces a substance which is capable of mimicking in other organs the action of sympathetic impulses. In the description care has been taken to state that the substance, sympathin, appears when nerve impulses are delivered to the affected tissues. Whether sympathin results as a sort of secretion from the minute nerve terminals or is a product of the responsive cells is not yet clear. Facts will be presented later which imply that these cells do contribute to the effective chemical mediator of the nerve impulses.

*The Question of Exhaustibility of Sympathin Sources.* The concept of chemical mediation of nerve impulses and the fact that remote effects occur when there is local production indicate that sympathetic impulses produce not only enough of the mediator for direct action but also an excess which, traveling elsewhere in the blood stream, has the indirect actions described above. There are, consequently, two signs of a lessening of the output of sympathin: a reduced action on the distant indicator that would measure the degree of exuberant production, and a reduced action on the organ immediately affected that would measure the production for local use. As will be explained later, both

the local and the remote responses are functions of the concentration of the mediator, and each nerve impulse, being uniformly maximal, gives rise to a minute quantum of the mediating substance (see p. 173).

Using the nictitating membrane as an indicator Orías (1932) stimulated preganglionic fibers of the cervical sympathetic 10 times a second for an hour without evidence of fatigue. Dye (1935) repeated this experiment and found that the membrane remained contracted to more than half its initial maximal contraction for three hours of continuous stimulation during which each nerve fiber received 108,000 shocks. The apparent partial exhaustion was due in some degree to "stimulation fatigue," since a shift of the electrodes to a fresh portion of the nerve induced an immediate rise in the response. Both sets of experiments involved the mediation of acetylcholine at the ganglionic synapses of the excited fibers. They tested, therefore, the possibility of exhaustion of acetylcholine as well as sympathin. Obviously both sources are relatively resistant—it is practically impossible to exhaust them by prolonged stimulation.

To learn whether there is reduction of the excess output of sympathin while the local supply continues Dye used the nictitating membrane to signal remote, and blood pressure to signal local action while he stimulated the splanchnic nerves of an adrenalectomized animal. In a fairly typical experiment splanchnic stimulation 15 times a second for 2 hours (108,000 shocks) resulted in a rise of blood pressure which, to be sure, was not kept at the first high rise of the start but which revealed continued local action by a drop of 40 mm. of mercury occurring when the nerve fibers were no longer excited. "Stimulation fatigue" was not present. The overflow of sympathin into the blood fell below the threshold for the nictitating membrane at the end of 70 minutes. Quantitative injections of adrenaline indicated that during the period of stimulation there was some reduction in the sensitiveness of smooth muscle in the membrane, but it was still responsive.

It is noteworthy that the smooth muscle of the splanchnic vessels and that of the nictitating membrane are both structures receiving tonic innervation. Whether the persistent delivery of impulses in natural conditions renders this neuromusculature more or less subject to exhaustion than a neuromusculature which is only occasionally active (*e.g.*, the pilomotors) is not known. In any case there is little indication that normally the viscera manifest signs of defective function because of an inadequate supply of sympathin.

## CHAPTER VII

### PRODUCTION OF THE CHEMICAL MEDIATORS IN NATURAL CONDITIONS

A consideration of the appearance of sympatho- and parasympathomimetic substances as a consequence of impulses discharged normally from the central nervous system might be regarded as superfluous. Surely the impulses aroused by stimulating nerve trunks are not different from those aroused reflexly, and the data derived from the simpler experiments might, therefore, be reasonably regarded as sufficient. Observations have been made, however, on the production of the chemical mediators in various natural circumstances, and the observations have brought forth facts not otherwise noted which lead to suggestive inferences. For these reasons they are presented.

*Reflex Liberation of Acetylcholine.* By use of the method of drawing blood from the coronary vein of an eserized dog (see 146), Krayer and Verney (1934) found that, if the vagi were intact, the rise of blood pressure caused by an injection of adrenalin evoked a reflex appearance of a substance in the coronary venous blood which reacted like acetylcholine, contracting leech muscle and lowering the blood pressure of a cat—an effect prevented by atropine. When quantified the acetylcholine in the blood was found to increase as the blood pressure was raised to greater heights. At its maximum it reached four times the initial concentration, although the coronary flow was nearly doubled.

Employing Tyrode solution which contained eserine Gollwitzer-Meier and Otte (1933) perfused mesenteric and splenic vessels in the dog, and found that when the carotid sinus nerves were stimulated, to produce reflex vasodilation, a substance appeared in the perfusate which acted as

acetylcholine acts. It reduced the contractions of the isolated frog heart and caused a fall of blood pressure in the cat, both of which effects were abolished by atropine. The perfusate taken before the stimulation had no effect.

*Reflex Liberation of Sympathin.* Although sympathetic stimulation sets free sympathin, a certain degree of stimula-

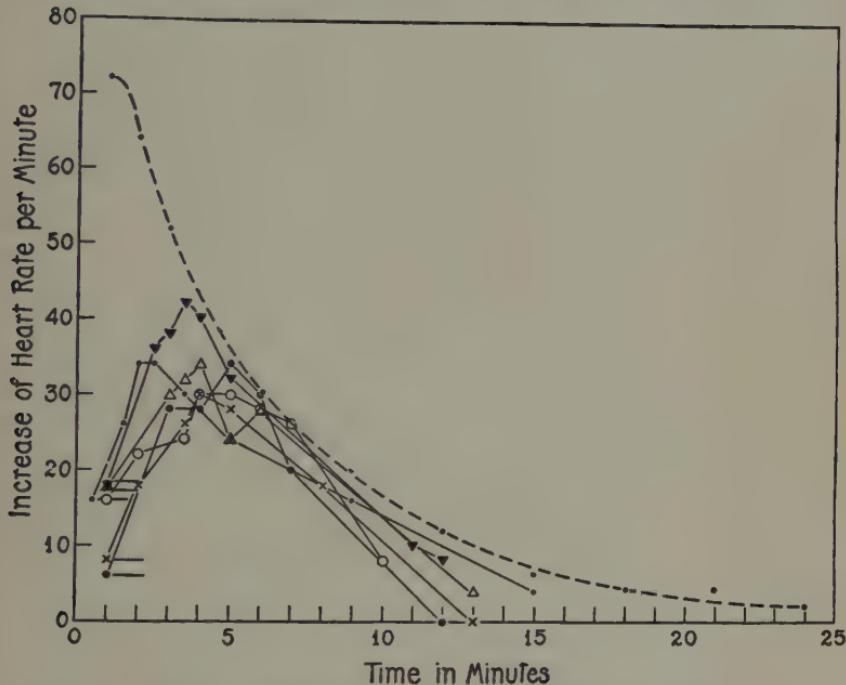


FIG. 16. Graphs (in solid lines) showing typical examples of the slow increase and decrease of the heart rate in cats after complete recovery from denervation of the heart and denervation of the adrenals and the liver. The base line represents the original heart rate while the animals were comfortable on a cushion; the first point in each graph indicates the heart rate when the animal had been transferred from the cushion and fastened back-downward on a holder; the short horizontal line at the right of this point shows the period of struggle and excitement. In the upper graph (in a dash line) is shown the immediate reaction of the denervated heart to one minute of excited struggle when the innervation of the adrenal glands is intact; the transfer of the animal from the cage, in which it was excited, to a comfortable cushion, occurred at O. (Newton, Zwemer and Cannon, 1931.)

tion is requisite for the development of sufficient concentration in the blood to produce secondary effects—*i.e.*, as shown by Rosenblueth and Morison (1934), there is a thresh-

old frequency of maximal stimulation below which sympathin does not have demonstrable effects on distant organs. Does sympathin evoked reflexly reach a concentration above the threshold? The answering of this query was undertaken by Liu and Rosenblueth (1935).

They found that when a sciatic or a brachial nerve was stimulated in adrenalectomized cats under urethane anesthesia, a delayed contraction of the denervated nictitating membrane could be evoked. By comparing effects before and after removal of the stellate ganglia in the course of an experiment they were led to conclude that the response is dependent on the number of sympathetic nerves which are intact and are involved in the reflex activation (cf. p. 65, *supra*).

*Liberation of Sympathin as a Result of Excitement and Struggle.* In Figure 16, taken from Newton, Zwemer and Cannon (1931), are shown (in solid lines) typical increases of heart rate, resulting from one minute of emotional excitement and struggle, in cats which had completely recovered from denervation of the heart, liver and adrenal glands. The maximal increase was reached about three minutes after the animals were fastened to a holder and the original rate was not restored until eight or nine minutes thereafter. Since the action of sympathin depends on its concentration (see p. 174), we may infer that the concentration began to increase in the blood near the start of the period of excitement; that although the animals, at the end of that period (one minute), were returned to a comfortable cushion, the concentration continued to rise for about two minutes more; and that sympathin disappears from the blood stream relatively slowly.

The observations in the denervated heart have been supported by Partington (1936) who used the chronically denervated nictitating membrane as an indicator, with the acutely denervated membrane as a control. The adrenal glands were rendered inactive. When cats thus prepared were excited by a barking dog or by being bound to a holder,

the consequent erection of hairs and dilation of pupils was accompanied by retraction of the sensitized, but not by the control membrane. The start of the secondary reaction came about 30–40 seconds after the excitement began. In sympathectomized animals the response of the membrane did not occur.

The dash line in Figure 16 represents the acceleration of the denervated heart of the cat when the adrenal glands are present and stimulated by one minute of excitement. The slower development of effects from sympathin as compared with adrenine indicates a slower passage into the blood. Cannon and Bacq (1931) offered the following suggestions to account for the phenomenon: (1) on the assumption that sympathin is formed inside the responsive cells (see p. 141), the membrane of the muscular units may be less readily permeable than that of the secreting units of the adrenal gland; (2) sympathin must diffuse in tissue fluid before reaching a capillary and must then pass the capillary wall, whereas adrenine, according to Sharpey-Schafer (1924), is probably secreted directly into the blood; and (3) in many regions where sympathin is produced vasoconstriction and lessened blood flow is the rule, as contrasted with vasodilation in the adrenal glands when the splanchnics are stimulated (cf. Biedl, 1897).

A phenomenon observed by Newton, Zwemer and Cannon (1931) in excited animals, that has not been duplicated in acute experiments, is a summation of sympathin effects as periods of excitement are repeated. Four one-minute periods recurring after rest intervals of one minute increased the rate of the denervated heart, first 22 beats, thence up to 33, and on to 39 and 44 beats per minute at the end of eight minutes. Continuance of the procedure for three more periods resulted in no further acceleration, but, instead, in a reduction of the increase to about 40 beats per minute above the initial resting level. It is clear that because sympathin remains for minutes in the blood stream and because also the sources of sympathin are not readily ex-

hausted, repeated sympathetic stimulation may cause an accumulating concentration of the substance in the circulating blood.

If a cat is quickly decorticated under brief ether anesthesia it manifests, after recovery from the anesthetic, periods or "fits" of activity characterized by a full display of the physiological features of great rage (81). After preparing cats by surgically denervating the heart and one adrenal gland and removing the other adrenal, Whitelaw and Snyder (1934) tested the influence of "sham rage" on sympathin coming from the liver. In animals with the hepatic nerves intact the percentage acceleration of the heart during active periods of rage was as a rule considerably greater than it was in animals with hepatic nerves previously severed; and when the hepatic nerves were cut acutely, after registering the effects of fits of excitement, the subsequent fits were invariably less effective in causing a faster heart rate. The average per cent acceleration in 9 cases of intact hepatic nerves was 7.7, and in 9 cases of severed hepatic nerves it was 3.1. There is little doubt that the residuary effect was due to sympathin from other sources than the liver. That the hyperactivity of the sympathetic system in the quasi-emotional state was producing a steady discharge of sympathin, which the fits increased, was indicated by a noteworthy reduction in the rate of the denervated heart immediately after the hepatic nerves were cut in the course of an experiment.

When sympathetic impulses are discharged in consequence of normal emotional expression or the expression which follows removal of the cerebral cortex, sympathin appears in the blood in quite effective concentrations.

*Other Conditions Liberating Sympathin in Unanesthetized Animals.* In studies on medulli-adrenal secretion it has been found that not only "painful" (*i.e.*, simple reflex) and emotional conditions evoke the secretion, but also that external cold, asphyxia and hypoglycemia are effective (cf. Cannon, 1928). Since all these states rouse the sym-

thetic system to activity it was to be expected that they would increase the concentration of sympathin in the circulating blood. In addition to reflex and emotional liberation of sympathin there is now evidence of its discharge when an animal is chilled or has a reduced blood sugar.

By use of the comparable chronically and freshly denervated nictitating membranes, as indicator and control respectively (see pp. 60, 189), Partington (1936) found that when cats were exposed to *cold* ( $2^{\circ}$  C.) there was no retraction of the freshly denervated membrane but that the other, more sensitive, was withdrawn, sometimes until it was only a slight rim at the edge of the eyelids. The withdrawal was not continuous; indeed, it was occasional and when it occurred it lasted not more than two minutes. The response to cold which marked the occasions for withdrawal was shivering. In animals completely sympathectomized and subjected to the same experimental procedure cold surroundings had no effect on the sensitized membrane.

When *hypoglycemia* was induced by injection of insulin, and the stage of gastro-intestinal disturbances and dilated pupils was reached, the sensitized membrane was retracted to a mere rim, but after two or three minutes it emerged and remained relaxed although the animal might go into convulsions.<sup>1</sup>

In experiments on the effects of cold and hypoglycemia on the secretion of adrenine (85, 88), the *continuous* acceleration of the denervated heart, during the whole period of response to the experimental conditions, pointed to continuous activity of the sympathetic system. The persistent erection of hairs while an animal is in cold surroundings likewise testifies to a tonic discharge of sympathetic impulses into the pilomotor muscles. From the results of Partington's observations it would appear that tonic im-

<sup>1</sup> In the experiments of Cannon, McIver and Bliss (1924) on the influence of hypoglycemia on adrenine secretion, convulsive spasms were accompanied by an increase of the already rapidly beating denervated heart, a change which indicated an extra discharge of adrenine, and therefore an extra activity of the splanchnic representatives of the sympathetic system.

pulses do not set free enough sympathin to produce remote effects. Experimentally, such effects appear only when a sufficiently large number of sympathetic fibers are stimulated at a sufficiently rapid rate (see pp. 65, 173). The remote effects recorded by Partington in specially accentuated behavior might have been due to either a spatial or a temporal increase of impulses, or to both.

*The Coöperation of Adrenine and Local and Circulating Sympathin.* Three distinct degrees of activity may be distinguished when sympathin is liberated: a maximal effectiveness in the innervated "key cells" (see pp. 17, 122), correlated with a maximal concentration there; an intermediate concentration and effectiveness in neighboring cells of the affected organ, cells which are either not innervated or, because of a shifting service of the nerve fibers, are not at the moment receiving impulses; and finally a minimal concentration and response in distant effectors to which sympathin is borne by the flowing blood. The last action occurs only when sympathin has been produced in such excess that it is carried through the "dead space" of the blood channels between source and indicator, and past the vascular smooth muscle which it encounters, in sufficient concentration to be effective. This remote influence resembles that of adrenine which, likewise carried in the circulation, exerts its influence at a distance from the source in the adrenal medullae. It is characteristic of the sympathetic system, when specially excited, to act as a whole; thus adrenine is secreted by splanchnic impulses at the same time that sympathetic impulses elsewhere in the body are liberating sympathin (cf. pp. 7, 75). It is reasonable to expect that adrenine, which is mimetic of the action of sympathin, would reinforce sympathin in both its local and its remote effects.

In 1932 Rosenblueth and Cannon demonstrated the co-operation of adrenine and sympathin. They selected a strength of stimulus for the splanchnics that produced a secretion of adrenine which was just below the require-

ment for contraction of the nictitating membrane. They also selected a strength of stimulus which, when applied to the lower abdominal sympathetic chains, caused only a slight contraction of the membrane. Simultaneous repetition of the stimuli in the two regions provoked a large contraction, much higher and longer than the separate stimulations would lead one to expect. Modifications of this experi-

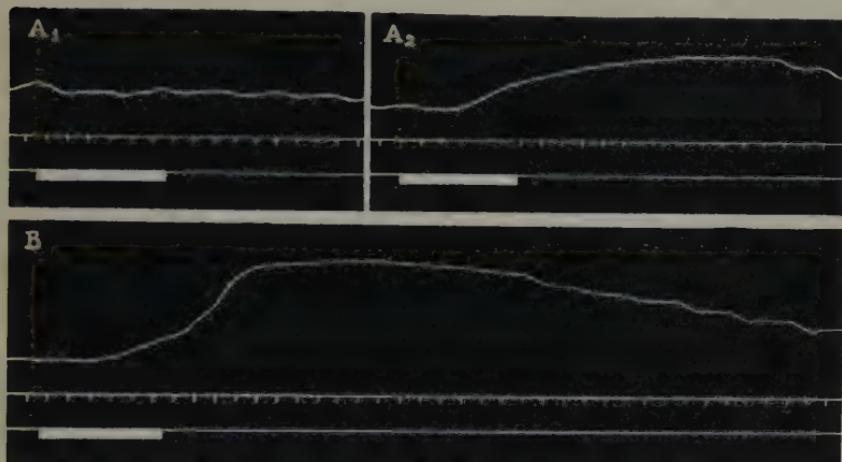


FIG. 17. A<sub>1</sub>, absence of contraction of the sensitized nictitating membrane on stimulating isolated splanchnic nerves (adrenal gland present), with coil distance at 10.5 cm. A<sub>2</sub>, slight contraction of the nictitating membrane when the lower abdominal sympathetic strands were stimulated, with coil distance 7 cm. B, contraction of the membrane when the two sets of nerves were stimulated simultaneously, with the same strength and duration of the stimuli as in the separate stimulations. (Rosenblueth and Cannon, 1932.)

ment, in which the stimulating current was just below threshold for sympathin and was just above threshold for adrenaline, or in which each current was only slightly effective, invariably confirmed the results shown in Figure 17, *i.e.*, adrenaline and sympathin collaborate in affecting structures innervated by sympathetic nerves.

The foregoing observations were extended by Liu (1935) who studied the additive effects of all three sympathetic components—local sympathin resulting directly from nerve impulses, sympathin from a distant source, and adrenaline. When tested in combinations of any two or when all three

were simultaneously employed they were found to co-operate. And even though circulating sympathin alone may have no obvious influence, being subliminal in the circumstances, it is capable of increasing the response of

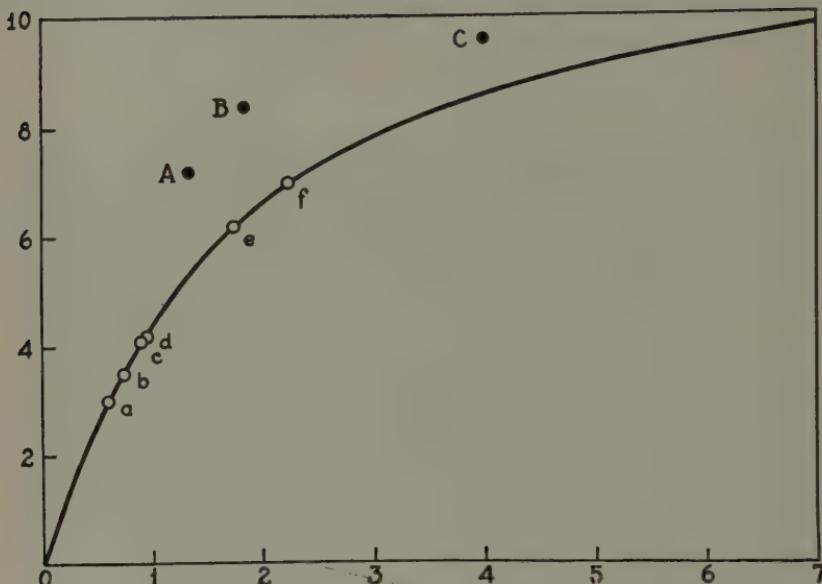


FIG. 18. Comparison of the effects of adrenalin and of sympathin from two sources. Ordinates: isotonic responses (cm. in the record) of the right nictitating membrane, denervated 6 days. Abscissae: doses of adrenalin (unit, 0.0004 mgm.). The curve resulted from 5 adrenalin injections (not marked). Points a, d and f are responses to cardio-accelerators; b, c and e, to right splanchnics (adrenals excluded), stimulated separately. The points are plotted on the curve at the corresponding ordinates, thus quantifying the sympathin in terms of adrenalin. Points A, B and C are responses to the simultaneous stimulation of the two sets of nerves with the same stimuli used in the separate stimulations, as follows: A = a + b, B = c + d, and C = e + f. A, B and C are plotted at the abscissae obtained by adding those of the separate stimulations. For explanation see text. (Rosenblueth and Morison, 1934.)

either of the other two chemical stimuli if operating at the same time.

In a quantitative examination of the effects of sympathin liberated from two distinct sources and working coöperatively on a denervated nictitating membrane, Rosenblueth and Morison (1934) found an unexpected relationship between the results of single and coupled stimulations. If

each of the two stimuli (applied, respectively, to the right cardio-accelerators and to the right splanchnics after adrenal removal) evoked *small* responses (*e.g.*, about a 2 cm. record of the nictitating membrane), simultaneous stimulation generally yielded a combined effect which was greater than linear (more than 4 cm., up to 7 cm.). The summation of *moderate* individual responses (about 4 cm.) was approximately linear (about 8 cm.). *Greater* individual responses (over 6 cm.) resulted in a summation less than linear (about 9 cm.). This last response to simultaneous application of strong and frequent stimuli, however, was higher than that resulting from a dose of adrenine equal to the sum of the two doses which matched the responses to separate stimulations of the nerves (see Figure 18). The summation of the effects of sympathin from two sources, therefore, exceeds the effects which would be predicted from a quantification in terms of adrenine. The excess of the combined sympathin effects decreases as the individual amounts of sympathin employed in the combination increase.

An explanation of the first of the three conditions mentioned above—that of the combined effect being greater than the sum of the effects from separate sources—may be found in the idea of a “dead space” in the circulatory system (see p. 78). Into this vascular channel between source and indicator sympathin must diffuse in sufficient quantity to make the concentration at the indicator rise above the threshold before there can be a response. Obviously the smaller the amount of sympathin produced the more important the dead space as a barrier, for so little may be the output that after passing the vascular channel little will remain for remote action. Now if two small discharges of sympathin—each previously proved effective—are made to occur at the same time, each will presumably deliver the same quantity of sympathin to the indicator as before, *i.e.*, the concentration will be summed. Since each was able to pass the dead space singly, however, there will be in the combined discharges an excess of the dead-space

component. That, added to the combined stimulating components, would account for the more-than-linear response. The theoretical bearings of the other observations made by Rosenblueth and Morison will be discussed later (see p. 101).

*The Significance of the Chemical Mediators in the Functioning of the Autonomic System.* In the foregoing pages most of the evidence for production of sympathin has been derived from experiments in which the substance was transmitted by the circulating blood. Bain (1933) has expressed regret that any stress has been laid on the transmissibility of the autonomimetic substances, for such stress may distract attention from the fact of cardinal importance, the production of chemical substances at the nerve endings. Furthermore, he states, "a mechanism exists which expressly tends to prevent such transmission being effective even if it takes place—the rapid destruction of neuromimetic substances by the blood."

Acetylcholine is, in fact, so rapidly destroyed that it cannot be regarded as normally having any action other than that at the source. Sympathin, on the other hand, diffuses into the blood stream, is carried to all parts of the body, and is not rapidly destroyed. As shown in Figure 16, it may continue to exert its secondary influence for ten minutes or more after the period of stimulation has ended (cf. p. 74). Moreover, sympathin differs from acetylcholine not only in being present in blood at times in concentrations which affect sensitized structures but also in finding simultaneously in the blood a special endocrine product—adrenine—with which it coöperates. When the sympathetic system goes into vigorous action, therefore, it liberates sympathin at perhaps all its myriads of endings—sympathin in such excess that it overflows and enters the circulation; and it liberates, also into the circulation, adrenine. These two substances, as has been shown above, have additive effects. To what degree sympathin may be important in the presence of a normal discharge of adrenine has not been

determined, but it is clear that locally produced sympathin, circulating sympathin and circulating adrenine all work together to unify and synchronize the operations of the sympathetic system. Even when an organ has been deprived of its sympathetic fibers the circulating agents will force it into coöperation with the other affected viscera.

The remarkable unlikeness in stability of acetylcholine and sympathin is related significantly to the neural organization of the sympathetic and parasympathetic divisions of the autonomic system. As pointed out elsewhere (77), to accomplish quickly, in emergencies, useful adjustments of bodily processes, the sympathetic division is arranged to work as a unit. The provision for diffuse discharge of sympathetic impulses, found in the overlapping of preganglionic fibers in their courses up and down the sympathetic chains (see p. 7), is reinforced by the simultaneous discharge of both adrenine and sympathin which, distributed diffusely, must have diffuse effects. Unlike the sympathetic, the parasympathetic neurones, reaching out from the brain or spinal cord directly to the effectors, are arranged for specific responses in separate organs—for such incongruous functions as secretion of saliva, narrowing of the pupil and slowing of the heart rate. If acetylcholine were a stable substance, persisting in the blood, it might, at a given time when an extensive region received parasympathetic impulses, induce changes in other regions that would be disturbing because the functions would be quite unrelated to one another. The possibility of such disturbance, however, is avoided by the extreme lability of acetylcholine.

## CHAPTER VIII

### THE NATURE OF CHEMICAL MEDIATORS

In earlier pages the parasympathomimetic substance has been referred to as acetylcholine, and the sympathomimetic substance as an adrenine-like agent, sympathin. The evidence that sympathin is not the same as adrenine has not been given. Further consideration of the nature of these two mediators of autonomic impulses will now be offered.

*Acetylcholine as the Mediator of Parasympathetic Impulses.* The demonstration by Dale (1914a) that acetylcholine duplicates the effects of parasympathetic impulses was accompanied by the hint that transitoriness of its action was due to a rapid hydrolysis wrought by an esterase. This inference was supported by the experiments of Loewi and by others. Loewi (1921) tested normal salt solution which had stood for some time in the chamber of the frog heart and found choline present. The choline was increased by vagal stimulation, but was too slight in amount and too ineffective to be regarded as the real mediator of vagal impulses. The true vagus substance was shown to possess characteristics of acetylcholine or a closely related ester of choline. According to Witanowski (1925), it resembles acetylcholine in being dialyzable, in being unstable in an alkaline but stable in an acid solution, and being soluble in alcohol but not in ether. Furthermore, Loewi and Navratil (1926) found that an aqueous extract of the heart inactivates both the "Vagusstoff" and acetylcholine, and that acetylation restores the original effectiveness. They concluded, therefore, that the change from highly potent and very unstable acetylcholine to the relatively impotent but stable choline is due to a hydrolytic cleavage of the acetylcholine into choline and acetic acid.

The destructive agent has the characteristics of a ferment;

it is destroyed by heating to 56° C. and by exposure to ultra-violet light—at least when it is present in salt solution. Galehr and Plattner (1928), who found that acetylcholine disappears from human blood (at 40° C.) in 15 seconds, reported that heating and radiation did not disturb this rapid action. They attributed the disappearance to adsorption of acetylcholine, presumably on the corpuscles, for serum was much less effective than whole blood. In support of the view that there is a cleavage of acetylcholine, however, and that it is brought about by an esterase, Engelhart and Loewi (1930) showed that blood protects against heat and ultraviolet light; in human blood serum the esterase of the frog heart is not affected by these physical agents. The demonstration by Stedman, Stedman and Easson (1932) that a purified preparation of choline esterase, made from horse serum, has a powerful action on acetylcholine and butyrylcholine, but only a weak action on methylbutyrate, supports the view that the splitting of acetylcholine depends upon a fairly specific esterase.

Further evidence that the parasympathomimetic substance is acetylcholine is found in the protective action of eserine. Loewi and Navratil (1926) observed that eserine greatly prolongs the effect of both acetylcholine and "Vagus-stoff" on the frog heart, but has no influence on two other vagomimetic substances, choline and muscarine. Furthermore, they learned that the effect is prolonged because eserine interferes with the activity of an esterase, which quickly breaks down acetylcholine. If eserine is added to blood (136, 240) or to various organ extracts (260), it acts similarly to shield acetylcholine from fermentative hydrolysis induced by the esterase contained in these fluids. Numerous examples, mentioned above as illustrations of the sources of the parasympathomimetic substance, revealed that that substance (acetylcholine) can exist and act outside its natural confines only when eserine guards it against attack by the widely distributed esterase. So direct and specific is this relation that Dale and Gaddum (1930) have argued

that the potentiation of nervous effects by eserine may be regarded as a reliable reason for inferring that the effects are produced through the agency of a highly unstable choline ester.

Three other considerations point directly to the conclusion that acetylcholine is the mediator of parasympathetic impulses. First is the fact that acetylcholine is the only ester of choline which has been chemically identified as existing in animal tissues; in extracting it from the spleen Dale and Dudley (1929) found that it was preserved for a considerable time in the isolated intact organ but was rapidly destroyed when the organ was minced. Secondly, when acetylcholine was compared quantitatively by Chang and Gaddum (1933) with various other choline esters (in effects on intestinal contractions, blood pressure, frog's rectus abdominis and eserinized leech muscle), the responses, expressed as per cent of the response to acetylcholine, as a rule varied widely in the different tests. Pyruvylcholine was an exception, for the ratio of its activity to that of acetylcholine was fairly constant, but there is no basis for assuming that this ester exists in the body. And third, there is the application of the fact just mentioned, first made by Dale and Feldberg (1934c), that when a dilute solution of acetylcholine is compared with a sample of the parasympathomimetic substance, in effects on various indicators, the two respond quantitatively in the same manner (see Figure 8).

All these evidential data, taken together, seem to make the proof that what Loewi first called "Vagusstoff" is really acetylcholine.

*The Nature of the Sympathomimetic Substance.* When Loewi, in 1921-22, stimulated the sympathetic fibers distributed to the heart of frogs and turtles, the Ringer solution in contact with the cardiac muscle during the acceleration acquired an adrenaline-like property; if applied to a second heart it made this heart, likewise, beat more rapidly. The great accumulation of evidence since the first experi-

ments, based on characteristic responses of a variety of indicators to the sympathetic mediator derived from a variety of sources (cf. pp. 60-69), points to the close resemblance of the mediator and adrenine. Moreover, in 1926 Loewi and Navratil found that, like adrenine, the effective agent was rendered ineffective when mixed with eosine and exposed to ultraviolet light; and Lanz (1928) reported that, like adrenine, it becomes inert on standing in air for 24 hours or on being heated to 100° C. Also, as shown by Rosenblueth and Schlossberg (1931), it resembles adrenine in having its influence in the organism much augmented by a previous injection of cocaine. Like adrenine, also, it gives the Viale color reaction, as discovered by Bacq (1933d). And furthermore, Loewi (1936b), employing a highly sensitive chemical test described by Gaddum and Schild (1934) (fluorescence of adrenine in the presence of strong alkali and oxygen), has reported that, like adrenine, the fluid from the frog heart after sympathetic stimulation yields the typical green fluorescence.

In coöperation with Henri and with Schepers, Bacq (27, 28) has studied the ultraviolet absorption spectrum of physiological salt solution perfused through the heart of the Hungarian frog until clear and then tested after stimulation of the cardiac nerves. The absorption by the test perfusate taken during sympathetic stimulation and acidified was found to be definitely greater than the control, and the region of absorption of the test perfusate indicated qualitatively the absorption bands of the polyphenols. When the perfusate was made alkaline the absorbing power was increased—a change which was not reversible by return to an acid reaction. These variations of absorption as a function of pH are such as are furnished by readily oxidizable polyphenols and therefore support the direct spectrographic testimony. Bacq interprets these observations as offering another type of evidence that the sympathomimetic agent and adrenine are identical and as suggesting that that agent has a polyphenol nucleus.

All these facts concerning the sympathetic mediator—its universal sympathomimetic action in the organism, its adrenine-like responses to physical and chemical tests—strongly sustain the conclusion that the mediator is actually adrenine, locally set free when sympathetic impulses arrive at an effector. Such was the view of Cannon and Bacq (1931), although they suggested the name "sympathin," because it has a different source than adrenine. There are facts, however, which indicate that sympathin differs from adrenine, and further that there are two kinds of sympathin. These facts will be presented under five headings.

1. Sympathin derived from a region where sympathetic impulses excite the effectors causes a rise of blood pressure after ergotoxine, whereas adrenine causes a fall. When, in an animal simply anesthetized, commercial adrenalin is injected in a sufficiently large dose there ensues a rise of blood pressure, or an initial brief sharp rise, then a fall and a final long rise, with gradual return to the former level. Now, if the lower abdominal sympathetic chains or the hepatic nerves (89) are stimulated for a short period, the sympathin which is evoked will likewise produce a characteristic prolonged rise of blood pressure, which may last four or five minutes. Here is evidence that adrenine and sympathin are similar in their effects. In 1906, Dale reported that preparations of ergot have the property of blocking the excitatory action of adrenine or sympathetic impulses, without affecting the inhibitory action. After an injection of such preparations, therefore, adrenalin or secreted adrenine, which normally mimics (in the cat, for example) both vasodilator and vasoconstrictor impulses, has only a vasodilator influence—the blood pressure falls. Cannon and Rosenblueth (1933), employing ergotoxine, readily confirmed this observation (see Figure 19A). On the other hand, they found that, although stimulation of the lower abdominal sympathetic chains causes, during the first half-minute, a brief fall, the fall is followed by a rise of pressure which lasts eight or nine minutes or longer (Fig-

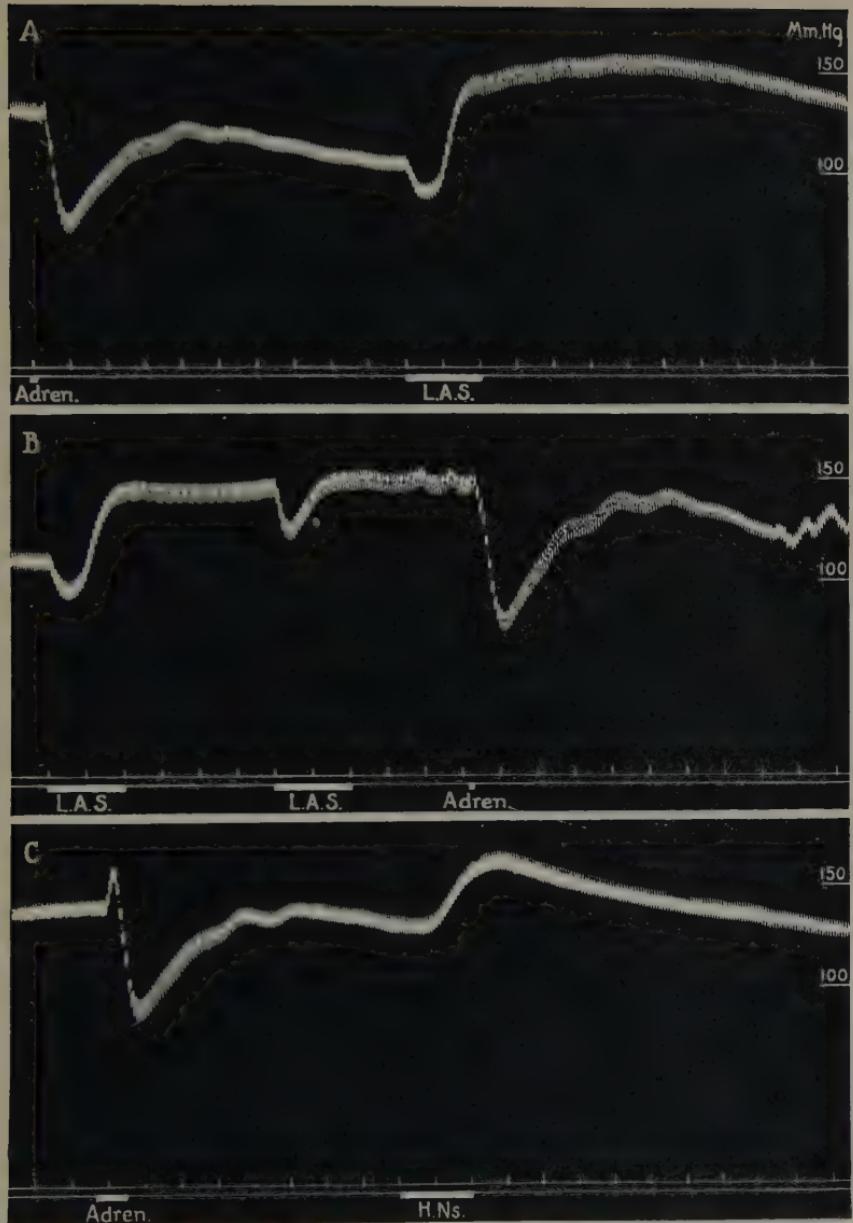


FIG. 19. The effects of adrenalin and sympathin on carotid blood pressure after ergotoxine (5 mgm. per kgm.). A, effects of injecting adrenalin (0.5 cc., 1:50,000), and stimulating the lower abdominal sympathetic chains (L.A.S.). B, after injection of cocaine (7 mgm. per kgm.). Effects of stimulating the lower abdominal sympathetic chains and of injecting adrenalin during the elevated pressure induced by the nerve stimulation. C, effects of injecting adrenalin and of stimulating the hepatic nerves (H.Ns.). (Cannon and Rosenblueth, 1933.)

ure 19A). If during the rise the nerves are stimulated again or adrenalin is injected, a pure fall results (Figure 19B). And if the hepatic nerves are stimulated, a simple elevation of blood pressure occurs, in marked contrast to the fall produced by adrenine (Figure 19C).

The fact that after ergotoxine stimulation of the sympathetic supply to the smooth muscle of the tail region and to the liver both made the blood pressure rise led to the inference that the "substance of special and unknown nature," reported by Cannon and Uridil (1921) as appearing in the blood when hepatic nerves were excited, was sympathin.

Whether the tail region or the liver region was stimulated after ergotoxine, the increased blood pressure was always associated with a faster heart rate. The difference between the effects of sympathin and adrenine were not due to that factor, however, for after sympathin from the liver had increased the rate of the denervated heart 44 beats per minute and raised blood pressure 20 mm. of mercury, adrenine, injected in such manner as to match the action on the heart, increased the beats by 42 per minute and lowered the blood pressure 34 mm. of mercury.

Confirmation of the results on blood pressure was obtained by tests made on the volume of the leg and the spleen. Hepatic sympathin and adrenine normally induce a contraction of both organs. After ergotoxine the elevation of arterial pressure due to sympathin is accompanied by only slight expansion of the leg—possibly the resultant of local contraction confronted by the higher pressure within the vessels. The fall of arterial pressure due to adrenine is accompanied by a marked expansion of the leg—an effect explicable as the consequence of vasodilator influence. The initial drop of blood pressure shown in Figure 19A and B, when the leg and tail regions were affected by sympathetic impulses after ergotoxine, can be accounted for as due to immediate vasodilation; the released sympathin, not blocked in its vasoconstrictor action by ergotoxine, promptly re-

places the drop with a pressure rise. The absence of the initial drop when hepatic nerves are stimulated (Figure 19C) indicates that there is no vasodilation in the hepatic area.

2. Sympathin derived from purely excitatory impulses, though like adrenaline in causing a contraction of the nictitating membrane, is unlike it in failing to stimulate the pupillodilator fibers of the iris. Struck by the similarity of action of the hepatic cardio-accelerator substance and adrenaline, Cannon and Uridil (1921) tested the substance for its effect on the iris. After removing the superior cervical sympathetic ganglion and allowing time for degeneration of the fibers they stimulated the nerves of the liver; typical effects were obtained on heart rate and blood pressure, but there was no change in the eye. Adrenal secretion had the first two effects and also dilated the pupil. In 1912 Elliott had noted, after excising the adrenal glands, that splanchnic stimulation caused slight dilation of the pupil—a result which he ascribed to the release of adrenaline from accessory depots; in 1923 Hartman, McCordock and Loder reported that in a cat excitement after adrenalectomy was followed by some retraction of the completely denervated iris; and Bacq (1933c) likewise found that stimulation of the lower abdominal sympathetic chains, the hypogastric nerves or the sciatics, induced iris retraction, *i.e.*, dilation of the pupil. It appeared, therefore, that sympathin from the liver might be different from that arising in other sources as well as different from adrenaline.

The problem presented by this anomaly was studied by Cannon and Rosenblueth (1935). They recorded from the same animal (cat) the action of adrenaline, hepatic sympathin, and sympathin from the cardio-pulmonary area, on the sympathectomized nictitating membrane and iris. When the three stimulating agents evoked nearly equal responses of the membrane adrenaline markedly dilated the pupil, sympathin from cardio-accelerator stimulation widened it slightly, and hepatic sympathin had only minor and inconsistent effects (see Figure 20). The discrepancy be-

tween hepatic sympathin and an equivalent amount of adrenine was especially striking, but that between cardiac sympathin and adrenine also was remarkable.

On the chance that the discrepancy might be due to simultaneous stimulation of constrictor and dilator fibers

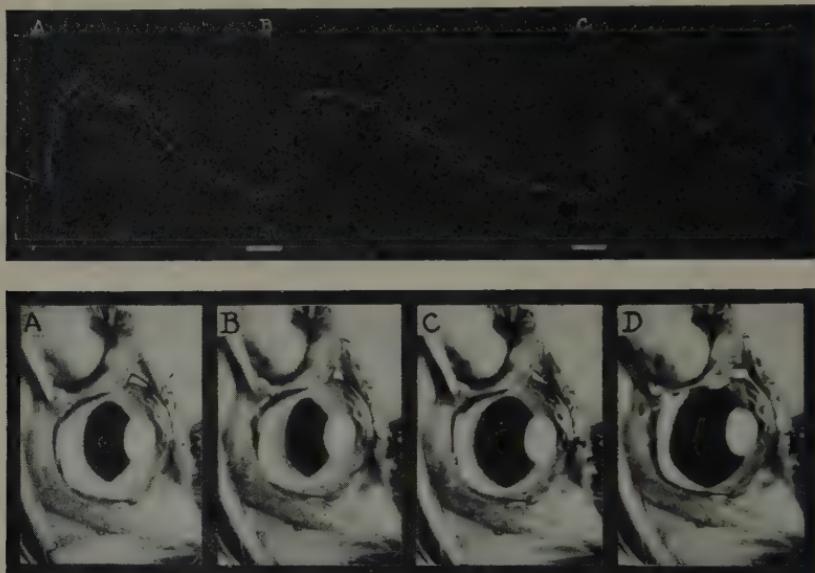


FIG. 20. A comparison of the effects of sympathin and adrenalin on the denervated nictitating membrane and iris. Above, the graphic records are responses of the nictitating membrane. A, to 0.15 cc. adrenalin, 1:200,000. B, to stimulation of the hepatic nerves. C, of the cardio-accelerator nerves. Time, half-minutes. Below, photographs of the reacting iris. A, control. B, the same stimulation as before of the hepatic nerves. C, of cardio-accelerator nerves. D, injection of adrenalin, 0.15 cc., 1:200,000. The white spot is due to reflection of light. (Cannon and Rosenblueth, 1935.)

of the iris by sympathin, whereas adrenine stimulated only the dilator fibers, Cannon and Rosenblueth interrupted the circular fibers by cutting the iris radially in one or two places. On the side of the operation the superior cervical sympathetic ganglion was removed. After the aqueous humor had become quite clear (about a month), the influence of hepatic and cardio-accelerator stimulation was tested as before, *i.e.*, by matching uniform contractions of the nictitating membrane with dilation of the pupil. It was

found that severance of the circular fibers of the iris brought about a condition which permitted hepatic sympathin to retract the iris, *i.e.*, to shorten the radiating fibers and widen the pupillary opening. The effect was rarely as great as that produced by an equivalent amount of adrenine (measured by contraction of the membrane), probably because of some remnant of contraction in the circular fibers, as shown by presence of the light reflex.

An explanation of the influence of sympathin on the iris will best be given later (p. 102). For the present the observations just detailed simply add to previous evidence that sympathin and adrenine are not identical.

3. Sympathin differs from adrenine because the effect of combined sympathin from two sources is greater than the effect of combined doses of adrenine which match the separate sympathin actions, *i.e.*, when equivalent amounts of the two substances are added the effects are not equal. Already this phenomenon has been briefly considered in relation to the nictitating membrane as an indicator (see pp. 80, 81). Its reliability as a criterion is obviously based on the reliability of the indicator as a quantitative measure. In comparing a delicate color reaction with Elliott's (1912) blood-pressure method of quantifying an unknown solution of adrenine Folin, Cannon and Denis (1913) found that Elliott's method was remarkably accurate. Rosenblueth (1932b) examined the responses of the nictitating membrane to varying doses of adrenine and came to the conclusion that it was as sensitive as the blood-pressure method, was "more accurate, simpler to prepare and equally lasting."

After sensitizing the right nictitating membrane of a cat by sympathetic denervation, Rosenblueth and Morison (1934) injected multiples of 0.0004 mgm. of commercial adrenalin (regarded as unity). The resultant contractions of the membrane, when plotted, yielded the curve shown in Figure 18. Points *a*, *d* and *f* in the curve mark the heights of contraction due to increasing amounts of sympathin from frequencies of maximal stimulation of the cardio-accelerator

nerves; and points *b*, *c* and *e*, the similar responses to increasing frequencies of maximal splanchnic stimulation (adrenals absent). The location of the points in the curve serves to quantify in terms of adrenalin the sympathin produced by each stimulation. Now, the cardio-accelerators and the splanchnics were excited simultaneously, precisely as they were stimulated before, when they caused contractions *a* and *b*, respectively. The amounts of adrenalin which separately would cause contractions *a* and *b*, would, if added, cause a contraction the acme of which would fall in the curve at an abscissa appropriate for the combined doses. The response to sympathin from the simultaneous stimulations, however, when plotted at that abscissa, reached A, well above the curve. Similarly B marks the response to simultaneous stimulation of the two sets of nerves with the stimuli used separately for *c* and *d*, and C likewise for *e* and *f*. In other words, if the response of the membrane to sympathin S, from source 1 was equal to that elicited by a dose of adrenalin A, and the response to sympathin S<sub>2</sub> from source 2 was equal to a dose of adrenalin A<sub>2</sub>, then, were sympathin the same as adrenalin, the response to A<sub>1</sub> + A<sub>2</sub> should be equal to that to S<sub>1</sub> + S<sub>2</sub>. This proved not true. In each instance the sympathin from two sources (S<sub>1</sub> + S<sub>2</sub>) evoked a contraction considerably greater than that evoked by combining the adrenalin doses (A<sub>1</sub> + A<sub>2</sub>) which matched the separate amounts of sympathin. This result indicates another difference between sympathin and adrenine.

4. Sympathin from a region where sympathetic impulses excite effectors is like adrenine in having a remote excitatory action, but unlike it because lacking in inhibitory action. It will be recalled that, after ergotoxine, stimulation of the hepatic nerves caused a pure rise of blood pressure, denoting the presence of only excitatory impulses (cf. p. 89). The "cardio-accelerators" also, although having inhibitory fibers for the coronary arteries and the smooth muscle of the bronchioles, are mainly excitatory. To test the action of sympathin from these sources on indicators having oppo-

site responses Cannon and Rosenblueth (1933) used the nictitating membrane as a contracting muscle and the non-pregnant uterus of the cat as a relaxing muscle. Both

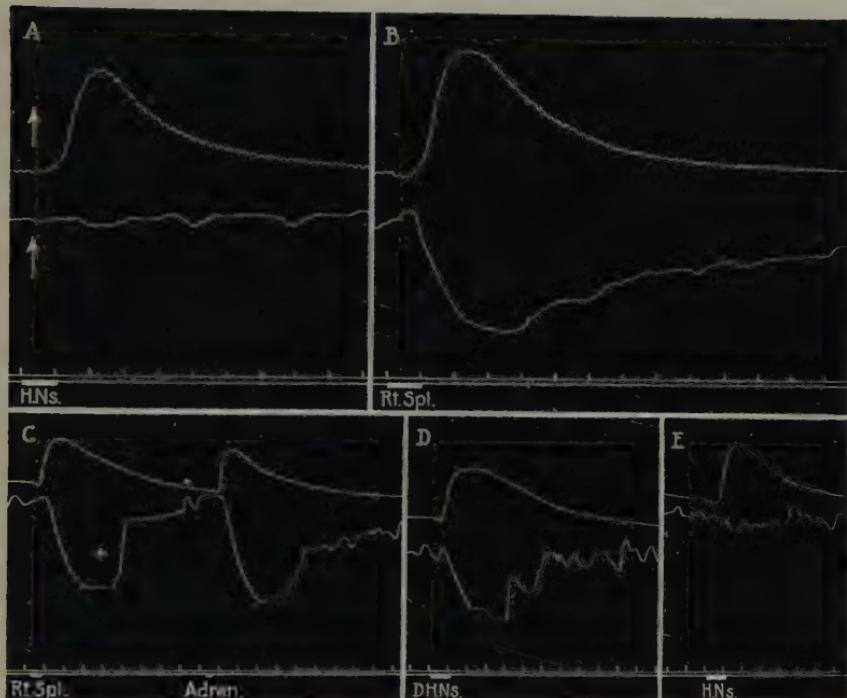


FIG. 21. Effects of sympathin from excited and inhibited sources on indicators which contract or relax. In each instance the upper record is the nictitating membrane with cervical sympathetic cut; the lower record, the denervated non-pregnant uterus. Arrows indicate contraction. Time, half-minutes. A, effects of stimulating the hepatic nerves (H.Ns.), purely excitatory. B, effects of stimulating the right splanchnic nerves (adrenals excluded), excitatory and inhibitory. C, effects of stimulating the splanchnic nerves (adrenals excluded) and injecting adrenalin (0.3 cc., 1: 200,000). D, effects of stimulating the duodeno-hepatic nerves, excitatory and inhibitory. E, after severance of the duodenal nerves (D.HNs., excitatory and inhibitory), stimulation of the hepatic nerves, excitatory only. (Cannon and Rosenblueth, 1933.)

structures were deprived of their sympathetic nerve supply. When adrenaline was injected it produced the typical opposed effects in these two indicators (cf. Figure 21C). If then the hepatic nerves were stimulated (Figure 21A and E), the nictitating membrane contracted, but the non-pregnant uterus did not relax. A weak electric stimulus applied to the

cardio-accelerators likewise provoked a contraction of the membrane but did not relax the uterus; the uterus was sensitive, for an amount of adrenine which duplicated the membrane record induced a sharp relaxation of the uterus. These results mark another difference between sympathin and adrenine.

5. Sympathin is unlike adrenine in having two separable effects. Although hepatic nerve stimulation has a positive but not a negative action, the stimulation of regions in which nerve impulses cause both contraction and relaxation, or have excitatory and inhibitory effects, results in the production of sympathin which has both positive and negative actions. Such a region is the splanchnic area, where sympathetic impulses contract blood vessels and relax long extents of the gastro-intestinal tract. When, after removal of the adrenal glands, the splanchnics are stimulated, the sympathin produced thereby contracts the nictitating membrane and relaxes the non-pregnant uterus (see Figure 21B and C). The duodeno-hepatic nerves are distributed to the liver and to the intestine, *i.e.*, to a part (the liver) where the action is positive and to another (the duodenum) where it is both positive and negative. Excitation of the duodeno-hepatic nerves, therefore, has the same secondary effects as excitation of the splanchnics (see Figure 21D). If now the nerves to the duodenum are cut and the same stimulus is applied as before, the nictitating membrane contracts, but the uterus does not relax.

Unfortunately no part of the body is known where sympathetic impulses cause solely a relaxation or inhibition—always there are attendant blood vessels which the impulses contract. And up to the present no means are available for isolating the negative from the positive excitation. Since the sympathin given off from regions simultaneously excited and inhibited has remote excitatory and inhibitory action, whereas that given off from an excited region alone has only an excitatory action, it would appear that there are two kinds of sympathin—excitatory and inhibitory.

All these observations taken together support firmly the conclusion that sympathin is not identical with adrenine. All of the earlier evidence linking sympathin with adrenine merely emphasized the similarity of their biological effects and physical and chemical reactions. The biological effects were such as not to discriminate critically between excitatory and inhibitory influences, and the physical and chemical reactions were not only much less delicate than the biological tests but also much less capable of yielding real insight into the nature of sympathin. Bacq (1933d), although arguing strenuously at one time for the identity of sympathin and adrenine, later (1935) admitted that they are not the same. The statements by Loewi (1936b), that no fact opposes the view that the substance set free by sympathetic stimulation is adrenine, and that there is complete identity of the physiological behavior of the sympathomimetic substance and adrenine, were made without evident consideration of the available pertinent data outlined in the foregoing pages.

## CHAPTER IX

### THE THEORY OF EXCITATORY AND INHIBITORY SYMPATHINS

The facts presented in the foregoing pages, indicating that sympathin may have two separable effects, led Cannon and Rosenblueth (1933) to postulate the existence of two kinds of sympathin, excitatory and inhibitory. This theory may be helpful and suggestive.

*The Argument.* Adrenine acts to stimulate some smooth muscle cells (*e.g.*, those of most blood vessels) and to inhibit others (*e.g.*, those of the intestinal wall). There is no reason for assuming two different types of these cells. An agent which modifies adrenine or a condition (*e.g.*, a specific excitability) which accepts adrenine peculiarly, is therefore present inside some muscle cells to make them shorten; and inside other cells another agent or condition is present, acting in an opposite sense, to make them lengthen. The adrenine-like substance, liberated by sympathetic impulses, also produces stimulatory and inhibitory effects, and, as in the case of adrenine, the differentiating agent or condition may be supposed to reside in the affected cells. An analysis of the action of adrenine and of nerve impulses on smooth muscle led Rosenblueth (1932c) to the view that a substance, A, from the outside (adrenine, *e.g.*), or M (a local product) unites in the cell with a hypothetical substance H, thus making a combination, AH or MH, which evokes a response proportional to the concentration of the compound (see p. 172). This concept presumes an agent rather than a condition as the differentiating factor. The sympathin which comes away from excited cells and has elsewhere only excitatory effects supports the concept. The hypothetical H, therefore, must be regarded as either E (excitatory) or I (inhibitory); and the combination, when nerves are stim-

ulated, would be ME in a contracting, and MI in a relaxing muscle. Sympathin may be defined, consequently, as the chemical mediator of sympathetic nerve impulses, ME or MI, which in the cell induces the typical response, contraction or relaxation, and which, escaping from affected cells into the blood stream, induces typical responses in remote organs controlled by the sympathetic system. Thus the liver region, stimulated, gives rise to sympathin E, having only excitatory effects (Figures 19C, 21A), and the gastrointestinal tract, producing sympathin E and I, has both excitatory and inhibitory effects, on remote organs (Figure 21B and C). Also the heart muscle seems to discharge a large amount of E, while the coronary vessels and bronchioles discharge less of I; when sympathetic stimulation is decreased, therefore, E remains effective and I does not (see p. 96).

The observations pointing to the existence of two kinds of sympathin are related to the views expressed by Langley in 1905. Confronted with the fact that a single substance, adrenine, stimulates in one region and inhibits in another, he assumed that differentiating "receptive substances" were present in the responsive cells, and that the agent (adrenine), by combining with one or other of the two substances, would have its action determined in one direction or the other. Although some internal physical or chemical change in the agent might be conceived as the reason for difference of action, no such alternative to Langley's hypothesis has been offered. Furthermore, the evidence for a chemical union (see p. 178) supports his hypothesis. Whether the mediator, M, is derived from nerve endings outside the smooth-muscle cell, as Bacq (1934a) has pictured it, or inside the cell as the histologists would have it (see p. 14), it is probably differentiated inside the cell and, if so, must diffuse out from the cells to enter the circulating blood.

*Some Theoretical Explanations.* A theory should be helpful not only in providing a structural system for facts, but also in offering a way of interpreting obscure phenomena. There

are a number of such phenomena which have been touched upon and which should have further consideration.

1. The action of ergotoxine. When, after ergotoxine, the lower abdominal sympathetic fibers are stimulated, there is first a brief initial fall (due to unimpaired vasodilators) and then a prolonged rise of blood pressure; on the other hand, as a consequence of adrenaline injection, there is simply a fall followed by a return to the previous level (see Figure 19A, p. 89). If the mediator, M, locally produced, is identical with A (adrenaline), the failure of adrenaline to make the blood pressure rise after ergotoxine cannot be due to the blockage of the action of AE, since ME (*i.e.*, sympathin E) is effective. Also it cannot be explained on the basis of extreme contraction of vascular smooth muscle by ergotoxine, since sympathin E causes further contraction and a rise of pressure. Moreover, it is not reasonable to assume a change in the nature of E, for M unites with E, and A should be able to do likewise. Cannon and Rosenblueth (1933) suggested the possibility that ergotoxine alters the structure of adrenaline, perhaps as it enters the cell, in such manner that it no longer combines effectively with E. Clearly this action of ergotoxine could not be uniform, because a given dose of the drug has different degrees of effect in different organs (Dale, 1906). This explanation of the mode of action of ergotoxine is admittedly speculative. Indeed, it has been justly pointed out by Newton (1936) that this is an obscure aspect of the problem. A simpler assumption would be to conceive that sympathin is a substance entirely different from adrenaline. This assumption, however, would disregard all the evidence, physical, chemical and physiological, discussed elsewhere (pp. 86-88), which quite strongly supports the view that the two substances are closely related. It is of interest to remark that, even if such an assumption were adopted, it would still be necessary to postulate at least two different types of sympathin to account for the observed facts.

2. Less-than-linear summation of responses. It will be

recalled that whereas simultaneous stimulation of two sources with relatively infrequent stimuli results in a response greater than simple summation of the individual responses, repetition with more frequent stimuli results in a summation less than linear (see pp. 80, 81). To explain this observation Rosenblueth and Morison (1934) brought forward the idea that in the circulating blood sympathin may not be exclusively the compound, MH (*i.e.*, M + E or I), but may be associated with some M which diffuses out. In these circumstances, as the frequency of stimulation rises, the law of mass-action operates and there are higher concentrations of M relative to MH, and consequently a greater diffusion of M into the blood. Since M is assumed to be the equivalent of adrenine, it would then tend to make the summation less than linear, as already noted in comparing the effects of sympathin from two sources with the effects of equivalent amounts of adrenine (see p. 94).

3. Why sympathin E does not inhibit. Cannon and Rosenblueth (1933) raised the question as to why ME, which induces contraction in the cell where it originates, does not induce contraction when it enters a cell inhibited by sympathetic impulses. They suggested that the I in the naturally inhibited cell offsets or neutralizes the action of the incoming ME. Thus foreign MI, but not foreign ME, could influence cells which relax. Analogously, foreign ME alone could influence cells which contract.

In considering that circulating sympathin might be MH + M, Rosenblueth and Morison (1934) carried this speculation further. If pure M (the equivalent of adrenine) reaches a smooth-muscle cell with the I receptor, MI is formed and relaxation ensues. If ME enters the I cells, however, it may dissociate and the free M may combine with I so that equal amounts of MI and ME are ultimately present and the elastic properties of the muscle do not change. A similar situation would develop if a small amount of M were added to the ME. On the other hand, if both ME and MI are produced at the source (*e.g.*, by splanchnic

stimulation of the gastro-intestinal tract and its vessels), either ME or MI will preponderate at the indicator, dependent upon the presence of E or I in the receptor, and contraction or relaxation, respectively, will occur.

4. The response of the iris. Of the two sets of smooth muscle in the iris, only the radial fibers, having a sympathetic innervation, are supposed to contain the hypothetical differentiating receptive substance, E; and when adrenine is administered it combines with this substance to form AE to which the fibers respond by contraction. Similarly, when the cervical sympathetic strand is stimulated, they respond to the equivalent of AE, that is, ME. It must be assumed that they respond also to circulating ME discharged into the blood stream when, for example, the hepatic nerves are excited. Why, then, does not sympathin E, like adrenine, cause dilation of the pupil?

Circular fibers of the iris subject to parasympathetic nerves may be assumed to have no E, or none like that in the sympathetic field, and therefore adrenine on reaching them has no defined effect. As a result, adrenine, active on the radiating fibers, causes pupillary dilation. ME from the liver region, however, by bringing the missing E, might provide a stimulus which could act on the circular as well as on the radiating fibers. Thus the two sets of fibers would become antagonists; sometimes one set, sometimes the other, would prevail slightly, and sometimes they would balance each other—and these are just the effects which Cannon and Rosenblueth (1935) observed when they stimulated the hepatic nerves.

It will be recalled that, although purely excitatory sympathin from the liver had little or no action on the iris, sympathin set free from regions of excitation and inhibition retracted it (see p. 91), *i.e.*, when ME and MI are both present the result differs from that produced by ME alone. In an attempt to explain theoretically this situation Cannon and Rosenblueth supposed that some of the MI would counteract the ME in affecting the *circular* (constrictor)

fibers of the iris, and therefore they would be stimulated less than if ME alone were acting. They supposed, further, that when MI enters the *radial* fibers, it dissociates in the presence of E, and the M is then combined with both I and E. But ME also enters the radial fibers, and being present there in greater concentration than in the constrictor fibers, the iris is retracted (the pupil is enlarged).

Poos (1927) has adduced evidence that sympathetic impulses and adrenine have some inhibitory influence on the constrictor fibers of the iris. According to the theory of two kinds of sympathin, the relaxation would imply the existence of I in these fibers. If the E carried by pure sympathin E should overwhelm the I present, contraction might ensue. But in any case there would be invariably larger amounts of ME in the radial than in the circular fibers. That the iris is not retracted in these circumstances is possibly due to the fact that the constrictor is anatomically and mechanically a more efficient muscle than the dilator. The occasional slight dilation which occurs when sympathin E is liberated may be the result of some I in the constrictor fibers.

The usual absence of any noteworthy effect of ME on the iris is a striking phenomenon because, apparently, only in that organ are two smooth muscles so arranged as to be capable of acting as direct antagonists. When the circular fibers are severed and the possibility of antagonism is abolished, ME has an effect which is comparable to that produced by an equivalent dose of adrenine (see p. 92).

5. The reversal of the response of the cat uterus. The uterus of the non-pregnant cat responds to adrenine and to sympathetic impulses by relaxation; when the animal becomes pregnant, however, it responds by contraction. In 1929 Van Dyke and Gustavson reported that a similar reversal can be produced by injections of lipoidal extracts of mature corpora lutea. Kennard (1937) has studied the course of this transformation from relaxation to contraction as produced by progestin, and also the subsequent return

to relaxation. He found that the reversal of the responses to nerve stimulation precedes that of the responses to adrenine, so that for a time the uterus contracts when the hypogastric nerves are excited, and relaxes when adrenine is injected (see Figure 22). And if small doses of progestin

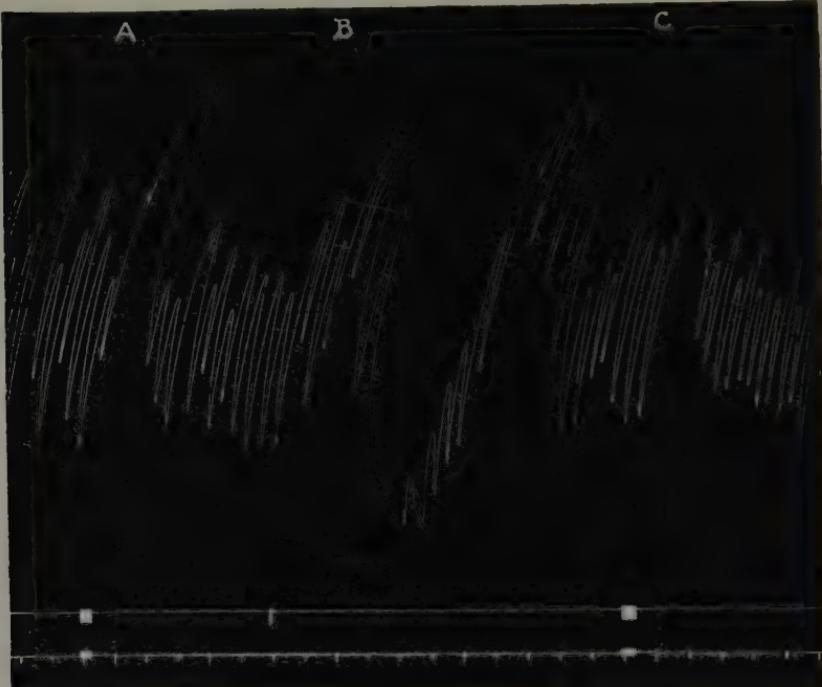


FIG. 22. Responses of the non-pregnant uterus of the cat, 4 days after being spayed, and 13 hours after injection of progestin ("Proluton," 1 unit). A and C, after 10 seconds of stimulation of the hypogastric nerves. B, after adrenalin ( $5 \gamma$ ). (Kennard, 1937.)

are employed the reversal may be restricted to that induced by nerve impulses.

These observations, at first glance, appear to upset the whole theory of chemical mediation. Kennard has given them, however, an explanation in terms of the theory of two sympathins. Assuming that in the non-pregnant state the uterine cells contain I, and therefore relax, and further that progestin converts I to E, he suggests that during the conversion there would be a stage in which I and E would

both be present. As stated earlier (p. 16), histologists have declared that not all smooth-muscle cells are innervated. If the change from I to E occurs earlier in the innervated, at some time in the transformation the differentiating substance E would predominate in them, and I would predominate in the non-innervated units. Then adrenine, affecting all cells simultaneously, would cause relaxation because the relaxed non-innervated are much more numerous than the contracted innervated cells. On the other hand, if the nerves are stimulated, sympathin E would be formed and would cause contraction. As it diffuses, it, unlike adrenine, has no effect on cells containing I (see p. 101).

*A Consideration of Some Criticisms.* Although Bacq (1935) has admitted that sympathin is not identical with adrenine, and although that conclusion is based in part on the evidence that sympathin exists in two forms, he has pointed out a number of objections to acceptance of the theory of sympathin E and I.

1. The theory implies the view that a smooth-muscle cell can respond in only one sense, by contracting or relaxing. That implication is, to be sure, involved in the theory. In accord with it ergotoxine does not reverse the action of adrenine; it blocks the positive action and leaves unaffected the negative. In other words, the smooth-muscle cells of some arteries and the alimentary canal, which adrenine relaxes, are not the same as those which adrenine excites. In terms of the theory one group of cells would contain the differentiating agent I; the other, E.

In support of the idea that adrenine may have opposite effects on the same cell Bacq cites the observation of Spaeth and Barbour (1917) that adrenine contracts the melanophores of Fundulus, but after ergotoxine it has quite the opposite effect. It is questionable, however, whether behavior true for a superficial cell of a fish is true also for an internal cell of a mammal. The fish is not closely related to the mammal, and the melanophores are not smooth

muscle. Until both excitatory and inhibitory effects of sympathetic impulses or adrenaline are demonstrated in the same muscle cells, the indications of specific responses in separate cells, according to the presence of a single differentiating agent therein, should be respected. Of course, the character of the agent may change, as when the smooth muscle of the cat uterus is altered by pregnancy; then adrenaline, which formerly caused relaxation, causes contraction. In the process of reversal, as Kennard (1937) discovered, adrenaline may have a diphasic effect, but that is explicable as a phenomenon due to a more rapid appearance of E in some cells than in others.

2. The nature of the differentiating agents, E and I, is not suggested. This charge is admitted. The statement, however, that search for the nature of these substances seems futile is not warranted. Pharmacological research may discover a means of chemically modifying adrenaline so that it would act like sympathin E in a discriminative manner. It could be used, for example, to stimulate the heart and raise blood pressure without inhibiting the intestine. And if modified into sympathin I it could relax bronchioles without disturbing the heart. Discoveries of that type might throw illumination on what occurs inside responsive cells. Just how a stimulus makes a muscle contract or relax is, of course, shrouded in darkness.

3. What is true for sympathin should be true of the parasympathomimetic substance. Bacq (1935) has pointed out that if sympathins E and I exist, "parasympathins" E and I also should logically be expected to exist, but that no published fact is known in favor of that conception. In spite of the lack of factual evidence, the problem of how acetylcholine acts to excite in one region and to inhibit in another is insistent. The possibility of a specific excitability in the responsive cells, differing in accord with the sense of the response, was suggested for sympathetic effectors (see p. 98); it was discarded because of the different behavior of an actual substance, sympathin, coming away from a

purely excited region, as compared with that from a region in which inhibition was added to excitation. Perhaps acetylcholine affects organs in opposite ways because of peculiar mechanisms—opposed excitabilities—in the organs. Or perhaps parasympathins E and I are formed, but, partaking of the extreme instability of acetylcholine, they flash instantly out of existence as soon as they have acted. Certainly the problem of the opposed effects of acetylcholine is not to be dismissed merely because no decisive data have been found.

4. Alternatives to the theory of two sympathins may be imagined. Bacq (1934a, 1935) has offered a number of suggestions in avoidance of the idea of sympathin E and sympathin I.

Sympathin I, he states, has not been demonstrated to be different from adrenine. Admittedly, when an inhibited area is stimulated, the remote result is both inhibition and excitation, *i.e.*, just such as adrenine produces. As already explained, however, that occurs because every known inhibitable area has within it excitable elements, and, according to the theory, sympathin E would invariably be produced along with sympathin I (see p. 96). Since sympathin from a purely inhibited organ has never been demonstrated, a difference between sympathin I and adrenine cannot at present be demonstrated. If sympathin I were the same as adrenine, however, the addition of I to excitatory sympathin E should make the positive action of the two equal to the action of adrenine on various structures. Such is not the case. A combination of E and I having on the nictitating membrane an effect equal to a given dose of adrenine, has a smaller effect than adrenine on the iris (see Figure 20.)

Another suggestion offered by Bacq (1934) is that when the sympathetic has an inhibitory influence the mediator is adrenaline; and when excitatory, noradrenaline, *i.e.*, non-methylated adrenaline, which, like all primary amines, has only a slight inhibitory action and has a stimulating action

not inverted by ergotoxine. In brief, sympathin I would be adrenaline or adrenine, and sympathin E would be noradrenaline. Obviously, on this basis adrenine, when secreted or injected, would have to be altered, at least in part, into noradrenaline, in order to cause stimulation. For that alteration there is no evidence.

Still another proposal espoused by Bacq (1934a) is related to Rosenblueth's (1932c) hypothesis that the intermediary sympathetic substance, M, is the equivalent of adrenine. Adrenine is fairly easily oxidized. Bacq suggests that as it undergoes oxidation, it loses its inhibitory more easily than its excitatory power and thus may become a purely excitatory agent. With regard to this idea the fact may be emphasized that the sympathomimetic substance from the liver begins to act after the shortest delay—in about 15 or 20 seconds (92)—and has solely a stimulating effect, whereas the substance from the tail region—after a considerably longer delay (80)—has both effects. In other words, the M which has most time to lose all its inhibitory influence by oxidation does not lose it, and the M which has least time loses all of it! Argument, however, is not necessary. The tests carried out by Blaschko and Schlossmann (1936), in which they used rabbit intestine as an inhibited indicator and cat's blood pressure as a stimulated indicator, revealed that at no stage in the progressive oxidation of adrenine was there a significant difference of effects. The hypothesis that sympathin E is a slightly oxidized adrenine is thus rendered unlikely.

As a comment on all suggestions that the differential action of adrenine depends on some extracellular change of its nature, the problem presented by the feline uterus may be stressed. When, with pregnancy, the response of that organ to adrenine is altered from relaxation to contraction, is it to be presumed that the essential character of the cat's organism is so profoundly transformed that it can suddenly metamorphose adrenine, as it circulates from the point of injection to the uterus, from an inhibitory to an

excitatory agent? Not a shred of evidence in favor of that view can be found. And there is much evidence, as surveyed in foregoing pages, that when a single agent evokes contraction or relaxation, the effect results from modifying circumstances in the affected cells.

*Chemical Mediation of Nerve Impulses as Related to Theories of Inhibition.* That a substance produced by nerve impulses can both directly and indirectly cause inhibition has an important bearing on theories of inhibition. Especially is this true if, as Howell (1925) has declared, "inhibition is fundamentally the same process in all tissues," because other theories (drainage, interference, anabolism) would thereby be supplanted. The chief support for the humoral or chemical theory of inhibition, cited by Howell, was that offered by Loewi's (1921) "Vagusstoff." When Loewi demonstrated that the peculiar substance liberated by vagal impulses while they are inhibiting the heart can stop another heart if applied to it, he transformed cardiac inhibition from an idea, expressed in vague phrases, into a concrete phenomenon ready for further study. The evidence for an inhibitory sympathin brings additional testimony in favor of the action of a chemical agent when a physiological process is inhibited.

## CHAPTER X

### ELECTRICAL PHENOMENA IN AUTONOMIC EFFECTORS

Although the appearance of action potentials in smooth muscle was demonstrated by Fuchs as early as 1908, knowledge of these phenomena is incomplete and fragmentary. The main purposes of the early studies on the subject were the establishment of the presence of electric changes in different cold-blooded and mammalian smooth muscles and the determination of the nature of the spontaneous rhythmic contractions of these muscles. It was assumed that the one-to-one or many-to-one correlation between the electrical and the mechanical cycles would differentiate twitches from tetani. Because of these limited interests the electric responses to nerve stimulation have been almost totally neglected.

In more recent studies, however, this aspect of smooth-muscle physiology has developed considerably. Different types of electrograms have been recognized. Pharmacological tests have been applied and interesting results have ensued. Sufficient evidence has been obtained to justify an analysis of the physiological significance of the several components of the electrograms. Such an analysis throws light on the problems of neuromuscular conduction and excitation.

*Electric Responses of Smooth Muscle to Single Nerve Volleys.* Many smooth muscles respond mechanically with typical twitches when single shocks are applied to their excitatory nerve supply (*e.g.*, the nictitating membrane, the pilomotors, the bladder). In other neuromuscular systems, however, single shocks fail to elicit any recordable contraction or relaxation (*e.g.*, the uterus). We shall call the muscles of the first class "single-volley" and those of

the second, "multiple-volley." In single-volley systems each stimulus can evoke electric responses, in addition to mechanical reactions. The multiple-volley systems studied thus far do not yield detectable electrograms after single shocks (287, 245).

The electric responses of the nictitating membrane, pilo-motors and bladder to single nerve volleys are complex (Figure 23). Lambert and Rosenblueth (1935) have dis-

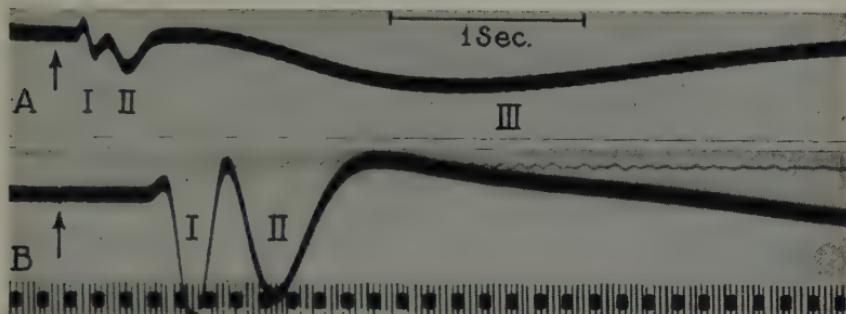


FIG. 23. Electric responses of the pilomotors. String galvanometer records. Single induction shocks applied to the lumbar sympathetics at the time indicated by the arrows. A, I, II and III. B, more amplification and faster film than A; I, II and beginning of III. Time: 10 msec.

tinguished at least three components, *I*, *II* and *III*, in these electromyograms.

The first component, *I*, is a monophasic spike which precedes contraction. Its latency varies in different neuromuscular systems and with the site of application of the stimulating electrodes on the nerve. After subtracting from the total latency the time of conduction in the nerve, a measure of the neuromuscular delay remains. These delays are considerable—at least 15 msec. for the nictitating membrane and 40 for the pilomotors (285).

Rosenblueth, Davis and Rempel (1936) have shown that the polarity of *I*—*i.e.*, its electrical sign in the records—depends exclusively on the orientation of the muscle cells with respect to the electrodes which lead the electric responses to the amplifier and recording instruments. Whether one or both of the leads are in contact with active tissue is of no influence on the polarity. These statements apply

also to the other components of the electrograms, *II* and *III*. The experimental basis for these conclusions is the following. When one of the leads is inserted into the skin of the tail at a given point and the other is successively inserted at points describing a semicircle around the first, beginning cephalad and finishing caudad, the electrograms of the pilomotors first decrease in size until they disappear when the two leads are in a plane perpendicular to the longitudinal axis of the tail; they then increase, but reversed in polarity,

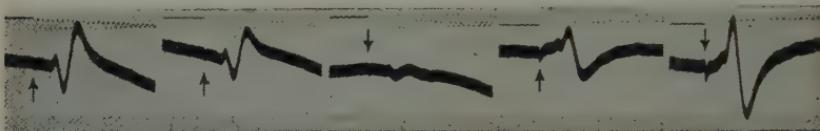


FIG. 24. Influence of the position of the leading-off electrodes on the sign of the pilomotor electromyograms. String galvanometer records. Single induction shocks applied to the lumbar sympathetics. The grounded electrode was kept fixed. The grid electrode described a semicircle around the grounded one, beginning cephalad and finishing caudad. (Rosenblueth, Davis and Rempel, 1936.)

so that finally a mirror image of the first response is obtained (Figure 24). Reversing the leads along the axis of the tail always reverses the polarity of the electrograms, whether the skin be intact, or removed at one or both of the points of insertion of the leads.

In the phenomena just described, smooth muscle differs strikingly from skeletal muscle. The source of this difference lies in the physical conditions imposed by the small size of the smooth-muscle cells. Failure to realize this difference, and the consequent interpretation of electrograms of smooth muscle in accordance with assumptions which are held valid for skeletal muscle may be a source of error. When, for example, Bacq and Monnier (1935) infer that the relaxation of the non-pregnant cat's uterus on injecting adrenine or stimulating the hypogastric nerves is attended by a *positive* variation of the muscle cells, similar to the Gaskell effect in the heart, they may be right, but they may be wrong, for their records do not permit conclusions as to the changes in individual cells.

In order to determine the absolute sign of an electric change occurring in a smooth muscle it would be necessary to record the responses of single cells, using a technique similar to that which has been employed in skeletal muscle, *i.e.*, placing a lead on a normal region of the cell and the other on a damaged region.

The magnitude of *I* is a function of the number of active muscle cells interposed between the recording leads. Thus, in the pilomotors, within certain limits, imposed probably by the lack of simultaneity of activation of the muscles when the lumbar sympathetic is stimulated, *I* increases as the distance between the leads is greater. It may be concluded that the electrograms of smooth muscle sum in series, as do the discharges from the elements of the electric organs of fishes.

The component *II* is more complex and variable than *I*. It is usually polyphasic. Its latency is difficult to determine, for *I* usually merges into *II* without a distinct break. Some evidence has been presented by Rosenblueth, Davis and Rempel (1936) that *II* may begin quite early during *I*.

The component *III* is a prolonged monophasic deviation roughly coincident with the mechanical response. Bacq and Monnier (1935) state that *III* precedes the contraction, and is its cause. The precise beginning of *III*, however, is difficult to determine, for it succeeds *II* without a marked transition. In the observations of Lambert and Rosenblueth (1935), contraction began in the nictitating membrane invariably during *II*. From the evidence available, therefore, no conclusion can be drawn as to whether *III* precedes contraction or not.

*The Electric Response of Smooth Muscle to Repetitive Stimulation.* The effects of repetitive stimulation differ in the single-volley and the multiple-volley systems. Separate descriptions are therefore necessary.

In the nictitating membrane and the pilomotors repetitive stimulation leads to the following results (285). With frequencies as slow as 1 per second, *I* shows usually a decline

in magnitude, while *II* may become more prominent toward the end of the series. As the frequency is increased the decline of *I* is more marked (Figure 25). At frequencies of 20 per second or higher, only a few initial spikes are dis-

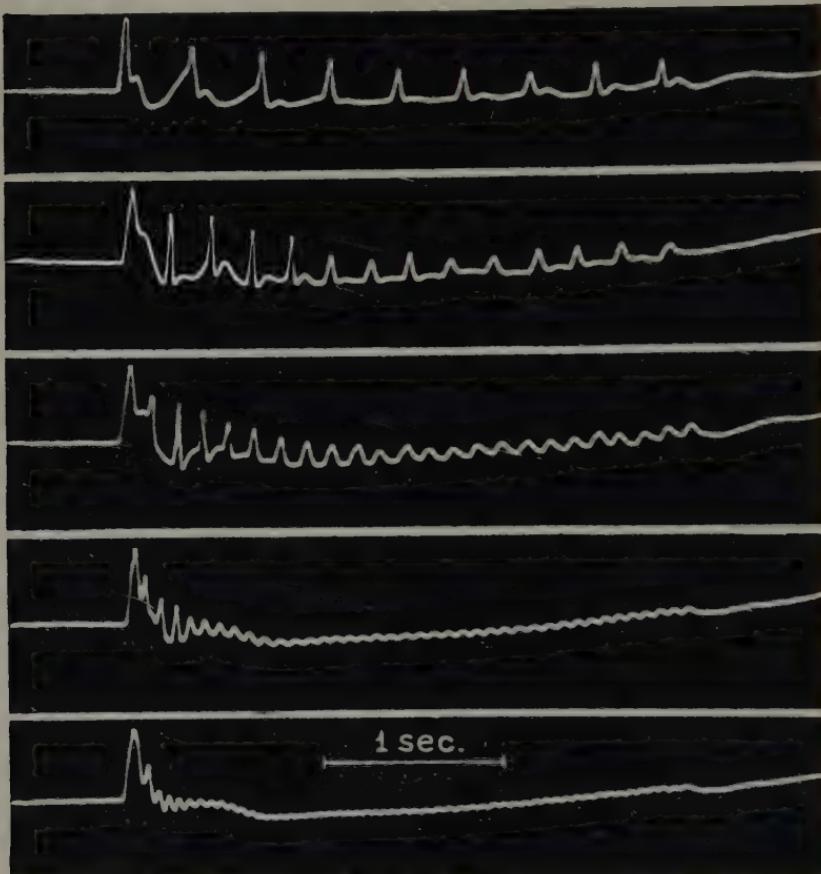


FIG. 25. Electric responses of the nictitating membrane to repetitive stimulation of the cervical sympathetic. Cathode-ray oscillosograph records. Frequencies: 2.3, 4, 8, 11 and 16 per second. (Rosenblueth, Davis and Rempel, 1936.)

cernible. Unlike *I*, both *II* and *III* are capable of summation—*i.e.*, the effects of succeeding shocks build on the preexisting responses if the intervals between the stimuli are sufficiently short. When series of stimuli at high frequencies are applied, a summated *II* is visible only at the

beginning of the electrograms (see 285), while *III* yields a smooth wave which persists throughout contraction (29).

The electric responses of multiple-volley systems are quite different from those just described. Thus, in the cat's pregnant uterus Rosenblueth, Leese and Lambert (1933) found that the individual shocks do not elicit any correlated potentials. When repetitive stimulation is applied, there occurs after a long latency and simultaneously with contraction, a series of rhythmic, relatively brief, complex disturbances, superimposed on a slow, smooth deflection (29). The brief, rhythmic potential changes resemble the component *II* of the single-volley systems; they are also similar in frequency and pattern to the "spontaneous" waves which appear in the same muscle (see below). The slow deflection corresponds in all probability to *III*. Analogous results have been observed in the retractor penis of the dog by Brücke and Oinuma (1910). The main difference between the single- and the multiple-volley systems appears, therefore, to consist in the presence of *I* in the former and its absence in the latter.

*The Electrograms of "Spontaneous" Rhythmic Contraction.* The electrical changes attending spontaneous activity of many smooth muscles have been recorded. As stated before, one of the questions which led to the study of electrograms was that of determining whether each contraction was accompanied by one or several "action potentials." If a one-to-one correspondence occurred, it was inferred that the spontaneous activity denoted rhythmically recurring twitches. Conversely, if several electric deviations were present during one mechanical cycle, a tetanus was postulated. The results of such observations are contradictory. Thus a one-to-one correspondence of electric and mechanical changes has been found in the intestine (4, 43), in the stomach (337, 165, 268), and in the ureter (251), while a many-to-one relation appeared in the retractor penis (70) and in the uterus (287).

It is possible that these contradictions might be correlated

with the single- or multiple-volley characteristics of the systems studied. On the other hand, the assumption that a one-to-one correspondence classes the mechanical effects as twitches is not justified; for, as previously reported (p. 114), the electrograms of single-volley systems may yield fused, smooth tracings, which do not bear evidence of repetitive activation, when sufficiently frequent stimuli are applied.

*Electric Phenomena of Relaxation.* The electric changes attending relaxation have not been extensively investigated. Relaxing neuromuscular systems may all belong to the multiple-volley class, since no responses to single nerve volleys have been observed. The effects of repetitive stimulation on the "spontaneous" waves of these systems is probably opposite to those which occur in the multiple-volley contracting systems described above. Thus, Brücke and Oinuma (1910) speak of a negative chronotropic and inotropic action of the pelvic nerves on the spontaneous electric background in the retractor penis of the dog. In the non-pregnant cat's uterus, similarly, stimulation of the hypogastric nerves induces a quiescence of the spontaneous waves, when these are present (unpublished observations).

A positive electrical variation of smooth-muscle cells when they relax in response to nervous impulses has been repeatedly observed. The earliest report is that of Reid (1895) on the constrictor muscle of the iris when the cervical sympathetic was stimulated. Orbeli and Brücke (1910), however, failed to confirm Reid's results. Reasons have been given (p. 112) for rejecting the sign of the recorded responses as an index of the sign of the changes in the cells. Further studies will be necessary, therefore, to establish whether relaxation of smooth muscles is correlated with increased positivity of the surface of the active elements.

*The Action of Certain Substances on the Electromyograms.* Certain substances, e.g., adrenine, cause smooth muscles to contract or relax. It is of interest to investigate whether these mechanical responses are attended by corresponding

electric changes. Rosenblueth, Leese and Lambert (1933) were unable to detect any *I* or *II* components when the nictitating membrane contracted in response to adrenaline. This observation was confirmed by Monnier and Bacq (1935), who found only *III* potentials in these circumstances.<sup>1</sup>

If the cervical sympathetic is stimulated while the nictitating membrane is contracted in response to adrenaline, the mechanical effects of the nerve impulses sum with those of the hormone, as shown by Rosenblueth and Rioch (1933a); Rosenblueth and Cannon (1936) found, however, that the



FIG. 26. Decrease of the electric responses of the nictitating membrane by adrenaline. String galvanometer records. Single induction shocks applied to the cervical sympathetic. The first record was obtained before the injection of adrenalin ( $40 \gamma$ ). The succeeding records were taken 30, 50, 85 and 240 seconds after the injection. (Rosenblueth and Cannon, 1936.)

electric responses (*I* and *II*) to the nerve volleys are decreased (Figure 26) or may disappear altogether. This impairing action of adrenaline on the electrograms does not occur exclusively when the recording muscles contract in response to the hormone, but may appear as an independent effect. Thus, the pilomotors of the cat do not contract when adrenaline (*e.g.*,  $20 \gamma$ ) is injected intravenously, unless cocaine has been administered previously. Yet such a dose of adrenaline, injected without cocaine, markedly decreases *I* and *II* in the pilomotors (Figure 27). Cocaine accentuates the depressing action of adrenaline on the electrograms.

The generally accepted membrane theory of excitation accounts for the spike potentials of skeletal muscle as the depolarization of a polarized interface. Analogously, *I* in smooth muscle may be due to the depolarization of the polarized surface of the muscle cells. The magnitude of *I*

<sup>1</sup> This failure to note rapid electrical changes when adrenaline is injected may have been due to insufficient amplification. Eccles and Magladery (*J. Physiol.*, **87**: 87P, 1936) have reported the appearance of minute repetitive electrical disturbances when the nictitating membrane is contracted by adrenaline.

would then be dependent upon the degree of polarization of this surface. The depressing influence of adrenine on  $I$  may be explained by assuming that the hormone partly or completely depolarizes the cells (245, 284).

The action of adrenine on the spontaneous electrogram of the intestine, studied by Berkson (1933a), is particularly interesting. Whether the observations be made *in situ* or *in vitro*, there is normally a cyclic electrogram corresponding closely to the cyclic mechanogram of rhythmic activity. If adrenine is injected or added to the bath the mechanical

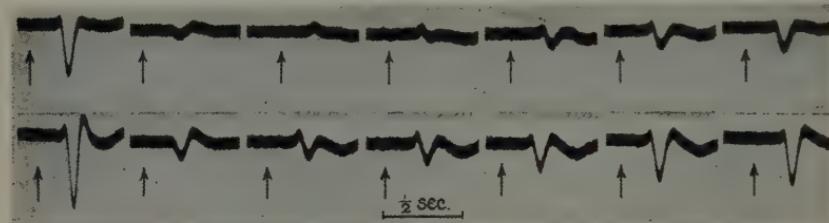


FIG. 27. Decrease of the electric responses of the pilomotors by adrenine. String galvanometer records. Single make shocks (upper records) and break shocks (lower records) applied to the lumbar sympathetics. The first records (upper and lower) were obtained before the injection of adrenalin ( $20 \gamma$ ). The succeeding responses were elicited 15, 45, 60, 115, 180 and 300 seconds after the injection. (Rosenblueth and Cannon, 1936.)

changes disappear, while the electrogram is unaffected. Atropine has a similar effect (42).

Ergotoxine paralyzes the contractions of smooth muscle in response to sympathetic nerve impulses. Its effects on the electrograms differ, depending on whether the muscle remains relaxed or exhibits a contracture when the drug is injected. Thus, in the nictitating membrane, which goes into a marked, prolonged contracture after a small dose of ergotoxine, both electric and mechanical responses are abolished. In the pilomotors, on the other hand, which do not contract after ergotoxine, Rosenblueth, Leese and Lambert (1933) found that nerve impulses still elicit characteristic  $I$  and  $II$  potentials, although no mechanical responses are detectable.

The mechanical responses to sympathetic nerve stimula-

tion, Rosenblueth and Rioch (1933a) noted, were considerably increased by cocaine; on the other hand, the electric phenomena *I* and *II*, according to Rosenblueth and Cannon (1936), are depressed by the drug. Yohimbine and 933F, which reduce considerably the mechanical responses of the nictitating membrane to adrenine, while leaving relatively unimpaired the contractions on stimulation of the cervical sympathetic, increase *I* and *II* in the membrane. They have the same effect on *I* and *II* in the pilomotors (245, 284).

*Preganglionic Denervation of the Nictitating Membrane.* The mechanical responses of the preganglionically denervated nictitating membrane to single shocks applied to the postganglionic nerve supply are greater and longer than those of the normal controls (p. 144). Rosenblueth and Cannon (1936) found, however, that the electric potentials *I* and *II* are smaller than in the normal membrane, and decrease far more readily with slow, short series of stimuli.

*Separability of the Electromyograms and Mechanograms.* In skeletal muscle a long controversy has been waged on the possibility of obtaining action potentials (conduction) without contractions and *vice versa*. In 1930 Gasser summarized the status of the problem by concluding that conduction probably cannot take place without contraction, while every contracture is an instance of contraction without conduction and the corresponding spike potential. Skeletal muscle is mentioned in this connection solely because the discussion of the relations between the electric and the mechanical events has been mainly concerned with this tissue. It should be borne in mind, however, that a strict analogy between skeletal and smooth muscle has not been ascertained; indeed, there are strong arguments against the view that *I* is the equivalent of the spike potential of skeletal muscle (see p. 121). The case of smooth muscle should, therefore, be discussed separately.

Thus far it has not been possible to separate *III* from contraction. If the view developed by Dubuisson (1935) is accepted, that the chemical phenomena underlying con-

traction lead to electric changes, and if it is assumed, further, that *III* is the manifestation of these changes, it may be concluded that *III* and contraction may not be obtained independently.

On the other hand, *I* and *II* may appear without contraction, and *vice versa*. Thus, as already shown, when the sympathetic nerves are stimulated after an injection of adrenine, contraction of the pilomotors, or further contraction of the nictitating membrane, occurs with greatly diminished or absent action potentials (p. 117). Berkson (1933a and b) has demonstrated that nicotine and curare abolish the spontaneous electrograms of intestinal muscle, while the rhythmic contractions persist. Conversely, action potentials without mechanical effects are obtained in the pilomotors after ergotoxine, and in the intestine after adrenine or atropine. Furthermore, *I* and *II*, and the mechanical changes may vary independently and even in opposite directions. For example, cocaine and preganglionic denervation enhance the mechanical while depressing the electric responses (pp. 119, 144).

It may be concluded from this evidence that, while *III* appears to be an inseparable concomitant of contraction, neither *I* nor *II* is indispensable for contraction or is invariably attended by it. Furthermore, the conclusion is warranted that activation of a muscle by a nerve may occur without the appearance of *I* or *II*.

*The Physiological Significance of the Electromyograms.* This discussion will be concerned mainly with *I* and *II*. Since *I* precedes contraction and appears in the records as a monophasic spike (Figure 23B), there is a temptation to consider it as analogous to the spike potential of skeletal muscle—*i.e.*, the electric index of a transmitted wave of depolarization responsible for conduction of the excitatory process in the muscle. The first objection to this view is that conduction, indispensable in the long fibers of skeletal muscle, appears quite unnecessary in the small elements of smooth muscle. If intercellular conduction, from the in-

nervated to the non-innervated cells, were effected by conducted depolarization, *I* might denote this process; but observations by Rosenblueth and Rioch (1933c) lead to the conclusion that all-or-none intercellular conduction does not occur in smooth muscle. Furthermore, the conducted disturbance of skeletal muscle is an indispensable step in the excitation of the muscle by a nerve impulse—*i.e.*, no contractions have been obtained by nerve stimulation without the appearance of the action potentials. In smooth muscle, on the contrary, as was shown (p. 120), nerve impulses may elicit contractions without detectable *I* potentials—*e.g.*, after adrenine. The component *I*, therefore, is probably not analogous with the spike potential of skeletal muscle and does not denote conduction of the excitatory process.

According to the theory of chemical mediation of autonomic nerve impulses, the chemical mediator is produced at some stage between the nerve action-potential and the mechanical response. The potential *I* occurs within these time limits; it might be correlated in some way with the production of the mediator—*i.e.*, *I* might be either a necessary step for the liberation of the mediator or a change induced by the latter after its production. From the evidence discussed above the conclusion seems warranted that the mediator can be liberated, as denoted by contraction, without a preceding *I* potential. Furthermore, the long latency of *I* (neuromuscular delay) in the nictitating membrane and pilomotors is not in favor of the interpretation that the nerve action potential directly activates *I*, whereas this delay is readily accounted for on the assumption that *I* is a consequence of the mediator.

The depressing action of adrenine on *I* (Figures 26 and 27) furnishes an important lead for the interpretation of the relations between *I* and the mediator. The suggestion has been previously offered that *I* may be the sign of a depolarization of the surface membrane of the cells and that adrenine impairs *I* by partially or completely depolarizing this surface

during the entire period of its action. But the primary chemical agent for the sympathetic nerve impulses is in all probability adrenine (p. 99). The potential *I* may then be the sudden depolarization induced by the sudden liberation of the mediator on the arrival of the nerve impulse.

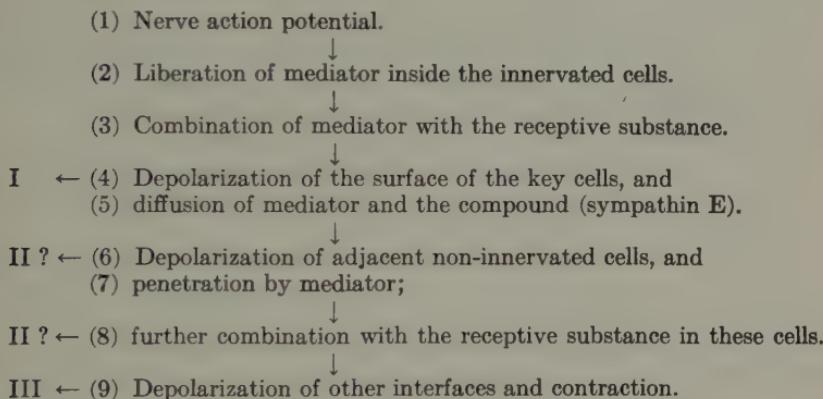
The rapid decrease of *I* on repetitive stimulation is readily explained by this hypothesis, as follows. The destruction of the mediator is a slow process. Successive quanta of mediator liberated by successive nerve impulses will therefore further depolarize an interface already partially depolarized because of the mediator remaining from the preceding impulses. If the shocks are frequent enough to insure the accumulation of the mediator to a concentration which will maintain the interface totally depolarized throughout the period of stimulation, no *I* potentials will be visible after a few shocks (Figure 25). A further discussion of the physiological significance of *I* will be found on pp. 142-144.

The significance of *II* is still obscure. Probably it is a composite phenomenon, resulting from a simultaneous occurrence or an overlapping sequence of several relatively independent changes. If the explanation suggested above for *I* is accepted, successive depolarizations of non-innervated cells should be expected to take place as the mediator diffuses from the innervated key cells; these depolarizations might be at least partly responsible for *II*. The resemblance between *II* in single-volley systems and the rhythmic potential changes of multiple-volley systems, previously described, may not be more than a gross analogy—*i.e.*, the two effects may in reality correspond to entirely different physiological processes. Further studies are necessary before any statements may be made that are not purely speculative.

The component *III* appears to be equivalent to the delayed potentials of cardiac and skeletal muscle. Dubuisson (1935) has suggested that the latter are the electric manifestation of the chemical changes associated with contraction. If this suggestion is applied to *III*, the last component of the electrograms would be an event inseparable from and

simultaneous with the mechanical effects. The data available do not warrant a decision as to whether a strict simultaneity occurs.

The general conclusion which may be reached is that the components of the smooth-muscle electrograms may not constitute fundamental, indispensable steps in the sequence of events by means of which nerve impulses lead to mechanical responses. The electric phenomena may be concomitants, not necessary links, in the excitatory chain. This suggestion is embodied in the following schematic summary of this chain:



*Electric Responses of Glands.* Bayliss and Bradford (1885) were the first investigators to report that activity of the salivary glands is attended by electric disturbances. The fact has been confirmed repeatedly since (see Rosenblueth, Forbes and Lambert, 1933, for references). Notwithstanding the early discovery of glandular electric phenomena, they have been studied even less than those of smooth muscle. Practically the only gland which has been observed from that standpoint is the submaxillary. It is an open question whether other glands will show electric disturbances during activity similar to those which occur during salivary secretion.

*Responses of the Submaxillary Gland to Single Nerve Volleys.* When single shocks are applied to the chorda

tympani and the electric changes in the submaxillary gland are recorded, complex responses appear (57, 40). They may be divided into two components, an initial "quick" excursion and a subsequent "slow" wave (Figure 28).

With adequate recording leads the quick component appears usually as a monophasic wave. Its latency is, however, variable; repetitive stimulation tends to shorten it. The duration of the deflection is likewise variable,



FIG. 28. Electric response of the submaxillary gland to a single induction shock applied to the chorda tympani. String galvanometer record. Q = quick effect. S = first phase of the slow component. (Rosenblueth, Forbes and Lambert, 1933.)

500 to 800 msec.; in general, big excursions last longer than smaller ones.

The slow component begins after the first one has ceased. Its duration in response to a single shock lasts from 15 to 60 seconds. It appears usually as a diphasic wave in the records. The independence of the quick and slow components is shown by the possibility of obtaining either separately, depending on the position of the leading-off electrodes or on the degree of previous stimulation of the gland. Further evidence of the independence of the two components will be described presently, in relation to the pharmacological aspects of the phenomena.

Single shocks applied to the cervical sympathetic do not lead to the appearance of any clear electric response in the submaxillary gland (286). Even after injections of cocaine, which increase the salivary secretion in response to sympathetic nerve impulses, single nerve volleys remain ineffective.

*Responses of the Submaxillary to Repetitive Stimulation.* The results of repetitive stimulation of the chorda tympani vary with the frequency employed. Single shocks repeated

at relatively long intervals (30 to 60 sec.) elicit quick responses of decreasing magnitude, which may finally disappear. Two maximal shocks delivered at shorter intervals, e.g., 1 sec., demonstrate the ability of the quick effect to sum; if the interval is shorter the two deflections may fuse into a single large wave. Similar remarks apply to the slow component, but the summation interval is much longer. It may be concluded that the two components sum independently (286).

With series of stimuli of higher frequencies (1 to 20 per sec.), as the frequency increases, the discrete waves merge



FIG. 29. Electric response of the submaxillary gland to tetanic stimulation of the chorda tympani. String galvanometer record. The stimulation, which lasted 1.2 sec., began just before the initial deviation and ended with the stimulus artifacts in the record. Large summed quick effect and first phase of the slow component. (Rosenblueth, Forbes and Lambert, 1933.)

into an initial large summated quick deflection succeeded by a slow effect, equally summated (Figure 29). The slow component is bigger and longer, the greater the number of stimuli applied. Occasionally, in undetermined conditions, Rosenblueth, Forbes and Lambert (1933) observed a series of rhythmic waves taking the place of the slow component.

Repetitive stimulation of the cervical sympathetic gives responses quite similar to, though smaller than, those obtained from the same stimulation of the chorda tympani.

Bradford (1887) devised the experiment of ligating Wharton's duct in order to determine whether the electric responses of the gland were due to the flow of saliva along the excretory ducts during secretion. The responses persist, however, for a long time (one hour or more) after the ligation (56, 62, 286).

*The Effects of Drugs on the Electric Responses of Glands.* Injections of pilocarpine, which elicit salivary secretion,

yield also electric responses from the glands. Usually prolonged slow waves appear, which are approximately coincident with the secretion and which are similar to the slow components on repetitive stimulation (62, 83). Occasionally, pilocarpine may evoke rhythmically recurring waves analogous to those recorded from stimulation of the chorda tympani (286). If the chorda is stimulated while the gland is under the influence of pilocarpine, no electric responses, quick or slow, are visible; the gland is apparently refractory.

Moderate doses of atropine (*e.g.*, 0.25 to 1 mgm. per kgm.), sufficient to paralyze secretion when the chorda tympani is stimulated, abolish the quick component of the electrograms both to single and to repetitive shocks (286); the slow component may be present, although reduced (56, 175). Larger doses of atropine (*e.g.*, several mgm.) abolish all electrical phenomena on stimulation of the chorda, although the responses to sympathetic stimulation may persist (56, 83, 286).

*The Significance of the Electrical Phenomena of Glands.* The sources of the potentials recorded during activity of the salivary glands might be *a priori* the following: excitatory waves in the secretory effectors, similar waves in the contractile elements of the blood vessels, chemical and physical changes in the cells during activity, changes in the rate of flow of blood or saliva. Although probably all these sources are effective, the most significant elements seem to be those associated with changes in the secretory cells. The potentials are not of muscular origin, for, as shown by Bradford (1887), atropine abolishes them while vasodilation is still present. The movement of saliva cannot be a significant source, since the potentials persist after ligation of the excretory duct.

Interpretations based on the shape and sign of the responses recorded are not illuminating. Some of the difficulties pointed out in connection with the corresponding features of the smooth-muscle electrograms (p. 112) are also pertinent as regards the glands.

The temporal correlation of the electric disturbances with the different events occurring in the glands has unfortunately not been made. The main obstacle to establishing this correlation is the impossibility of determining accurately the time-course of secretion.

In comparing the glandular electrograms with those of smooth muscle, the quick component of the former might be homologized with *I* or *II* in the latter and the slow element with *III*. The quick component differs from *I* and resembles *II* in its ability to sum (cf. pp. 114, 125). Even if this homology should be accepted, however, no satisfactory understanding of the phenomena would be gained, for the significance of *II* is quite obscure (p. 122).

The analogy between the slow component and *III* may be only gross; after atropine slow components may occur without secretion, whereas no analogous separation of contraction and *III* has been reported.

## CHAPTER XI

### THE EXCITABILITY OF AUTONOMIC EFFECTORS

Since nerve impulses follow the all-or-none principle, the gradation of responses in any effector depends on the number of nerve impulses delivered per unit of time, not on variations in the magnitude of a constant number of impulses. Variation in the number of nerve impulses delivered per unit of time may occur in two ways. A smaller or greater number of nerve fibers may discharge at a constant frequency; or a constant number of nerve elements may deliver impulses at different frequencies. In describing similar phenomena in the central nervous system Sherrington (1929) suggested the use of the terms "spatial" and "temporal," respectively, to denote these two modes of variation. The use of these terms may be extended to cover the two types of gradation of responses in the effectors. By temporal summation is meant that due to variable frequency of nerve impulses delivered through a constant aggregate of fibers, and by spatial summation that due to a variable number of fibers activated at a constant frequency. Needless to say, in the physiological responses both modes of gradation may occur.

*An Examination of Spatial and Temporal Summation.* Spatial gradation of responses has been studied by varying the intensity of stimulation of the nerve supply to an effector at a constant frequency. As the intensity is increased a greater number of nerve fibers will be activated by the stimuli until all the fibers supplying the effector will participate in the responses—*i.e.*, these responses will have reached a maximum for the particular frequency employed. Certain precautions are necessary for such a study to yield fruitful results. The effector selected should be such as to

permit an exact judgment of the responses elicited; if, for instance, a smooth muscle were used which exhibits large intrinsic rhythmic changes, the estimation of the responses

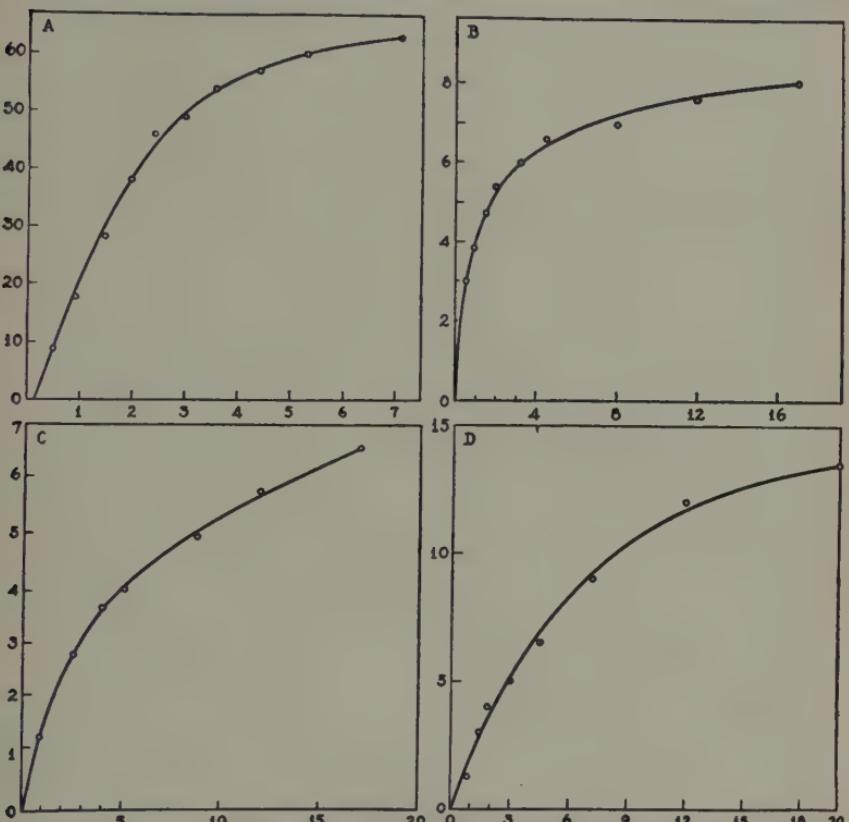


FIG. 30. Frequency-response curves of sympathetic effectors. A, abscissae: frequencies of stimulation of the lumbar sympathetics. Ordinates: angles of erection of a hair in the tail of a cat. B, abscissae: frequencies of stimulation of the cervical sympathetic. Ordinates: heights of the records of isometric contractions of the nictitating membrane 15 seconds after the beginning of stimulation. C, as in B, but isometric contractions of the nictitating membrane. D, abscissae: frequencies of stimulation of the right cardio-accelerator nerves. Ordinates: maximal increases of heart rate per 15 seconds. (Rosenblueth, 1932c.)

would be difficult. The type of response recorded—*i.e.*, isometric or isotonic, for muscle—should be such as to yield accurate information about the changes in the effector as a whole.

This method has been applied to several autonomic effec-

tors (177, 292). The results for both isometric and isotonic contractions of the nictitating membrane of the cat are represented graphically by S-shaped curves which denote a skewed distribution of the thresholds of the nerve fibers in the cervical sympathetic around a mean excitability. These curves are quite similar to those obtained from isometric contractions of skeletal muscle when its motor nerve supply is stimulated with varying intensities (267, 292). Such response-intensity curves depict the electrical excitabilities of the nerve fibers concerned, as a function of the intensity of the stimuli applied.

The relations between the responses obtained and the number of nerve fibers activated are assumed to be linear. The basis for this assumption will become clearer when later the concept of motor units will be discussed.

Temporal summation may be investigated by applying stimuli at variable frequencies but with constant intensity. The purpose is to activate always the same number of nerve fibers. To eliminate the possibility of a summation of subliminal stimuli at high frequencies, which would tend to increase the number of fibers activated, it is preferable to employ a maximal intensity, thus stimulating all the fibers in the nerve. The application of this method to a large variety of autonomic effectors yields curves which closely approximate rectangular hyperbolas (Figure 30). These curves are discussed from other standpoints in pp. 172-177. It is beyond the scope of this book to analyze the relations between these hyperbolas and the curves yielded in similar experimental conditions by skeletal muscles; the interested reader is referred to the discussion by Rosenblueth and Rioch (1933c).

An interesting feature revealed by the response-frequency curves is the strikingly low frequencies of nerve discharge at which very nearly maximal responses of autonomic effectors are obtained. Optimum frequencies may be regarded as the lowest which yield a maximal response for a given number of nerve fibers. Some of these optimum frequencies

are tabulated below (Table 1). They range from 20 to 30 per second for the majority of autonomic effectors. But a frequency of 10 per second elicits as a rule more than 80 per cent of the maximal response.

Temporal summation appears to furnish a wider range of gradation in smooth than in skeletal muscles. Thus, if the

TABLE 1

APPROXIMATE FREQUENCIES OF STIMULATION OF THE PRE-GANGLIONIC FIBERS WHICH PRODUCE MAXIMAL RESPONSES  
(Rosenblueth, 1932b)

SYSTEMS	FREQUENCY PER SECOND
Sympathetic	
Pilomotors	15
Nictitating membrane	20
Pregnant uterus	20
Intestine	20
Adrenal medulla	25
Heart (postganglionic)	25
Parasympathetic	
Heart	30
Submaxillary gland	35
Stomach	25

heights of maximal tetani are compared with those of maximal single twitches, the ratio of these two magnitudes is usually much greater for smooth than for skeletal muscles.

The relations between temporal and spatial summation were studied by Rosenblueth and Rioch (1933c) as follows. Response-frequency curves to maximal stimuli were constructed before and after cutting a fraction of the total number of nerve fibers supplying an effector. When such an experiment is carried out on a *skeletal muscle* (Figure 31) the ratio of the responses obtained with an intact nerve supply ( $R_i$ ) to those obtained after cutting some of the nerve fibers ( $R_c$ ) is constant at all frequencies (Table 2). On the assumption previously stated, that the response to a given frequency is directly proportional to the number of nerve

TABLE 2

RATIOS OF THE RESPONSES OBTAINED WITH AN INTACT NERVE SUPPLY  
TO THOSE OBTAINED AFTER CUTTING SOME OF THE BRANCHES  
OF THE MOTOR NERVE OF A SKELETAL MUSCLE

CURVES IN FIGURE 31A										
F	1	2	3	4	6	8	10	14	18	60
Ri/Rc	1.5	1.5	1.5	1.6	1.6	1.7	1.7	1.7	1.7	1.7
CURVES IN FIGURE 31B										
F	1	2	3	4	5	6	8	10	14	60
Ri/Rc	1.7	1.7	1.6	1.6	1.7	1.7	1.7	1.7	1.7	1.7

fibers activated, the ratio  $Ri/Rc$  is a measure of the fraction of fibers intact after the section—i.e., if one-half the nerve fibers were cut, the responses would be one-half those

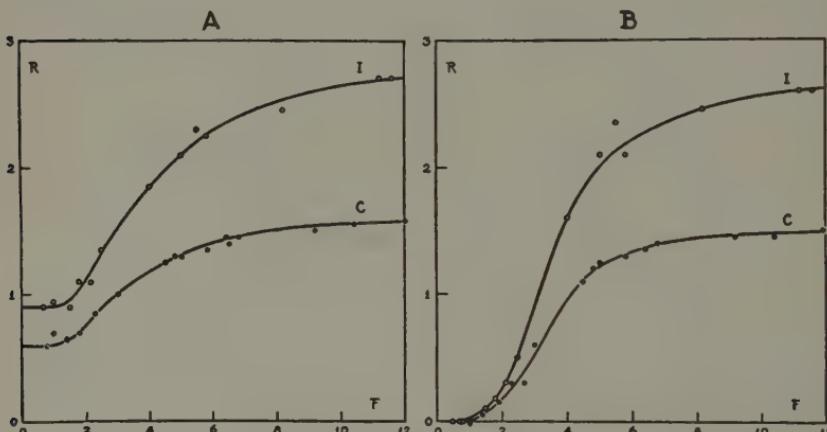


FIG. 31. Isometric contractions of the soleus muscle on maximal stimulation of its motor nerve at varying frequencies, I before, and C after cutting some of the branches of the nerve. Abscissae: frequency per second. A, ordinates: highest tension developed at equilibrium—after a few seconds' stimulation. B, ordinates: lowest tension developed at equilibrium. Always incomplete tetani. (Rosenblueth and Rioch, 1933c.)

originally obtained for any given frequency. The ratio  $F_C/F_I$  of the frequencies which give the same responses after and before the section is, on the other hand, variable, increasing as the responses increase (Table 3).

Similar experiments performed on *smooth muscle* yield very different results (Figure 32A). The ratio  $Ri/Rc$  de-

TABLE 3

RATIOS OF THE FREQUENCIES IN FIGURE 31 WHICH YIELD THE SAME RESPONSES BEFORE AND AFTER CUTTING SOME OF THE BRANCHES OF THE NERVE

CURVES IN FIGURE 31A										
R	0.2	0.6	1.0	1.1	1.2	1.3	1.4	1.45	1.5	1.55
Fc/Fi	1.1	1.2	1.2	1.3	1.5	1.6	1.9	2.4	2.8	3.6
CURVES IN FIGURE 31B										
R	1.0	1.1	1.2	1.3	1.4	1.45	1.5	1.55	1.6	1.65
Fc/Fi	1.8	1.8	1.9	2.0	2.3	2.4	2.8	3.2	4.3	5.0

creases as the frequency increases, showing a tendency to approach 1 as a limit (Figure 32B). This means that if, e.g., one-half the fibers were cut, single twitches would be

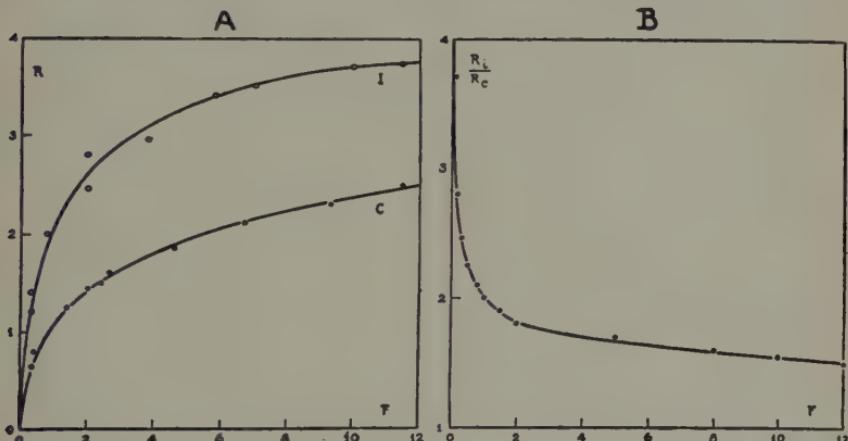


FIG. 32. Influence of cutting some branches of the superior cervical ganglion on the isometric responses of the nictitating membrane to maximal stimulation of the preganglionic nerve at varying frequencies. A, ordinates: tension. I with an intact nerve supply; C after section of some of the nerves. Abscissae: frequency per second. B, ratios of the ordinates of curves I and C in A plotted against the corresponding frequency. (Rosenblueth and Rioch, 1933c.)

one-half as high as before the section, but as the frequency of stimulation is increased the responses tend to approach the magnitude of those obtained before the section. A simple explanation for this tendency is furnished by the hypothesis that the chemical mediator liberated by the nerve impulses may diffuse from the site of production to neighboring cells

and activate them. Thus, even after cutting a relatively large fraction of the nerve fibers, those remaining intact may influence the whole effector. In skeletal muscle, if there is a chemical mediation similar to that of smooth muscle, lack of diffusion of the mediator would account for the restriction of the response to the innervated elements.

In smooth muscle the ratio  $F_c/F_i$  is constant for all responses (Table 4). This means that if, for example, one-half

TABLE 4

RATIOS OF THE FREQUENCIES IN FIGURE 32A WHICH YIELD THE SAME RESPONSES BEFORE AND AFTER CUTTING SOME OF THE BRANCHES OF THE GANGLION

R	0.5	0.75	1.0	1.25	1.5	1.75	2.0	2.25	2.5
$F_c/F_i$	6.5	6.0	5.8	5.6	5.9	6.2	6.8	6.6	6.5

the fibers were cut, doubling the frequency which elicited a given response with the intact nerve supply would yield an identical response after the section. It follows, therefore, that this ratio measures the fraction of fibers cut. This fraction should also be measurable from the ratio  $R_i/R_c$  for single twitches. Experimentally the values obtained agree satisfactorily.

Since the ratio  $F_c/F_i$  is constant for smooth muscle, and equal to the ratio  $R_i/R_c$  for single twitches (which measures the reciprocal of the fraction of the total nerve supply activated), it follows that, in this type of muscle, temporal and spatial summation are quantitatively interchangeable. That is, the responses are a function of the number of nerve impulses which reach the effector per unit of time, independent of the number of nerve fibers concerned. This conclusion has been reached by several observers (46, 160, 178, 293).

The possibility that a few autonomic nerve fibers may influence a large number of the elements of an effector is a striking feature in the organization of smooth in contrast to skeletal muscles. In the striped tissue a single motor fiber branches to supply 100 or more muscle fibers (98), and it can only activate the elements to which it distributes.

Such a group of muscle fibers must act as a physiological unit, the motor unit of Liddell and Sherrington (1925). In smooth muscle, on the other hand, there are no sharply defined motor units. The chemical mediator liberated at a given cell will diffuse to more and more of the neighboring cells as its concentration is raised by increasing the frequency of discharge. Since each preganglionic autonomic fiber may establish synaptic connections with many peripheral neurones there is a first multiplication of effects in ganglia; but a further increase is obtained from the diffusion of the mediator at the periphery. The sphere of influence of a preganglionic fiber may thus become quite extensive.

The direct and indirect response of smooth muscle is in accord with the innervation of only some of the cells (p. 16); in skeletal muscle a non-innervated fiber would be an inactive element. The two modes of organization provide for accurate and localized action of skeletal muscle (*e.g.*, the possibility of extending separately the third and fourth fingers of the hand by the same muscle), and for diffuse action of smooth muscle (*e.g.*, the emptying of a hollow viscus).

*Electrical Excitability.* According to current theories the study of the electrical excitability of a cell or tissue should throw light on other physiological characteristics. For example, conduction in a nerve axon is assumed to occur because of the electrical activation of the adjacent segments of the cell by the potential changes in an active region (cf. Lillie, 1922). The externally applied electrical stimulus is supposed to behave in a manner resembling that of the action potential when it activates the axon. From these assumptions it follows that if a tissue possesses the property of conduction by reason of a mechanism similar to that in nerves or in striated muscle fibers, it should be electrically excitable; conversely, if the cells do not respond to the electrical stimuli they do not possess a conducted disturbance similar to that of nerve or striated muscle.

The propagated disturbance becomes manifest as the

spike potential which may be recorded in nerves and striated muscles when conduction occurs. The electrical excitability of a tissue is thus correlated with its electrogram. If there is a genuine spike potential in the electric response the tissue should be electrically excitable; if it is inexcitable no spike potential should be expected.

When electric shocks are applied directly to an innervated effector and responses are obtained, these responses may be due to activation *via* the nerves—*i.e.*, the electric pulse may stimulate the nerves, and these in turn activate the effector. The mere response to direct application of electric stimuli to muscles or glands does not justify, therefore, the conclusion that the effectors are electrically excitable. Three methods are available to circumvent this difficulty. *First*, the excitability of the two tissues may be determined. The threshold intensity of an electric stimulus varies with its duration of application. The relations between these two variables—*i.e.*, the strength-duration curve—are quite typical for a given excitable structure, when studied in standard experimental conditions, and may differ considerably from one structure to another. If the electrical excitability of the nerves is different from that of the muscle in a given neuro-effector system, direct stimulation of the innervated muscle may yield strength-duration curves denoting the two different excitabilities; instead of a smooth single curve such as is obtained from a nerve axon, a break may be present, decomposing the curve into two segments.

A difference in the electrical excitability of nerve and muscle can also become manifest when the distribution of intensity or duration thresholds is studied, as explained in pp. 128–130. Here, again, the difference will be indicated by a break in the corresponding strength-response or duration-response curves. This method was applied by Simeone and Rosenblueth (1934) to the innervated nictitating membrane, immediately after severance of the cervical sympathetic. All the curves obtained, strength-response, duration-response and strength-duration, showed breaks denoting

two distinct excitabilities. Guttman and Wilber (1936) have also recently reported double strength-duration curves in the response of the musculature of the frog's stomach.

The conclusions which may be derived from the results obtained by this method are limited. If there is a break in the curves the existence of two distinct electrical excitabilities in the system is highly probable. The method does not furnish, however, any indication as to what are the distinct excitable elements. The curves from smooth muscle might be due to a difference in the excitability of the autonomic axons and that of some differentiated structure at the nerve endings, possibly the structure concerned with the liberation of the chemical mediator; if so, the muscle might or might not be electrically excitable.

A second method in the study of electrical excitability of smooth muscle was used by Rosenblueth and Cannon (1934). Any muscle, when responding directly to an electrical stimulus, might be expected to contract. Direct stimulation of muscles which respond to nerve impulses by relaxation might cause relaxation if the nerves were activated, but contraction if the stimuli were such as to act directly on the muscle cells. The non-pregnant uterus of the cat, which has exclusively an inhibitory sympathetic nerve supply, was tested. The stimuli yielded invariably relaxation, more marked with stronger or more frequent shocks. Short induction shocks and also long direct-current waves (up to 1 sec.), which might be expected to affect the muscle fibers directly, were tried at various intensities, but no contractions appeared. Even stimuli sufficient to produce visible injury of the tissues near the electrodes failed to evoke contraction. Such results suggest that the muscle is electrically inexcitable.

The third method consists in blocking or eliminating the nerves to the effector, so that the electrical stimuli will necessarily act directly on the responsive cells. Denervation can be readily performed in muscles devoid of nerve cells (for instance, the nictitating membrane, uterus or the pilo-

motors), by excising the proper sympathetic ganglia and allowing time for degeneration of the postganglionic axons.

Direct stimulation of such denervated muscles may lead to contractions. These contractions, however, differ sharply from those of normally innervated muscles when responding to nerve impulses or adrenine and also from those of the denervated muscles responding to adrenine. Unlike typical

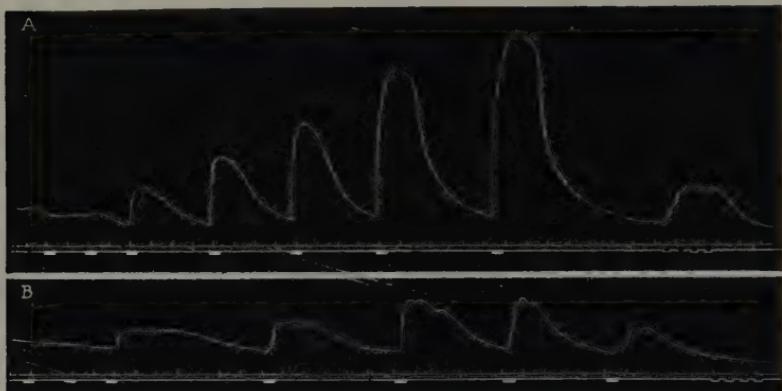


FIG. 33. Direct electrical stimulation of the nictitating membrane. Cat, dial anesthesia, cocaine, curare, adrenals ligated. Time: 30 seconds. A, stimulation of the innervated membrane with induction shocks. The first 7 signals mark a tetanizing frequency of stimulation; the coil distances were 9, 8, 7, 6, 5, 4 and 2 cm. The last record registers responses to single shocks (coil distance 0), as shown by the signal. B, stimulation of the denervated nictitating membrane with induction shocks. The first 7 signals mark a tetanizing frequency; the coil distances were 7, 6, 5, 4, 2, 4 and 5 cm. The last record registers the absence of effects of single shocks (coil distance 0), applied as shown by the signal. (Rosenblueth and Cannon, 1934.)

physiological reactions, changes in the intensity and frequency of the stimuli applied to the denervated effectors do not lead to corresponding spatial and temporal variations (Figure 33). A further difference lies in the inability of single shocks to elicit twitches, no matter how long or intense the current. Finally, the maximal contractions which electric stimuli evoke from the denervated nictitating membrane are much smaller than those which adrenine may evoke (Figure 34).

From these results Rosenblueth and Cannon (1934) came to the conclusion that denervated smooth muscles are electrically inexcitable. The shortenings which are produced by tetanic stimulation might be the direct effect of the current on the contractile system, *i.e.*, a passive effect, not a physiological response. Monnier and Bacq (1935) have confirmed the electrical inexcitability of the denervated nictitating membrane. They reported, further, that injections of 933F (see p. 157) rendered the membrane sensitive to the electrical stimuli; Rosenblueth, Davis and Rempel (1936) were unable, however, to confirm this observation.

Rosenblueth and Cannon (1934) studied also the electrical excitability of the cat's adrenal medulla, denervated by section of the splanchnic nerves and excision of the upper lumbar sympathetic chain. The nictitating membrane, sensitized by cocaine, was used as an indicator of secreted adrenalin. Wire electrodes were placed tangentially on the surface of the gland, at a distance of about 6 mm. on either side of the lumbo-adrenal vein, thus insuring a relatively large contact.

The innervated adrenal gland secretes readily when stimulated by means of weak induction shocks at tetanizing frequency. The same type of stimulation, even with much stronger shocks, was ineffective on the denervated gland. Direct current stimuli were likewise ineffective; even though the length, intensity and frequency of the pulses were increased till visible damage of the gland occurred. The de-

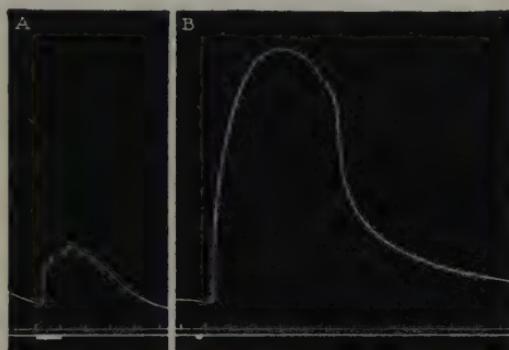


FIG. 34. A, highest contraction elicited by direct electrical stimulation of a denervated nictitating membrane. B, contraction of the same membrane caused by adrenalin (1 cc., 1:200,000) injected intravenously at the end of the experiment. (Rosenblueth and Cannon, 1934.)

nervated gland, however, was capable of secretion, for injections of acetylcholine elicited responses of the nictitating membrane which were due to secreted adrenine.

The electrical inexcitability of the denervated adrenal medulla has been confirmed by Sgrosso (1935). On the other hand, Hermann, Jourdan, Morin and Vial (1936), working on dogs, have recently reported that adrenine is secreted when the stimulating electrodes are introduced into the denervated gland, bringing them in direct contact with the secreting cells. They attribute the negative results of previous observers to the shunting action of the cortical tissue when electrodes are placed on the gland surface. Cannon and Rosenblueth (unpublished observations) have repeated their previous experiments and extended them to dogs. In the cat, although the cocainized nictitating membrane does not contract obviously on stimulation of the denervated adrenal medulla, the more sensitive chronically denervated membrane is capable of detecting the small amounts of adrenine secreted. In both cats and dogs some adrenine may be obtained, but very strong stimuli are necessary and the secretion is extremely small as compared with that produced from the innervated glands. Introducing the stimulating electrodes into the medulla usually elicits a persistent discharge of adrenine as a consequence of the injury; when this secretion is not very marked the results of electrical stimulation do not differ significantly from those obtained when the electrodes are applied to the surface of the gland.

As a procedure alternative to denervation, a drug may be administered which can block the transmission of the nerve impulses when an effector is stimulated directly. The advantage of this method is the elimination of the possible objection that when after severance the nerves degenerate, changes occur at the effector, among which might be included the disappearance of an electrical excitability, present in the normally innervated tissues.

Ergotoxine paralyzes the action of the cervical and lumbar

sympathetics on the nictitating membrane and pilomotors, respectively (see p. 155). The membrane goes into a marked and sustained contracture when the drug is injected, so that any possible effects of direct electrical stimulation of the muscle are masked. Observations on the pilomotors, however, are not impossible, for the hairs are not erected by ergotoxine. Such observations (unpublished experiments) show that the pilomotors are electrically inexcitable after ergotoxine, much as after denervation.

In summarizing the evidence obtained by the three methods it may be stated that there is no direct proof of the electrical excitability of the smooth muscles tested, and that the results may be reasonably explained without postulating that excitability. It appears safe, therefore, to conclude that these smooth muscles are electrically inexcitable. Pierce (1933) has found that the amnion of the chick, physiologically devoid of nerves, responds to repetitive electrical stimulation, applied at a point, by several waves of contraction which spread throughout the membrane. Local mechanical stimulation yielded similar responses. Possibly the electric pulses may have originated a local contracture which could then act as a mechanical stimulus.

In relation to the remarks which introduce this section, it is probable that conduction similar to that in nerve or striated muscle is absent in smooth muscle and that the quick components of the smooth-muscle electrograms are therefore not analogous to the spike potentials of nerve and striated muscle. These conclusions are further discussed on pp. 121, 122.

*The Theory of Double Excitation; Chemical and Electrical.* This theory was developed by Monnier and Bacq (1935) to account for the effects of the dioxane derivative, 933F, on the responses of the nictitating membrane to sympathetic stimulation and to adrenine (Bacq and Fredericq, 1935a; see p. 157).

Monnier and Bacq argued that since 933F impairs considerably the responses of the membrane to injected adren-

ine, it should also prevent the action of the locally produced mediator, assumed to be identical with adrenaline, when the nerves are stimulated. Yet the responses to nerve impulses are only slightly decreased by the drug. They concluded, therefore, that the nerve impulses possess another mode of exciting the muscle than by liberating the mediator, and suggested that this other mode is electrical, the nerve action-potential behaving as an electric stimulus. As arguments in support of the existence of an electrical transmission at the neuromuscular junction, still other features in the responses of the nictitating membrane after 933F were emphasized. Since these features uphold the theory only in an indirect way, they will not be regarded here, in order to avoid a too elaborate discussion.

Monnier and Bacq did not state explicitly how many and what steps were assumed as intermediate between the nerve action-potential and the final response. Obviously, however, they accept the muscle action-potentials (*I* and *II*, pp. 111-113) as being among these intermediate steps in the process of excitation, for in the development of their theory they postulate that electric transmission can occur only in effectors where *I* and *II* are present in response to single nerve volleys. The sequence should then probably be the following: nerve action-potential → *I* and *II* → contraction; with no chemical steps between these events.

Electric transmission seems improbable in view of the following facts. There is a long time-interval between the arrival of the nerve impulses at the effector and the appearance of *I* (p. 111). This long neuromuscular delay is unaccounted for if the nerve action-potential activates *I* directly. Furthermore, if *I* stimulates contraction directly, parallel variations in the magnitudes of the two phenomena should be expected; there is abundant experimental evidence, however, that *I* and contraction not only may vary independently, but even in opposite directions (see pp. 119, 120). If an electric step were involved in the transmission, an electrical excitability of smooth muscle should be readily

demonstrable, yet the data (p. 141) point to an absence of electrical excitability.

Electrical transmission, however, is not the only hypothesis which can account for the paralysis of the responses of the nictitating membrane to adrenine by 933F, and the relative immunity of the contractions elicited by nerve impulses. An alternative explanation is suggested elsewhere (p. 157). Injected adrenine reaches the muscular cells from the *outside*, while the chemical mediator is in all probability liberated *inside* the cells. If 933F should render these cells relatively impermeable to adrenine, the injected hormone could not reach the contractile system, while that liberated locally would still be effective.

Several tests of this hypothesis are available, and they support the suggested explanation. Thus, according to the membrane theory, the impermeability of a cell determines its polarization; and the latter may be judged by the magnitude of the action-potential, since this is assumed to be due to a transient depolarization. Injections of 933F should then increase  $I$ . More generally, if the hypothesis is correct, any drug or condition which increases the permeability of smooth muscle, as shown by increased responses to adrenine, should decrease the magnitude of  $I$ , and, conversely, drugs or conditions which render smooth muscle less permeable should decrease the responses to adrenine and also increase the action-potentials. The relevant experimental evidence is summarized in Table 5, together with the inferred changes in polarization and permeability. There is a satisfactory accord between the data and the inferences. Thus yohimbine and 933F, which are assumed to decrease the permeability of the muscle cells, reduce only slightly the mechanical response to nerve stimulation but greatly lessen or abolish the response to injected adrenine; the action-potentials are increased. Cocaine, adrenine and preganglionic denervation, which are assumed to increase the permeability, have opposite effects on both the mechanical and the electrical responses.

TABLE 5

	MECHANICAL RESPONSES		MUSCLE ACTION-POTENTIALS (I AND II)	POLARIZATION	PERMEABILITY
	Nerve Stimulation	Adrenine			
Yohimbine	-	--	+	+	-
933F	-	--	+	+	-
Cocaine	+	+	-	-	+
Adrenine	+	+	-	-	+
Preganglionic denervation	+	+	-	-	+

Further evidence for the interpretation here adopted is the following. If changes in the permeability of the muscles are postulated, such changes may not be specific for adrenine. The effects of other substances capable of inducing contraction may, therefore, increase or decrease as do those of adrenine. This expectation is confirmed experimentally. Thus, cocaine and denervation increase the responses of the nictitating membrane not only to adrenine, but also to acetylcholine, histamine and to potassium or calcium chloride (274). Similarly, 933F decreases the responses not only to adrenine, but also to acetylcholine, and to potassium or calcium chloride (30).

Finally, it may be mentioned that the specific paralysis of the responses to adrenine by 933F is not limited to single-volley systems such as the nictitating membrane, as the dual theory demands. Ross (1936) has recently obtained evidence that in the pregnant cat's uterus, a multiple-volley system in which no action-potentials I occur (p. 111), the drug blocks the contractions to adrenine more readily than those to stimulation of the hypogastric nerves, much as in the nictitating membrane.

*The All-or-None Principle.* A response follows the all-or-none principle when its magnitude is not influenced by the intensity of the stimulus which elicits it. Thus, as shown by Bowditch in 1871, when electrical stimuli of varying intensity are applied to cardiac muscle, a critical threshold-intensity is found. If the shocks are weaker than this thresh-

old no contraction occurs, but strengthening the stimuli above the threshold does not yield correspondingly greater responses. Similarly, nerve impulses (cf. Davis, 1926) and twitches of single striated muscle fibers attended by a propagated disturbance (263) do not vary in magnitude with the intensity of the electrical stimuli which activate them.

All-or-none reactions are also designated as quantal reactions; the two terms will be used here interchangeably. Responses in which the magnitude varies with the intensity of the stimuli are designated "graded responses."

In many physiological observations the effects recorded are not immediate direct consequences of the stimulus applied, but result only after several causally linked interposed events have developed. Thus, when an electric shock elicits a twitch from a skeletal muscle fiber, at least one intermediate step is known—the conducted action-potential which precedes the contraction. Other more complex instances involving several links could be readily cited.

Adrian (1914) has pointed out that in such chains of linked events which lead to a given response, if any step is all-or-none in character the relation between the stimulus and the response will likewise be all-or-none in character, although some of the other steps may be graded in nature. In examining the applicability of the all-or-none principle to smooth muscle one is confronted, therefore, with two questions: is there any evidence of quantal behavior in these effectors; and if so, to which of the several events involved in the excitatory process is this all-or-none character to be attributed?

In order to answer these questions it is necessary to analyze each step of the excitatory process. From commonly accepted evidence, some of which has been presented in foregoing pages, we may assume the following probable sequence of events:

- 1) electric shock → 2) local excitatory state in the nerve → 3) nerve impulses → 4) liberation of the chemical mediator → 5) combination of the mediator with the recep-

tive substance → 6) mechanical reaction (contraction or relaxation).

This is admittedly a simplified statement of the excitatory sequence. If the electrical phenomena which occur in the muscle are included, a much more complex schema ensues (see p. 111). It appears preferable, however, to discuss first the simplified outline and to analyze later the bearing of the electromyograms on the problem at hand.

The first three steps occur in nerves. The muscular events of the excitatory process begin only at link 5 in the sequence. The nerve impulse, as mentioned before, is known to be a quantal reaction. According to Adrian's dictum, therefore, when smooth muscles are activated through their nerves an all-or-none behavior should be expected.

To verify experimentally this prediction it is necessary to study a group of muscle cells supplied by a single nerve fiber. For if a nerve trunk, containing several axons, is stimulated, spatial summation (p. 128) will determine an apparent gradation of the responses when the intensity of the stimuli is varied. A suitable indicator is found in the pilomotors of the cat's tail. If the lumbar sympathetic chains are stimulated by single shocks, with gradually increasing intensity, and the erections of an isolated hair are observed or recorded, a sharp threshold is found and further intensification of the stimulus does not lead to an increased mechanical excursion (276).

In order to test the applicability of the all-or-none principle to smooth muscle, it is indispensable, therefore, to eliminate the participation of the nerves in the observations. The results which have been obtained in muscles deprived of their nerves have already been reported and discussed (pp. 137, 138); it was concluded that denervated smooth muscle is electrically inexcitable. Consequently from these observations it cannot be concluded that a quantal step exists in the effectors. With reference to the schema of the excitatory events given above, it is apparent that observations on the electrical excitability of smooth muscle do

not fit the physiological steps assumed, for there is no electrical step postulated beyond the nerve impulse.

According to this schema the applicability of the all-or-none principle to autonomic effectors must depend entirely on the mode of action of adrenine and acetylcholine. On pp. 168–172 reasons are given for concluding that the action of these substances is graded, not quantal. The inference seems justified, therefore, that smooth muscle does not follow the all-or-none principle.

Even when the nerves are stimulated, thus including the all-or-none step 3 in the observations, it is possible to demonstrate graded peripheral effects. The experiments of Rosenblueth and Rioch (1933c), described in detail on p. 133, are quite incompatible with a quantal interpretation. These data would appear to violate Adrian's dictum that an all-or-none relationship between a stimulus and a response must exist even if only one of the steps involved is quantal. The violation is not real, however, for the results in question do not depend on the intensity of the stimuli applied, but on their frequency. The all-or-none law refers exclusively to the magnitude of the individual responses to each shock; it is relevant, therefore, only in connection with problems of spatial summation, and irrelevant in experiments governed by the laws of temporal variation.

As regards the bearing on the present discussion of the electrical phenomena which attend the responses of smooth muscle, reference should be made to pp. 119–123. Therein arguments are adduced which suggest the inference that the electromyograms are phenomena which are only incidental to the process of neuromuscular transmission, and not indispensable links in the chain of excitatory events. The electrical inexcitability of smooth muscle corroborates this view, for if an electrical step were fundamentally involved in the activation of the effector we should expect electrical stimuli to elicit responses.

The conclusions stated in this chapter involve fundamental differences between striated and smooth muscles.

Skeletal fibers possess a propagated disturbance which insures rapid conduction, they are electrically excitable and follow the all-or-none principle. Smooth muscles appear to be devoid of these characteristics.

A uniform working hypothesis may be established if, in development of a suggestion made by Elliott in 1907, it is assumed that the conducted disturbance of skeletal muscle is the physiological equivalent of the postganglionic nerve impulses in the autonomic neuromuscular systems. A close correspondence of physiological properties can then be found step by step in the two systems, somatic and autonomic (279).

## CHAPTER XII

### THE PHARMACOLOGY OF AUTONOMIC NEURO-EFFECTOR SYSTEMS

Pharmacological evidence has played an important rôle in the development of knowledge of chemical mediation in autonomic systems. Pharmacological methods have been the basis of far-reaching physiological observations and conclusions. On the other hand, physiological knowledge furnishes a solid basis on which pharmacological systematizations and theories should rest. In the following discussion such data will be presented as appear to be specially important from both the physiological and the pharmacological points of view.

For the purposes of analysis, it will be useful to schematize the process of neuromuscular transmission into linked steps; the action of different substances may then be localized at certain of these steps. The following schema will be used: 1) preganglionic nerve impulse → 2) postganglionic nerve impulse → 3) chemical mediator → 4) receptive substance → 5) specific response. This schema, while omitting details of the process of transmission, leads to a relatively simple concept of the pharmacodynamic actions in question.

*Substances Which Mimic the Effects of Autonomic Nerve Impulses.* It is well known that injections of a number of substances reproduce some of the reactions obtained by stimulating different autonomic nerves. It was early recognized that some of these substances are quite undiscriminating in their effects with respect to the innervation of the responsive structures. Thus, barium salts or histamine elicit contractions of most smooth muscles, independently of whether the excitatory innervation is sympathetic or parasympathetic.

Other substances appear to exert a lesser or greater degree

of selectivity in their action, tending to reproduce only sympathetic or parasympathetic responses. These selective actions have led to the classification of the autonomomimetic substances into two groups: sympathomimetic and parasympathomimetic. The fidelity with which the effects of either division of the autonomic system are reproduced varies for different substances. Several qualitative and quantitative degrees of mimetism are thus recognizable.

Responses of heterogeneous effectors supplied by either of the divisions of the autonomic nervous system can be elicited by a single substance; for instance, ephedrine causes contractions of the nictitating membrane, dilation of the bronchioles and acceleration of the heart. With effectors differing thus widely in nature and having apparently in common only a uniform sympathetic innervation, it has been assumed that such a substance acts by stimulating the corresponding nerves, the site of action being usually localized at the nerve endings.

In the detailed study of any one of the autonomomimetic substances invariably some discrepancies, qualitative or quantitative, are found between its actions and those of the corresponding division of the autonomic nerves. Two substances stand out, however, as the most nearly perfect examples of sympathomimetism and parasympathomimetism—adrenine and acetylcholine, respectively.

The newer knowledge of the chemical mediation of autonomic nerve impulses clears up observed discrepancies and leads to revised views as to the site of action of the mimetic substances. The schema on p. 149 suggests that drugs may elicit responses by stimulating the excitatory chain at any of the steps and that a distinction between them should be made from this standpoint. Thus, as shown by Dale in 1914(a), acetylcholine may activate the chain at step 2, the synapse in the ganglia; in this respect acetylcholine behaves like nicotine. This action, which leads to rise in blood pressure by stimulation of constrictor nerves, is usually masked by the peripheral vasodilating action exerted prob-

ably at step 4. In the latter effect acetylcholine resembles muscarine. To demonstrate the nicotine-like action it is necessary to eliminate the muscarine-like effects by administration of atropine.

Other substances are known which activate the autonomic peripheral neurones; besides acetylcholine and nicotine the quaternary ammonium bases, studied by Burn and Dale (1915) and by Hunt (1926), as well as potassium salts studied by Brown and Feldberg (1936a), might be mentioned. These substances may stimulate both sympathetic and parasympathetic neurones; their action on the effectors with a dual, antagonistic nerve supply, will depend on the doses employed and on the experimental conditions.

Adrenine and acetylcholine act at step 4 in producing their usual sympathomimetic and parasympathomimetic effects. All the qualitative discrepancies which have been encountered when comparing their effects with those of stimulating sympathetic and parasympathetic nerves, respectively, disappear if one keeps in mind the concept (see p. 45) that some sympathetic nerves liberate acetylcholine peripherally while some parasympathetic fibers may have adrenine for their chemical mediator (see p. 46). The quantitative discrepancies may well be due to the variable ease with which these substances, injected into the blood stream, reach the various reacting structures observed. Thus, only very large doses of adrenine produce as a rule erection of the hairs, while stimulation of the corresponding sympathetic nerves readily yields marked erections. If cocaine is injected, however, which probably favors the diffusion of adrenine from the blood into the tissues (p. 143), adrenine is rendered more efficient in causing contractions of the pilomotor muscles.

Adrenine and acetylcholine do not mimic the action of autonomic nerves by stimulating the corresponding nerve endings, as is still quite generally stated. The nerve impulses lead to the liberation of these substances and whether the chemical mediators be conveyed by the circulating blood

or produced locally in response to the nerve impulses, their action is exerted on the effector cells.

Within the cells of the effector the schema postulates two systems, the receptive substance 4 and the reacting structure 5. Adrenine and acetylcholine are assumed to act by combining first with 4, not directly on 5. This assumption seems to be the only plausible explanation for the fact that both mediators produce contraction in some muscles and relaxation in others (see pp. 98, 106).

The schema suggests, *a priori*, that other chemical agents may affect directly the contractile system 5 and might uniformly produce, therefore, either contraction or relaxation. Such is probably the site of action of barium salts and histamine, which, as mentioned before, usually cause contractions of smooth muscles, independently of whether they possess single, double or no innervation, and independently of the nature of the innervation, excitatory or inhibitory.

With the data available a further attempt at systematization of the autonomomimetic drugs would be hazardous. It would likewise be indulging in pure speculation to attempt to localize the action of other substances in this group; chemically, the group is quite heterogeneous.

In summarizing the foregoing discussion, the following statements appear justified. Some autonomomimetic substances act by stimulating postganglionic nerve impulses, *e.g.*, nicotine; these substances may mimic sympathetic and parasympathetic effects indiscriminately. Other autonomomimetic substances act probably by stimulating the effector system directly, *e.g.*, barium salts; this group, likewise, shows no systematization corresponding to sympathetic or parasympathetic innervation. Adrenine and acetylcholine act precisely as do the corresponding sympathetic or parasympathetic nerve impulses, because the nerve impulses exert their effects through liberation of the same substances. It is possible that other drugs, *e.g.*, ephedrine or some of the amines studied by Barger and Dale (1910), may act similarly

to adrenaline and acetylcholine, by previous combination with a differentiating receptive substance which conditions the sign of the response. Such drugs may be expected to show a lesser or greater degree of sympathomimetism or parasympathomimetism. Some substances may act at several of the steps indicated, *e.g.*, acetylcholine and potassium ions. These two substances, specifically, and others as well, may further complicate their effects by eliciting a secretion of adrenaline from the adrenal medulla when injected intravenously (30, 149). These multiple effects should be borne in mind in analyzing recorded responses.

*Substances Which Block Autonomic Responses.* This group of substances, like the previous group, may be subdivided into classes according to the different steps in the process of neuromuscular transmission at which the block occurs. The schema suggested on p. 149 is not sufficient, however, for the analysis of all the blocking agents. It has been commonly assumed that adrenaline and acetylcholine are identical with the chemical mediators liberated by the nerve impulses at the effectors (pp. 32, 86, 98). According to the schema it would be expected, therefore, that any drug which blocks the responses to adrenaline or acetylcholine acts beyond step 3; hence such a drug would also be expected to prevent equally the reactions to the corresponding nerve impulses. The experimental data, however, show that some drugs are more efficient in blocking responses to injected adrenaline or acetylcholine than responses to autonomic nerve impulses. To account for this selective paralysis other features in the organization of the systems than those covered by the schema will have to be considered.

Some substances block the sequence of transmission between steps 1 and 2. To this class belongs curare, as shown by Langley and Anderson (1895). Nicotine, acetylcholine and potassium, whose ability to excite at this point in the chain has been mentioned (p. 151), are also blocking agents if the dose is sufficiently large (215, 68, 69). When a block is obtained by any of these drugs the preganglionic nerve

impulses do not lead to responses from the effectors, while postganglionic stimulation or injections of adrenaline or acetylcholine can still produce typical effects (181).

The first two steps in the schema are not specific. Nerve impulses may differ quantitatively as regards electrical threshold, rate of conduction and amplitude of the spike-potential, but, as far as is known, they are all qualitatively identical, subserving indiscriminate conduction. The specificity of the responses in different systems begins at step 3, with the possibility of two different chemical mediators. It should therefore be expected that specific blocking agents should exert their action beyond step 3, and such is indeed the fact.

Atropine is the best known drug which paralyzes parasympathetic responses. All the muscarine-like actions of acetylcholine are readily blocked by atropine. The responses to cholinergic nerves, mainly parasympathetic, on the other hand, vary considerably in their resistance to the paralyzing influence of the drug. Thus, while cardiac slowing, pupillary constriction and salivary secretion, obtained by stimulating the corresponding parasympathetic nerves, are easily blocked by small doses of atropine, gastro-intestinal motility and contractions of the fundus of the bladder persist after large doses.

Even when atropine produces a complete block of a given response to stimulation of parasympathetic nerves it has been demonstrated that the chemical mediator is still liberated by the nerve impulses. Loewi and Navratil (1924) showed this liberation after atropine paralysis in the frog's heart, and Henderson and Roepke (1933b) confirmed the phenomenon in the salivary glands. It may therefore be concluded that the block of the responses to nerve impulses, when present, occurs beyond step 3 in the schema.

A reasonable explanation of the cases in which atropine blocks the responses to acetylcholine while leaving those to parasympathetic stimulation relatively unimpaired was suggested by Dale and Gaddum in 1930. They argued

that when the nerves are stimulated the chemical mediator may be liberated in intimate contact with the reacting structure and that atropine might not be able to oppose this reaction, although it could prevent the effects of injected acetylcholine, carried to the tissues by the blood stream. This view is quite plausible when it is remembered that autonomic nerves are found ending intracellularly (cf. p. 14). It may then be concluded with Gaddum (1936) that atropine merely acts by preventing the mediator from reaching its site of action, when the mediator is not liberated intracellularly.

Many blocking substances acting on sympathetic effectors have been studied recently, but only two will be considered in detail here—ergotoxine and piperidinomethylbenzodioxane (933F). In contrast to atropine, which prevents both the excitatory and the inhibitory effects of acetylcholine and of some parasympathetic nerves, the substances of the present group show as a rule a greater degree of specificity; they not only limit their action to the adrenergic domain but usually block selectively excitatory or inhibitory responses only. Both ergotoxine and 933F belong to the class of drugs which block only excitatory responses; their effects, however, differ markedly.

Ergotoxine was shown by Dale (1906) to paralyze the excitatory responses of smooth muscle both to adrenine and to stimulation of sympathetic nerves. When the blood pressure is used as an indicator the progressive stages of the paralysis with increasing doses of the drug are interestingly demonstrated. A given dose of adrenine yields, before ergotoxine, a pure rise of blood pressure. If a small amount of ergotoxine is injected, adrenine elicits a sudden rise, then a fall and finally a slow rise of blood pressure. When more ergotoxine is administered both the initial and the delayed rises to adrenine decrease while the fall becomes more marked. Finally a stage may be reached when practically only falls of blood pressure are recorded (see Figure 19, p. 89).

As in the case of atropine, even when a response to sympathetic nerve stimulation has been completely paralyzed by ergotoxine, liberation of the chemical mediator may still be demonstrated (247, 80); indeed, the effects of sympathin on the heart rate may be increased after ergotoxine (Figure 14, p. 65). It is to be concluded, therefore, that the paralytic action is exerted beyond step 3 of the schema. Evidence has been presented which indicates that the substance which diffuses into the blood stream from the stimulated paralyzed source is not adrenine, but sympathin, the compound of the mediator, supposedly adrenine, with the corresponding receptive substance (pp. 98, 99). The blocking would occur, then, at the end of the excitatory sequence, between steps 4 and 5.

Dale (1906) showed that the paralysis of the responses to adrenine by ergotoxine runs a course practically parallel to that of the reactions to nerve stimulation; only minor quantitative differences are observable. In this respect ergotoxine differs from atropine in its mode of action, for atropine may block the effects of acetylcholine without preventing the responses to nerve impulses (p. 154). It might be objected that if the explanation suggested previously for the effects of atropine were accepted, the different action of ergotoxine might be due to a difference in the mode of ending of sympathetic and parasympathetic nerves in the effectors. It will be shown, however, that 933F, a sympathetic blocking substance, has effects closely resembling those of atropine.

The action of ergotoxine on the contractile system of the paralyzed cells varies. While usually a marked persistent contracture is induced by the drug (100, 19), a block may occur when no contracture is apparent, as in the pilomotors. Furthermore, Dale (1906) demonstrated that even in structures contracted by ergotoxine, *e.g.*, the blood vessels, injections of barium salts or pituitrin elicit further contraction. These phenomena can be reasonably interpreted by assuming that the paralytic effects are due to a barrier interposed

by ergotoxine between steps 4 and 5, the excitatory compound and the contractile system, as suggested above, whereas the contractures, when present, denote an independent direct effect of the drug on the contractile system 5. No explanation is available for the fact that only some smooth muscles contract on injections of ergotoxine, while others remain relaxed.

Piperidinomethylbenzodioxane (933F) is a synthetic drug recently prepared by Fourneau. Its effects have been investigated by several workers in different laboratories. After injections of this drug the most striking result is a decline of the excitatory responses of the sympathetic effectors. The reactions to nerve stimulation are relatively little decreased as compared with the great reduction of the responses to adrenaline (117, 25, 285). In this regard 933F resembles atropine as it influences some autonomic effectors, and differs from ergotoxine.

The physiological demonstration of sympathin on stimulation of sympathetic nerves is prevented by 933F (26). Sympathin is probably liberated, however, for the local response at the source may be only slightly impaired by the drug. It appears reasonable to infer that the action of 933F, like that of atropine, consists in preventing the active substances in the blood stream from reaching the reactive structures—*i.e.*, in rendering some smooth-muscle cells impermeable to these substances (cf. pp. 141, 155).

Other drugs are known which exert a similar influence on sympathetic effectors, *e.g.*, yohimbine (23, 284), but their study is not as complete as that of 933F. It is interesting to note that 933F may block the excitatory sequence not only at the effectors, but also between steps 1 and 2, at sympathetic ganglia, as shown by Bacq (1936b). Other effects of 933F are mentioned on pp. 141–144; it is unnecessary to discuss them here. For present purposes, suffice it to say that 933F is typical of the agents which block the excitatory effect of adrenaline. It has perhaps the most specific paralytic action known, being selective not only in

the domain of the autonomic system which it affects, but also in the stimulus and the sign of the response which it prevents.

As summarizing this analysis of the autonomic blocking agents, the following statements may be made. Some substances act by preventing transmission at the ganglionic synapse, *e.g.*, curare; this paralysis may affect sympathetic and parasympathetic systems indiscriminately. Another autonomic blocking substance prevents the *action* of the mediators on the responsive systems, *i.e.*, ergotoxine; this drug affects equally well the responses to sympathetic impulses and those to adrenine. Other paralyzing agents, finally, seem to act by preventing *access* of the stimulating mediators to the responsive systems, *e.g.*, atropine; these agents may block the effects of injected adrenine and acetylcholine while not hampering the responses to the chemical mediators liberated intracellularly when the nerves are stimulated. A given substance may possess more than one of these blocking modes, *e.g.*, 933F.

*Substances Which Increase Autonomic Responses.* These augmenting or adjuvant substances differ from those which mimic autonomic effects in that they do not themselves elicit such effects. Their action is observed when, on stimulating autonomic nerves or injecting adrenine or acetylcholine, greater responses are obtained than normally—*i.e.*, greater than without the adjuvant drug. The substances in this group can be divided into several classes, according to the different events which they influence in the process of neuromuscular transmission.

There are three ways in which an adjuvant action occurring at sympathetic ganglia may be demonstrated. (1) Normally the postganglionic fibers deliver only one volley of nerve impulses when a single shock is applied to the pre-ganglionic fibers, *i.e.*, in physiological conditions the ganglion does not demonstrate after-discharge (46, 126, 64, 63). If a drug were injected which gave rise to repetitive discharges of the postganglionic nerves for every preganglionic

volley, obviously the responses to nerve stimulation would be increased. (2) Eccles (1935b) has reported evidence that when submaximal single stimuli are applied which activate only some of the preganglionic fibers distributing to a sympathetic ganglion, some of the neurones in the ganglion fire and others are subliminally excited. If a drug should permit this subliminal excitation to reach threshold values the responses of the effector would again be correspondingly augmented. (3) Acetylcholine may stimulate autonomic ganglia (143). A drug which potentiates this action of acetylcholine will also increase the corresponding responses.

Eserine may increase autonomic responses by exerting at the ganglia—*i.e.*, between steps 1 and 2 of the schema—probably all three influences mentioned (151). Since acetylcholine is the chemical mediator of the preganglionic nerve impulses (see pp. 52–54) these influences may be due to the well-known ability of eserine to protect acetylcholine from destruction by the choline esterase present in the ganglia.

Other adjuvant actions occur at the periphery, beyond step 2. For instance, cocaine augments the responses of sympathetic effectors to stimulation of the postganglionic nerve fibers after destruction of the ganglia, and to injections of adrenine after complete denervation, the ganglionic synapses being thus excluded as a source of the increment (162, 281).

The precise mechanism of the augmenting action of cocaine is unknown. Some possibilities are suggested by facts at hand. When nerves are stimulated larger quanta of mediator might be liberated per nerve impulse; this explanation would not account, however, for the increased responses to adrenine. The reactivity of the contractile mechanism in the muscles for a given dose of mediator might be enhanced by the drug; this view does not seem to account readily for the facts that both excitatory and inhibitory (273) responses of muscles, and cardiac acceleration, and also the secretory activity of the salivary glands are in-

creased by cocaine. The drug might protect the mediator, retarding its destruction in the tissues; this seems a reasonable explanation of the marked prolongation of sympathetic responses after cocaine. Finally, the drug might increase the permeability of the cells, thus favoring the diffusion of adrenine from the blood and the passage of the mediator from the innervated to the non-innervated elements in the effectors. This last suggestion is further developed elsewhere (p. 143).

Eserine increases specifically the responses to cholinergic postganglionic nerve impulses and the muscarine-like effects of acetylcholine, through the protective action mentioned above. It is not known whether eserine influences otherwise the effector cells, *e.g.*, by modifying their permeability.

Increased responses to adrenine, due to protection of the hormone from being destroyed by the oxydizing systems in the tissues, have been recently demonstrated by Bacq (1936a).

For the sake of completeness other autonomic adjuvant substances may be mentioned; they have not been extensively studied and their mode of action is quite obscure. For example, ergotoxine appears capable of augmenting some vagal effects (100); also sodium bicarbonate (321, 322, 99, 182, 139, 237), amino acids (1, 2), calcium salts (336, 306, 198) and thyroxine (302, 303) have been shown to increase the responses to adrenine in certain experimental conditions.

*General Considerations Regarding the Pharmacology of the Autonomic System.* Several interesting features stand out from the analysis made in the previous three sections. For instance, no substance was mentioned as acting on steps 1 and 2—*i.e.*, as either stimulating or modifying the nerve impulses in the axons. In 1906 Sherrington emphasized the point that the central synapses are much more susceptible to drugs than are the peripheral nerve trunks. It may be added that the neuro-effector synapses are also relatively very sensitive to pharmacological influences. Some of the

drugs which have been mentioned are known to modify the properties of axons, *e.g.*, yohimbine (179); but the blocking action of this substance on the responses to adrenaline occurs with doses which do not modify the nerve impulses, as judged by velocity of conduction (284). This quantitative difference of susceptibility between synaptic excitation or transmission, on the one hand, and conduction on the other, points to a difference in the processes involved. The peculiar chemical mode of transmission at synapses may provide a basis for this difference.

The effects produced at ganglionic synapses occur alike in the sympathetic and the parasympathetic systems. It is noteworthy that the pharmacology of the synapses is, so far as known, similar in all respects to that of the junctions of the somatic motor nerves with striated muscles. Both relay points are interposed between two waves of conduction; acetylcholine is the chemical mediator (see pp. 48, 52) and its action at these points is similar to that of nicotine. These facts provide a satisfactory explanation for the non-specific pharmacodynamical influences which are encountered.

Specific influences on colinergic or adrenergic systems, respectively, occur with drugs which exert their action beyond step 3, the postganglionic nerve impulse. The specificity of the chemical mediator in the two systems may suffice to explain such discriminatory effects. Further specificity of action within each system, such as the exclusive blocking of adrenergic excitatory or inhibitory systems, should occur, according to the present schema, beyond step 4; it might be accounted for by the specificity of the two receptive substances, E and I, concerned with the corresponding excitatory and inhibitory responses.

The instances of difference in the effects of drugs between the responses to nerve stimulation and to injected adrenaline or acetylcholine may be explained by the evidence that autonomic nerves frequently end inside the cells of the corresponding effectors. A further knowledge of the peculiarities of innervation of each effector and of its pharmacology

may support or invalidate this view. It is interesting to note, however, that although several drugs are known to block markedly adrenine or acetylcholine while not checking significantly the nerve impulses, the opposite effect has not been encountered—*i.e.*, no drug is known to paralyze the nerves while allowing adrenine and acetylcholine to elicit their usual effects. This is precisely what would be expected with an intracellular termination of nerve fibers.

The specificity of action of some of the drugs mentioned is stressed because that is one of the significant points which any pertinent theory should cover. It is important, however, to keep in mind the fact that this specificity is by no means strict. For instance, atropine antagonizes not only the effects of acetylcholine, but also the contraction of the intestine to barium (297), the contraction of the spleen to adrenine (195), the response of skeletal muscle to veratrine (270, 271) and the secretion of the submaxillary gland on stimulation of the cervical sympathetic (214). Great caution should be exercised, therefore, in drawing physiological inferences based exclusively on pharmacological evidence.

*The Anesthetics.* The preceding sections deal with substances which have a preferential action on autonomic systems. Anesthetics, as an incident in their quite generalized effects, may deeply modify the experiments which deal with autonomic responses. Although no systematic study has been made of these changes, some of the actions which have been observed may be briefly reported.

Ether anesthesia may prevent the possibility of demonstrating the liberation of sympathin into the blood stream on activation of sympathetic nerves (80, 250). Rosenblueth and Cannon (1932), working with the cat's nictitating membrane, found that as ether anesthesia became deep the membrane entered into sustained contraction and its responses to sympathin decreased correspondingly.

Amytal has also been found to interfere with the liberation of sympathin (80). The reason for this action is unknown.

Sympathin is readily demonstrated under dial anesthesia (281). Yet dial is not without influence on sympathetic effectors. Thus, under that anesthetic, much greater doses of ergotoxine are necessary in order to change the blood-pressure responses to adrenine from rises into falls than in unanesthetized spinal animals (89). Similarly, the abolition of the responses to sympathetic vasodilators requires larger doses of atropine in dogs under dial anesthesia than in spinal, unanesthetized dogs (283).

Urethane anesthesia, finally, was found by Engelhart (1931) to interfere with the liberation of acetylcholine from parasympathetic systems. On the other hand, urethane has been used successfully in the studies on the production of sympathin (230).

## CHAPTER XIII

### THE MODE OF ACTION OF THE CHEMICAL MEDIATORS

Several methods are available by means of which attempts may be made to analyze the mode of action of drugs or hormones on tissues. These methods have been applied to adrenine and acetylcholine and, although the evidence is in no case direct, the uniformity of the results obtained permits strongly presumptive conclusions.

*The Minimal Effective Amounts.* The amounts of adrenine and acetylcholine which evoke detectable responses on reaching a smooth-muscle cell furnish arguments which eliminate certain possible explanations of the mode of action of these substances. An illuminating discussion of this method is given by Clark (1933). Thus, acetylcholine may inhibit the frog's heart when as little as 0.02 γ per gm. of moist tissue enters the muscle. Similarly, 0.06 γ of adrenine per gm. of frog's stomach will induce clear inhibition. The number of molecules of the hormones per gm. of tissue is then approximately  $10^{14}$ . On the assumption that the area covered by a molecule adsorbed on the surface of a cell is about  $100 \text{ } \text{\AA}^2$ , the total area which a monomolecular layer of the minimal effective amount of the hormones would cover would be  $10^{16} \text{ sq. } \text{\AA}^2 = 1 \text{ sq. cm.}$  From the figures given by Clark, the total area of the cells in 1 gm. of heart and stomach is about 6,000 and 20,000 sq. cm., respectively. The doses of acetylcholine and adrenine mentioned before can therefore cover only a small fraction of the total cell area. From these figures Clark concludes that it is practically certain that the drugs must exert their action by uniting with certain specific receptors in or on the effector cells, and these receptors must form only an insignificant proportion of the total volume or surface of the cells.

The previous figures permit also a calculation of the number of molecules of adrenine or acetylcholine which are effective per cell. The number of muscle cells per gm. of heart and stomach is estimated as approximately  $10^8$  and  $10^{10}$ , respectively. From the figures for minimal effective amounts per gm. of tissue, therefore, about  $10^4$  to  $10^6$  molecules of acetylcholine and adrenine may elicit definite responses in the single cells.

The figures obtained from the minimal doses effective in the intact mammal are of the same order of magnitude. Thus, Bacq (1934b) calculates that  $4 \times 10^{15}$  molecules of adrenine injected into a 3-kgm. cat have obvious effects and that the total number of cells in such a cat is approximately  $10^{13}$ . Assuming a uniform distribution of the hormone injected intravenously, each cell would receive about 400 molecules. The last assumption may not be legitimate, but still relatively low figures are obtained if only a limited distribution of the substances injected is assumed. For instance, an intracarotid injection of  $10^{-10}$  gm. of adrenalin in a cat under dial anesthesia may provoke a contraction of the nictitating membrane sensitized by previous denervation and cocaine. If one assumes only 1 gm. of tissues to which this adrenalin will be distributed and an average volume for the cells of 10,000 cu.  $\mu$ , a higher figure than that suggested by Bacq, each reacting cell will receive only 100,000 molecules. The figures assumed being probably too low for the amount of active tissue, and too high for the cell volume, 100,000 is an upper limit for such an observation. Any explanation of the mode of action of adrenine and acetylcholine should be such, therefore, as to cover the foregoing approximate estimations.

*The Comparative Method.* Information regarding the mode of action of drugs may also be obtained from a comparative study of the effects of the drug under consideration and other physically or chemically related substances. Such a study was made for adrenine by Barger and Dale (1910) in their classical work on sympathomimetic amines. Although

a catechol nucleus was not found to be an essential constituent of the molecule of a sympathomimetic substance, and although catechol itself was found inactive, it was concluded that a catechol nucleus is an optimal condition for activity. Increased activity with diminished resistance to oxidation was only broadly true, and required much qualification. Approximation to adrenine in structure was attended by increased sympathomimetic activity. Inhibitory and motor activity varied to some extent independently.

Barger and Dale concluded that their results were compatible with Elliott's (1904) suggestion that the "myoneural junction" receives the nerve stimulus or the chemical stimulus and is responsible for the determination of the response in the direction of augmentation or inhibition. As regards the physical or chemical mode of action of the amines which they studied, they agreed that the least unsatisfactory view is that which refers stimulant activity to the possession of some chemical property, and refers the distribution and, in the main, the intensity of the activity to a physical property.

The information derivable from this method of comparison is obviously limited. In the nictitating membrane, for instance, contractions may be obtained from adrenine and numerous related sympathomimetic amines, from acetylcholine, ergotoxine, histamine, pituitrin, thyroxine, pilocarpine, from potassium, calcium and barium salts, and probably from many other untested substances. An attempt to find a common physical or chemical characteristic in such a heterogeneous group of stimulants would be futile. It is possible that some of the substances listed act by liberating other agents; thus, in the superior cervical ganglion both acetylcholine and potassium ions act as effective stimuli, and potassium liberates acetylcholine in the ganglion (68). Furthermore, the normal process of activation of an effector, either through its nerves or by a hormone, involves a complex sequence of events, and widely different substances might impinge on the process at different steps in the sequence (see pp. 149, 153).

Yet a systematic study of series of chemically related compounds, such as Barger and Dale's, is of significance. One may well stress their conclusion that similarity to adrenine in structure is attended by increased sympathomimetic activity. Necessarily in their study only a few sympathomimetic reactions were recorded; and yet clear differences appeared with all the substances investigated. From this and from other similar reports, it seems justifiable to conclude that the slightest molecular deviation from the unique substances, adrenine and acetylcholine, will induce a recognizable qualitative or quantitative change in the physiological effects obtained.

This conclusion would appear to be more consonant with a chemical than a purely physical process. It is pertinent in this respect to mention the suggestion made by Tiffeneau (1934), that there should be an optically active constituent of the tissues with which adrenine would combine, in order to explain the difference of action between the dextro- and levorotatory isomers.

*The Quantitative Method.* A third method which has been applied to the investigation of the mode of action of adrenine and acetylcholine is that of quantifying the responses elicited by various doses. A formula correlating the two variables is obtained in this manner and the hypotheses adopted for the mode of action should be in accord with this formula. This method is especially applicable to the problem at hand, for the range of responses that may be studied is very wide, practically from 0 to 100 per cent. Experimental curves which cover only a narrow range of effects may be adequately described by several mathematical expressions, but as the range of variation is extended the number of adequate formulae becomes limited (cf. Clark, 1933).

The concentration-action curves of acetylcholine for the inotropic effect on the frog's heart has been extensively studied by Clark (1926, 1927). The curves correlating the responses with the doses are accurately represented by the

formula  $R = \frac{x}{k - k'x}$ , where R is the response; x, the concentration of acetylcholine; and k and k', constants. This formula corresponds to a rectangular hyperbola; its significance will be discussed later. Parabolic and exponential formulae do not describe satisfactorily these curves and the deviations are consistent. The concentration-action curve of acetylcholine on the nictitating membrane is entirely similar to that for the heart (246).

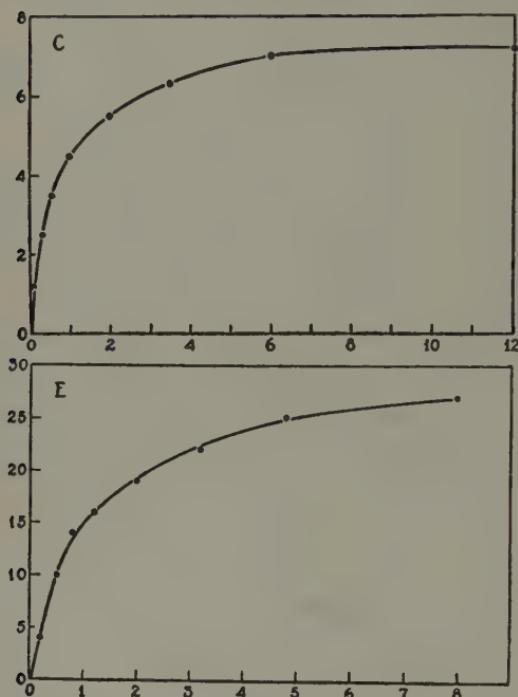


FIG. 35. Concentration-action curves of adrenalin. C, abscissae: doses of adrenalin. Ordinates: isometric contractions of the denervated and cocainized nictitating membrane. E, abscissae: doses of adrenalin. Ordinates: increases of the rate of the denervated heart. (Rosenblueth, 1932b.)

results which are adequately described by the formula for a rectangular hyperbola mentioned above. Rosenblueth (1932b) studied heart rate, isotonic and isometric contractions of the nictitating membrane and isotonic relaxations of the non-pregnant uterus in intact cats, and found in all these widely different instances precisely the same relations between the responses and the doses of adrenalin injected (Figure 35). Here again tests for parabolic and exponential formulae

The concentration-action curves of adrenalin have been repeatedly studied; the results are singularly consistent. Wilkie (1928) investigated the isotonic and isometric responses of arterial strips and obtained

revealed systematic deviations. The data reported by Schackell (1924) with arterial rings and by Gaddum (1926) with rabbits' uteri are also adequately described by rectangular hyperbolas.

The concentration-action curves of adrenine on the blood pressure are not consistent, some being rectangular hyperbolas (275, 341), while others are not (200, 244, 238). It should be remarked, however, that the experimental conditions have not been uniform, for in some cases the circulatory proprioceptors, depressors and carotid-sinus nerves were intact and opposing the effects of the hormone, while in others they were cut. Furthermore, the blood pressure is a complex indicator, since adrenine has both vasoconstrictor and vasodilator influences, and since the variations recorded may not be a linear function of the responses of the contractile elements of the vessels. The results obtained in the simpler systems mentioned above are therefore much more trustworthy.

Claims have been made that the concentration-action curves of adrenine are exponential and that the responses therefore follow the Weber-Fechner law (225, 223, 238, 8). It is interesting that these claims are mainly based on blood-pressure reactions, which, as was explained above, are unreliable. Moreover, when the hyperbola of the type concerned in these experiments is plotted with a logarithmic scale for the abscissae, an elongated S ensues, which is approximately a straight line in the middle range. Incomplete determinations might therefore suggest a logarithmic relation, but such a relation fails if the whole range of the curves is explored.

It may be concluded that the responses of smooth muscle (contractions and relaxations), of cardiac muscle and of the heart rate bear a hyperbolic relation to the concentrations of acetylcholine and adrenine that evoke them. Such a relation eliminates certain explanations of the mode of action of these substances, while leaving others open.

An all-or-none mode of action has been claimed (311, 167),

the concentration-action curves then representing variability in the threshold of different elements of the effector. Although it is possible *a priori* to conceive *any* distribution of thresholds among the elements, some distributions are quite unlikely, and hyperbolic curves belong to these unlikely distributions, for the following reasons. The usual integrated curve denoting individual variations in a homogeneous group of biological elements is an S-shaped curve, not a hyperbola. These hyperbolas only become S-shaped when plotted semilogarithmically. The range of variation in such a group is usually relatively small—*i.e.*, the highest threshold is usually less than, say, 100 times greater than the lowest threshold. For adrenaline and acetylcholine, however, graded responses are obtained within ranges which involve doses thousands of times greater than the minimal effective amounts. It is quite unlikely, therefore, that the concentration-action curves of adrenaline and acetylcholine represent distribution curves of individual variations in elements responding in an all-or-none manner. Quite probably, however, individual variations may account for slight deviations from the hyperbolas in certain preparations.

From the nature of the effector an all-or-none mechanism is highly improbable in the case of the heart rate. For if only some elements in the pace-maker were slowed by a small dose of acetylcholine the heart rate would persist unchanged, following the pace of the unslowed elements. Thus, to account for the graded effects recorded, it would have to be assumed that the variability of the rates of the elements covers the whole range of the responses and that the distribution of these rates and of the susceptibility to acetylcholine is identical. A similar reasoning applies to the accelerating action of adrenaline.

Straub's (1903, 1907) studies on the mode of action of veratrine and muscarine on the heart of aplysia led him to conclude that the effects were a function of the differences in concentration inside and outside the cells. This theory

has been generalized under the name "potential theory," a gradient of concentration or potential being postulated as requisite for action. The theory has been applied to the effects of acetylcholine and adrenine, but this application is probably not valid. There is no evidence that the action of these substances disappears when equal concentrations occur inside and outside the reacting cells, nor are there any data which demonstrate that an increase of action occurs when the potential gradient is reversed—*i.e.*, when fresh Ringer's solution is substituted at any time for the one to which the substance was added—as Straub's theory would demand. The potential theory is also invalidated for adrenine by the fact that stimulation of the cervical sympathetic during the rising phase of a response of the nictitating membrane to adrenine causes further contraction (291). Such stimulation, according to evidence already cited, liberates an adrenine-like mediator *inside* some cells and would therefore decrease the gradient of concentration; this decrease should lead to relaxation instead of contraction, if the theory were applicable.

The effector cells rapidly destroy adrenine and acetylcholine, especially the latter. It might be assumed that the concentration-action curves merely denote an increased rate of destruction with the higher concentrations. If such were the case, however, the curves should not have an upper asymptote, *i.e.*, the responses should increase until a limit imposed by other factors in the tissue would be attained, whereupon a break would be observed in the curves. But the curves for adrenine are smooth and asymptotic, even when substances such as cocaine are added to the tissue, which presumably retard the destruction of the hormone, as indicated by the prolonged effects obtained (p. 160).

When adrenine or acetylcholine evokes a given response a series of interposed events probably occurs between the arrival of the agent and the final phenomenon. For instance, when the response is contraction of a muscle, we may suppose that adrenine or acetylcholine induces some change

which in turn brings about a break-down of phosphocreatine, and the chemical energy thus set free suffers further transformations which eventually result in the development of tension by the muscle. The step responsible for the hyperbolic concentration-action curves might be any one in the series mentioned, and the others, including the immediate change induced by adrenine or acetylcholine might be directly proportional to the preceding event in the series. Or else the hyperbolas might be the smoothed-out resultant of several other curves describing the quantitative relations of each of the steps. The fact, however, that the curves of as widely different effectors as the cardiac pace-maker, the salivary glands (see below), the nictitating membrane and the non-pregnant uterus of the cat, are all similar, favors a placing of the common feature responsible for this similarity at the excitatory step and not in the range of the responsive events.

The most probable explanation of the hyperbolic concentration-action curves appears to be that they manifest a chemical reaction or adsorption (97, 191, 275). The mass-action law and the simplest formula put forward by Langmuir for adsorption (cf. Clark, 1933) have precisely the form which the experimental data reproduce consistently. In both cases the process would be reversible, for Langmuir's formula is based on the hypothesis that the adsorbed drug forms an easily reversible combination with a limited number of receptors on the surface of the adsorbent.

*The Mode of Action of the Chemical Mediators of Nerve Impulses.* In concluding the argument as developed thus far, it may be pointed out that all the methods applied to the analysis of the mode of action of adrenine and acetylcholine agree in suggesting the probability that these agents combine with other substances in the effectors, which, with Langley (1921), we may call the *receptors*. The purely physiological argument is even stronger in this connection. Both adrenine and acetylcholine contract or relax different smooth muscles. If it is assumed that the contractile system

is identical in all muscles—and this is the most reasonable assumption—then there should be some differentiating factor in the muscles which determines whether contraction or relaxation shall ensue. This differentiating factor is Langley's receptor. No other satisfactory hypothesis has come forth to explain contraction and "inhibition" produced by the same substance.

The next step in this inquiry is the analysis of the effects of nerve stimulation in the light of the previous conclusions as to the mode of action of adrenine and acetylcholine. If these substances are released by the nerve impulses on reaching the effectors, similar quantitative relations between the concentrations and the responses should be expected. The experimental data confirm these expectations.

Rosenblueth (1932c) studied the responses of several effectors when their autonomic nerves were stimulated maximally at varying frequencies. If the all-or-none character of nerve impulses is assumed to lead to the liberation of a fixed quantum of chemical mediator per impulse, then the concentration of the mediators will be proportional to the frequency of stimulation, provided that the rate of destruction does not grossly distort this simple relation. That the last assumption is probably safe is shown by the consistency of the effects obtained with adrenergic and cholinergic nerves, although adrenine is far more stable than acetylcholine. Some of the systems studied (blood pressure, stomach, duodenum and pregnant uterus) might be regarded as too complex or irregular for accurate interpretation, and, indeed, the results obtained from them would not be significant were it not that they yield curves identical with those found in the simpler and accurately quantifiable systems: the nictitating membrane (isotonic and isometric records), the pilomotors, the heart (slowing and acceleration) and the submaxillary gland.

Repetitive stimulation differs from injections of adrenine and acetylcholine, for in the latter case the substances are

brought continuously to the cells by the blood stream, whereas in the former the mediator is produced discontinuously, with a rhythm imposed by the frequency. This difference is particularly striking with slow frequencies of stimulation (*e.g.*, less than 1 per sec.) and tends to be smoothed out by the higher frequencies at which the successive quanta add to the remainder of the mediator persisting from previous stimuli. For this reason the responses to slow frequencies are not considered suitable for comparison with the concentration-action curves.

In all the systems mentioned, the frequency-action curves (omitting very slow frequencies) were rectangular hyperbolae, having the same formula as the concentration-action curves (Figure 30). Indeed, the frequency-action curve of the nictitating membrane coincides strictly with the concentration-action curve for adrenine when the units of the abscissae are properly selected.

Only one system was found in which the frequency-action curve was not a hyperbola: the system splanchnic-adrenal medulla. Here the output of adrenine from the gland was directly proportional to the frequency of stimulation of the splanchnic nerves—*i.e.*, the frequency-action graph was a straight line. This exception is interesting in two respects. The nerve supply to the gland is preganglionic, not postganglionic, as in the other effectors considered thus far. The chemical mediator of these preganglionic fibers is acetylcholine (149). But, unlike its action on other effectors with which the present discussion is concerned, the action of acetylcholine on the adrenal medulla is “nicotine-like,” not “muscarine-like” (see p. 150). The linear frequency-action relationship may be due to a direct effect of acetylcholine, not preceded by a combination with a receptor. Possibly in other “nicotine” systems, such as the sympathetic ganglia, a similar straight-line relationship will be found, although at least one “nicotine” effect of acetylcholine is known which yields a hyperbola, that on the rectus abdominis of the frog (95).

The second interesting aspect of this particular frequency-action relationship in the adrenal medulla is the contrast it offers to the other gland studied, the submaxillary. In the latter the usual hyperbola is obtained. The different relationship in the two glands supports the view that the hyperbolic curves, when present, occur because of some feature of the excitatory process (p. 172).

A hyperbolic frequency-action curve for the atrial inotropic effects of vagal stimulation in the turtle was found by Gilson (1933); and, as stated previously, Rosenblueth found the same relationship for the chronotropic effects in the cat. Recently Brown and Eccles (1934a) have raised doubts as to the reliability of the "supposed hyperbolic relationship" in this and other systems. They do not state their reasons for these doubts, nor did they repeat the experiments necessary to confirm or invalidate the relationship. Furthermore, their results are consistent with the theory advocated here.

If the effects of single vagal volleys on the heart rate are plotted as lengthenings of the cycles, curves are obtained which describe the time-course of inhibition. When the curves derived from maximal and submaximal volleys were compared, Brown and Eccles observed that the *ratio* of the inhibitory effects, at first approximately linear, became progressively greater until the maximal effects were reached. Similar variations were found in the ratio of the effects of single shocks applied to the right and left vagus, respectively (Figure 36). From these observations they concluded that the inhibitory lengthenings of the cycle are directly proportional to the concentrations of the acetylcholine liberated by the vagal impulses, *provided that the lengthenings be small*; larger concentrations of acetylcholine, they stated, would produce a more than proportional inhibitory lengthening.

These conclusions are not well justified. There is no criterion in such an experiment for determining which part of the curves represents the true ratio of the concentrations

of acetylcholine liberated by the two volleys. Brown and Eccles choose to assume that the early part of the curve, instead of the peak, represents the true ratio, but they publish no experimental evidence to support this choice. Indeed, the data they present in a subsequent paper (1934b) contradict their previous conclusions. In the later paper they report that two maximal volleys accurately sum their

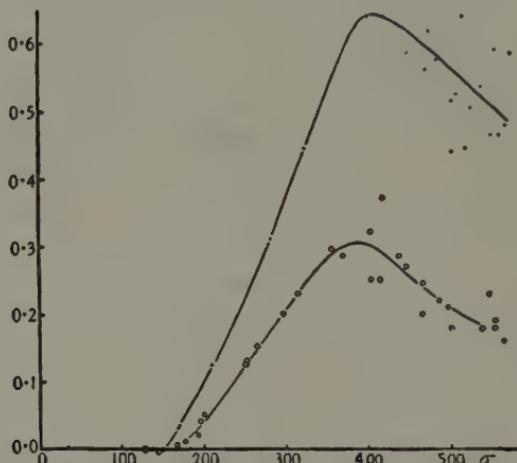


FIG. 36. Two inhibitory curves obtained by applying single maximal shocks to the right vagus (upper curve) and left vagus (lower curve). Abscissae: time in milliseconds. Ordinates: lengthening of each cardiac cycle, i.e., amount by which it exceeds a normal cycle, expressed as a fraction of the normal cycle. (Brown and Eccles, 1934a.)

lengthening effects up to the peak of the response. This lineal summation leads to the justifiable conclusion that the lengthenings are directly proportional to concentrations *as high as those obtained from two maximal volleys*. Since these concentrations are necessarily higher than those evoked by a single volley, it follows that the true ratio in the early experiments was the one corresponding to the peaks of the curves. The deviations from this ratio in the lower parts of these curves might be due to differences in the rates of liberation, diffusion and destruction of the acetylcholine. The results of Brown and Eccles support, therefore, the conclusion that the lengthening of the cycles

is directly proportional to any concentration of the acetylcholine liberated.<sup>1</sup>

Further elaboration of the mode of action of the cardiac nerves is beyond present purposes. Any views which might be suggested would be mainly speculative because of the scantiness and lack of uniformity of available evidence. For instance, it seems premature to discuss the theory put forward by Brown and Eccles (1934b) for the mode of action of acetylcholine. They assume that the heart beat occurs when a critical value of a hypothetical "excitement" is attained at the pace-maker, and suggest that acetylcholine cancels some of the excitement, so that more "excitement" has to be built up for the beats to occur. In order to test this hypothesis in relation to the data reported above it would be necessary to have some knowledge of the rate of formation of "excitement." But, while Eccles and Hoff (1934) conclude that "the excitement of the pacemaker increases relatively more slowly at first and then more and more quickly until it attains threshold and so sets up a beat," Brown and Eccles (1934b) state contrary conclusions, *i.e.*, that "for about 0.3 of a cycle after the normal time of a beat the excitement increases linearly . . . but thereafter the excitement increases at a progressively slower rate."

The frequency-action curves of autonomic effectors agree, therefore, with the concentration-action curves for adrenine and acetylcholine. This agreement substantiates the conclusion that the autonomic nerves act exclusively by liber-

<sup>1</sup> If we call A this concentration, C the duration of a basal cycle, and  $\Delta C$  the lengthening, we may then write  $\Delta C = kA$ .

The heart rate for a given concentration ( $HR_A$ ) is equal to the reciprocal of the duration of the cycles:

$$HR_A = \frac{1}{C + \Delta C} = \frac{1}{C + kA}.$$

The basal heart rate ( $HR_B$ ) is  $HR_B = 1/C$ .

And finally, the slowing ( $\Delta HR$ ) is

$$\Delta HR = HR_B - HR_A = \frac{1}{C} - \frac{1}{C + kA},$$

$$i.e., \Delta HR = \frac{A}{C^2/k + CA},$$

a rectangular hyperbola of precisely the same formula which Brown and Eccles question.

ating adrenine or acetylcholine at the effectors. The uniform explanation adopted, that the hyperbolic relationships are due to a reversible combination with receptors, has two main physiological supports: it accounts for the similarity of results in as different systems as muscles, glands and the cardiac pace-maker; and it explains adequately the complex problem of excitatory *versus* inhibitory effects. Further evidence for the theory, together with a further discussion of this problem, will be found on pp. 88-99.

*The Quantification of Acetylcholine, Adrenine and Sympathin.* The present section is not concerned with a detailed description of the several procedures available for quantifying these substances, but with a discussion of the general principles involved in the use of physiological methods.

As regards acetylcholine and adrenine, physiological quantification is carried out by selecting an indicator and matching its response to a given amount of the unknown solution with its response to a standard known concentration of the substance tested. Several doses of the standard solution have to be injected usually for a satisfactory match of the responses.

The concentration-action curves of adrenine (Figure 35) and acetylcholine suggest several points of interest in relation to the quantifying techniques. The height of the responses to be matched should occupy a position in the rapidly ascending portion of the curve for greater accuracy in the determination. In this region of the curve small changes of concentration or dose lead to relatively considerable changes in the responses; this is not true of the flat asymptotic portion of the curve.

Since the concentration-action curves can be satisfactorily described in many effectors by the formula of a hyperbola, involving only two constants, it follows that two points will determine the curve in such effectors—*i.e.*, two different injections of adrenine or acetylcholine would be sufficient to calculate the response that any other dose would yield. In practice, however, because of inevitable experimental

errors, it is desirable to determine three or four points. The doses which would elicit the responses obtained with the unknown solutions can then be safely interpolated in the resultant curve; this procedure may reduce the number of observations necessary for a given quantification.

The potency of an autonomomimetic drug is usually judged by comparison of the dose of this drug necessary to match the response to a given dose of adrenine or acetylcholine as a standard. From the fact that the concentration-action curves may be two different hyperbolas and not straight lines, it follows that the ratio of the doses of the standard and the test substances which yield the same response may not be constant, but may depend on the magnitude of the response. For example, when the drug is less potent than the standard, if the response is small (*e.g.*, less than 50 per cent of the maximal) the ratio of the dose of the drug to that of the standard will be smaller than if the response is large. On the other hand, if the responses are practically maximal the ratio may be equal to 1—*i.e.*, the same very large doses of two autonomomimetic substances may yield equal responses, although the potency of the substances may differ considerably.

Obviously, then, the method of comparing the efficacy of a substance with that of adrenine or acetylcholine by matching any single response is inadequate. The potency can only be judged by determining the complete concentration-action curves and then comparing the constants of these curves. The constant  $k$  of the formula on p. 168 is determined by the slope of the hyperbola at the origin; this is a parameter which may vary for different substances.

The quantification of the amount of sympathin liberated into the blood stream when a given stimulus was applied to the lower abdominal sympathetic chains was made by Rosenblueth and Schlossberg (1931) by matching the corresponding blood-pressure response with injected adrenine. Such a method assumed tacitly that adrenine and sympathin were identical. The newer data, however, have

revealed significant differences between adrenine and sympathin (pp. 88-97). A quantitative study of the production of sympathin was made by Rosenblueth and Morison (1934); it confirmed the difference between adrenine and sympathin and fitted reasonably with the assumptions that sympathin is a compound of adrenine with a receptive substance (p. 99) and that the responses are directly proportional to the concentrations of sympathin in the effector (p. 172). The following observation, previously mentioned (p. 80), is illustrative.

If sympathin were adrenine the summation of the responses obtained from two different sources of sympathin should be the same as that occurring by the addition of the corresponding doses of adrenine—*i.e.*, if stimulation of source 1 ( $S_1$ ) elicits a response of the indicator equal to that evoked by a dose of adrenine  $A_1$ , and if source 2 ( $S_2$ ) matches the response to the dose of adrenine  $A_2$ , then simultaneous stimulation  $S_1 + S_2$  would elicit a response equal to that of an injection of  $A_1 + A_2$ . Experimentally the effects of  $S_1 + S_2$  were invariably greater than those of  $A_1 + A_2$  (Figure 18), as the properties assumed for sympathin would lead one to expect.

The accurate quantification of sympathin requires, therefore, either the use of sympathin itself as a standard, which will not be possible until its exact composition shall be determined, or else a calculation of the amount of adrenine which the compound sympathin contains, if sympathin is recognized as being such a compound. It is unnecessary, however, to enter here into the details of such a calculation. It should be borne in mind, furthermore, that an estimate of the concentration of sympathin in the blood after stimulation of some sympathetic nerves will give no measure of the total amount of sympathin produced by such stimulation, for only a fraction of this total overflows into the circulating fluids (p. 69).

*Antagonisms.* In organs with a dual autonomic nerve supply the action of the sympathetic is usually antagonistic

to that of the parasympathetic. The quantitative interrelations of the two antagonistic influences have only been studied extensively in the heart; the present discussion will deal, therefore, mainly with this organ, and especially with changes of heart rate.

In 1875 Baxt stimulated simultaneously the vagi and the accelerators and recorded the heart rate. He noted a slowing during the stimulation period, succeeded by an acceleration

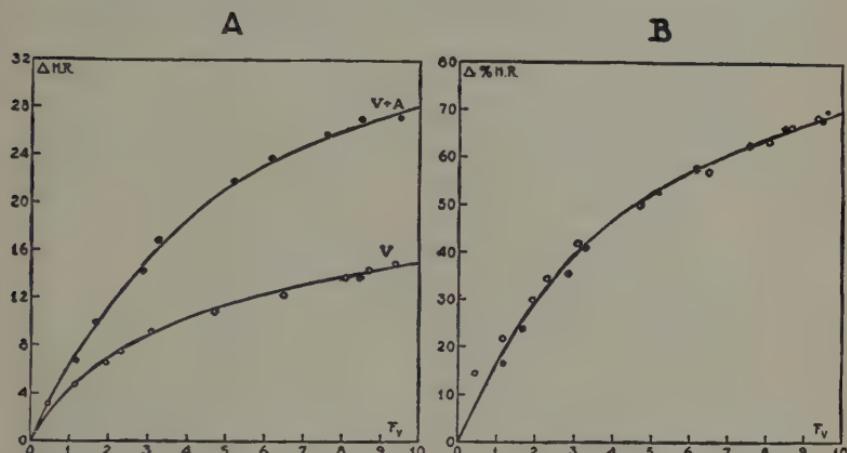


FIG. 37. Vagal slowing of the heart with and without simultaneous activation of the accelerators. Heart disconnected from the central nervous system. A, curve V: stimulation of the left vagus alone. Curve V + A: stimulation of the vagus during continuous stimulation of the right cardio-accelerators at a frequency of 8 shocks per second. Ordinates: slowing of the heart rate in beats per 10 seconds. Abscissae: frequencies (per second) applied to the vagus. B, the two curves of A, plotted as per cent slowing of the basal heart rates (ordinates). (Rosenblueth and Simeone, 1934.)

after the stimuli had ceased acting. A comparison of the effects of this joint stimulation with the responses to isolated excitation of the vagi and accelerators respectively, revealed that the slowing action of the vagus, measured as number of beats per unit time, was enhanced by the simultaneous stimulation of the accelerators. He also showed that during the acceleration which succeeded the application of the joint stimuli the heart rate followed closely the time-course and values which stimulation of the accelerators alone would induce.

Hunt (1897) confirmed Baxt's observations as regards the time-course of the responses to simultaneous stimulation of the vagi and the accelerators. He further noted that the heart rate during stimulation depended on the relative intensities applied to the two nerves, and concluded that, as a rule, the arithmetical mean of the rates evoked by the isolated stimuli was obtained during conjoint activation.

More recently Rosenblueth and Simeone (1934) have shown that the percental effects of any given stimulus ap-

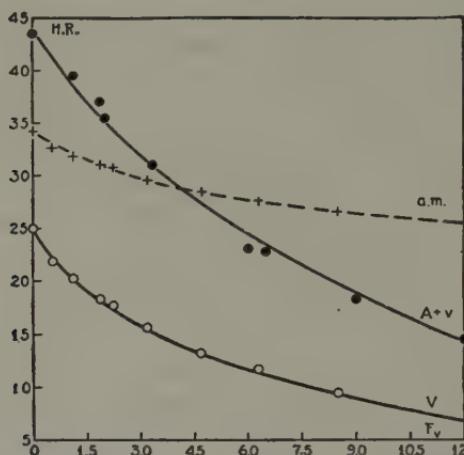


FIG. 38. Ordinates: heart rate per 10 seconds. Abscissae: frequencies (per second) applied to the left vagus. The curve V (circles) is that obtained by stimulating the vagus alone. The curve A + V (dots) results from stimulation of the vagus during persistent stimulation of the accelerators at 8 shocks per second. The curve a.m. (crosses) denotes the rates which would obtain if the effects of simultaneous activation were the arithmetical mean of the responses to separate stimulation. (Rosenblueth and Simeone, 1934.)

plied to either the accelerator or the decelerator nerves are invariably the same, independent of the simultaneous stimulation of the other set of nerves. Thus, the number of beats per unit time by which the heart rate slows down on stimulating the left vagus at a given frequency is greater if there is a continuous accelerator discharge than if the accelerators are inactive; the percental slowing, however, is the same in the two cases (Figure 37).

In Figure 38 the heart rates relative to frequencies of

stimulation of the vagus are plotted, first without any accelerator discharge, then during continuous stimulation of the sympathetic cardiac nerves at a constant frequency. The dotted line denotes the rates which would have occurred in the latter case if the heart rate during simultaneous stimulation were the arithmetical mean of the rate induced by the two separate stimuli. It is readily seen that the experimental values and the arithmetical mean are the same only at the point where the two curves cross—*i.e.*, for a certain degree of activation of the accelerators, only a certain frequency of stimulation applied to the vagus yields the arithmetical mean of the responses. It is probable that Hunt (1897) made his observations with stimuli which were close to the points where the lines intersect, and found, therefore, the arithmetical mean relationship.

It may be concluded that the increments or decrements of heart rate evoked by accelerator or vagal impulses are a function of the preexisting basal rate; this accounts for the differences in the numerical values recorded. But, since the percental effect is constant for a given stimulus, whether the antagonistic nerves be simultaneously activated or not, it may be further concluded that the two nerves affect the pace-maker independently and that the rate obtained is the resultant of the two opposite influences.

The relations between the heart rate and the degree of activation of the vagi and accelerators can be completely described by a nomogram (Figure 39). A straight line joining any two points in the scales which indicate the degree of activation of the two nerves ( $F_a$  and  $F_v$ ) will show what heart rate would have been obtained in the cat from which this nomogram was constructed.

The independence of action of vagal and accelerator impulses has a bearing on some problems of cardiac physiology. Thus, the observation of Baxt, that on simultaneous activation of the nerves a slowing may be succeeded by an acceleration, has been frequently interpreted as proof that the two nerves do not act on the same structure, but that the

"points of attack" are different. The data may, however, be interpreted otherwise. The two substances liberated by the nerve impulses may indeed both affect the same structure, *i.e.*, the pace-maker. It is well known that acetylcholine is more rapidly destroyed than the adrenaline-like sympa-

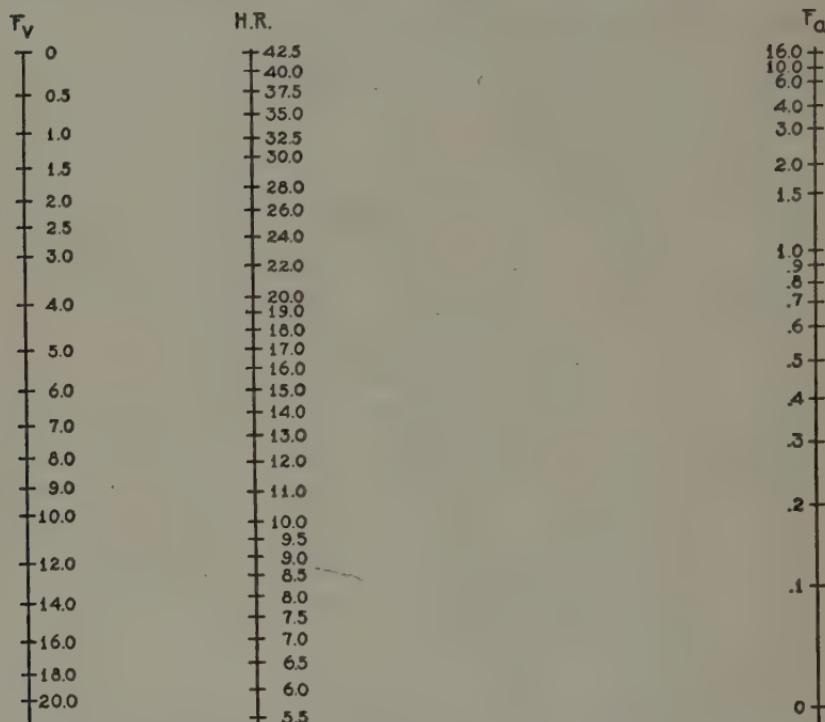


FIG. 39. Scalar nomogram depicting the heart rates occurring in a cat on simultaneous stimulation of the vagus ( $F_v$ ) and the accelerators ( $F_a$ ) at various frequencies. (Rosenblueth and Simeone, 1934.)

thetic mediator (p. 82). Even with a common point of attack the increased heart rate which succeeds the slowing could then be a consequence of this differential rate of destruction of the mediators. The problem of the antagonism between the two nerves is intimately related to the problem of the point of attack. Since the influences may be exerted on the same structure and the responses bear opposite signs, the effects may be called strictly antagonistic.

It is interesting to contrast the independence of effects

of the excitatory and inhibitory influences in the heart with the dependence of inhibition on previous excitation in the central nervous system. In the motoneurones, as shown by Sherrington (1925), the "central inhibitory state" does not have any direct effects, but acts by cancelling the "central excitatory state." The motoneurones differ from the pacemaker, however, because, unlike the latter, they do not possess an intrinsic activity. It will be important to study the interrelations between excitation and inhibition in neurones such as those in the respiratory center which probably do possess an intrinsic rhythmic activity.

Since stimulation of the vagi or the accelerators is tantamount to liberation of acetylcholine or adrenine, one might expect the interrelations between stimulation of one of the nerves and injection of the antagonistic substance, or between injections of the two substances, to be similar to those just described for the two nerves. The only one of these interrelations which has been extensively studied is the effect of vagal stimulation during the action of adrenine. The results of these studies have been quite consistent, showing that with relatively small doses of the hormone (up to 10  $\gamma$  per kgm.) the responses to vagal stimulation are increased, when the slowing is measured as number of beats per minute (see Samaan, 1935, for references). If the responses are measured as per cent of the accelerated heart rate elicited by adrenine, however, a fairly constant degree of slowing obtains for a given vagal stimulus. These results are in satisfactory accord, therefore, with the inferences drawn above for the vagal and accelerator interrelations.

When unphysiologically large doses of adrenine are injected (*e.g.*, more than 20  $\gamma$  per kgm.), on the other hand, the influence of the vagi is reduced or may even be abolished (300). These results contradict apparently the previous conclusions; the data at hand are not sufficient to justify an attempt at explaining this contradiction.

In the nictitating membrane of the cat an unusual situation occurs. The sympathetic nerves and, as would be

expected, adrenaline, induce contractions. Acetylcholine is also a stimulating agent (274), although Rosenblueth and Bard (1932) found no parasympathetic nerve supply. Morrison and Acheson (unpublished observations) have recently investigated quantitatively the concentration-action curve of acetylcholine and the summation of the responses to the two substances when injected simultaneously. The concentration-action curve of acetylcholine is a hyperbola, entirely similar to that of adrenaline. The upper asymptote—*i.e.*, the maximal response obtainable—is the same for the two substances. By selecting suitable scales for the doses in the abscissae the two curves superimpose. The responses to simultaneous injections of the two agents sum along the same curve—*i.e.*, the two substances are quite interchangeable. Such results agree entirely with the conclusion reached for the heart rate, that the action of the mediators is independent and that the final response is the resultant of the separate influences.

## CHAPTER XIV

### SENSITIZATION OF AUTONOMIC EFFECTORS TO THE CHEMICAL MEDIATORS

Two mysterious phenomena, now recognized as being related to chemical mediation of nerve impulses, were handed on from the physiologists of the nineteenth century to those of the twentieth century. These were the paradoxical pupillary reaction and the Vulpian phenomenon. Since 1900 these mysteries have been explained; the explanation has significance for present interests and perhaps also for future development of physiology.

*The Paradoxical Pupillary Reaction.* It has long been known that when the radial fibers of the iris, innervated by sympathetic axons, have been paralyzed by removal of the superior cervical ganglion the pupil on the paralyzed side is more widely dilated under certain conditions than on the normal side, or when the normal side has been freshly denervated. The phenomenon was described by Budge in 1855; it was reported by Schiff in 1868 as a "phénomène suprenant"; and in 1900 Langendorff gave it the name "paradoxical pupillary dilation."

Although many explanatory theories were offered by various investigators, the first to analyze the phenomenon carefully and to start towards the modern understanding of it was Anderson (1903). He not only showed that the paradoxical effect occurred as an accompaniment of excitement, dyspnea, anesthesia and death, and that it was due to some sort of local stimulation of the radial fibers of the iris, but that a similar excessive contraction, under the same conditions, occurred in the denervated nictitating membrane. These observations ruled out a number of rather ill-conceived explanations which had been presented by earlier workers. Anderson attributed the paradoxical effect to an increased

excitability of the denervated structures. He made no suggestion, however, regarding the nature of the exciting agent.

The next step was taken by Meltzer and Auer (1904) when they observed that a given dose of adrenine caused a marked dilation of the pupil of a rabbit twenty-four hours after the superior cervical ganglion had been removed, and a constriction of the denervated blood vessels of the ear, whereas on the normal side there was no effect. In the cat they confirmed the effect on the iris after it had been denervated for two days. The report of the important observations by Meltzer and Auer was marred by an elaborately erroneous theory. Elliott (1905) made the next advance when he established the law which had been suggested by Lewandowsky (1898) and Langley (1901*b*), that adrenine mimics the action of sympathetic nerve impulses. He confirmed Meltzer and Auer's observations that blood vessels, the nictitating membrane and the dilator fibers of the iris, after being denervated for some days, become sensitized to adrenine. He also confirmed Langley's observation that chronically denervated pilomotor muscles of the head and neck are likewise sensitized; and he noted, furthermore, sensitization of the retractor penis muscle after destruction of its sympathetic nerve supply. In 1912 Elliott reported that slight excitement, in a cat deprived of the superior cervical ganglion on one side, evoked the paradoxical pupillary reaction on the denervated side, and that after removal of the adrenal glands the phenomenon was absent. This observation confirmed that reported by Cannon and de la Paz (1911) that emotional excitement causes a discharge of adrenine into the blood stream. It is noteworthy that other conditions—asphyxia, anesthesia—which had been early associated with the paradoxical retraction of the sympathectomized iris, were later shown to evoke a secretion from the adrenal medulla (77). That the smooth muscle of blood vessels in man is rendered more readily responsive to adrenine by sympathectomy was shown by Freeman, Smith-

wick and White (1934), who used insulin hypoglycemia as a stimulus for medulli-adrenal secretion (85).

Further progress in understanding the sensitizing effect of sympathetic denervation was made by Hampel (1935) who traced the course of increasing sensitivity of smooth muscle after severance of its sympathetic fibers. He found that during the first week after removal of the superior

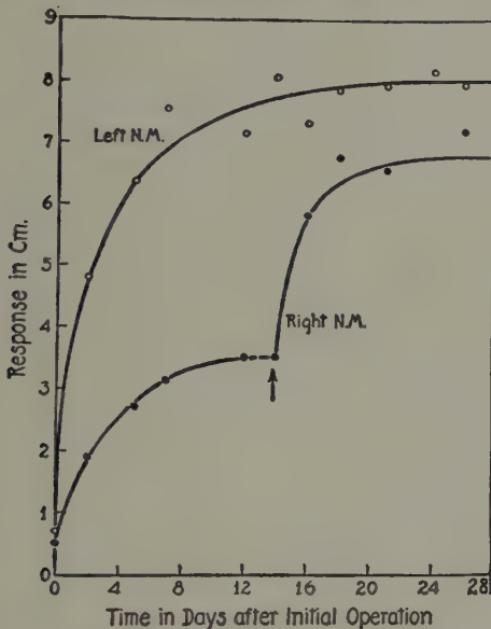


FIG. 40. Course of sensitization of the nictitating membrane to adrenalin after section of sympathetic fibers. The circles indicate isotonic contractions of the left membrane (with severed postganglionic fibers), and the dots, similarly, the right membrane (with severed preganglionic fibers), when adrenalin (1 cc., 1:100,000) was injected intravenously. The right postganglionic fibers were severed on the fourteenth day after the first operation, as indicated by the arrow. (Hampel, 1935.)

cervical ganglion the nictitating membrane became more and more responsive to standard intravenous injections of adrenalin, and that it continued to become more sensitive, but at a slower rate, during the second week, until a maximum was reached at the end of 14–16 days. Cutting the pre-ganglionic fibers caused some increase of responsiveness to adrenalin, and when thereafter the postganglionic fibers

were destroyed there was a further increase (see Figure 40).

What is true of sensitization to adrenine is true also for sympathin. It may be recalled that the experiments of Partington revealed that when conditions were so arranged that sympathin, but not adrenine, was liberated in the unanesthetized cat, the chronically denervated nictitating membrane was contracted thereby, whereas the acutely denervated membrane was not affected (see p. 77).

*The Vulpian Phenomenon.* The puzzling fact reported by Philipeaux and Vulpian in 1863, that after severance and degeneration of the hypoglossal nerve stimulation of the lingual nerve (ordinarily without effect) causes a slow contraction, has already been described (see p. 44). Also the generalization of the phenomenon by the observations of Rogowicz and of Sherrington have been cited. The evidence is now well grounded that it results from the production of acetylcholine where cholinergic fibers affect the smooth muscle of blood vessels which are distributed to the muscles deprived of their motor nerve supply.

For present interest the important fact is that skeletal muscle is sensitized by denervation. An injection of acetylcholine which has no effect on muscles normally innervated evokes the typical slow contracture in muscles deprived of their motor fibers. Simonart and Simonart (1935) found that, in a cat with right sciatic nerve cut a week previously, local application of 0.2 cc. of a solution of acetylcholine (1:10,000) to the exposed surface of the gastrocnemii caused fibrillary contractions of the muscle on the right side but had no effect on the left. Injection of the same amounts of the solution into the two muscles produced the same diverse results. And even when 1 per cent acetylcholine was injected the innervated muscle failed to respond, though the denervated one reacted as before.

The indications that acetylcholine performs a mediating function for motor nerve impulses delivered to skeletal muscle have already been detailed (see p. 48). It appears

that just as smooth muscle becomes sensitized to adrenine and sympathin when its sympathetic nerves have degenerated, in much the same manner skeletal muscle becomes sensitized to its chemical mediator when the motor nerves have degenerated.

*The Sensitization of Smooth Muscle to Acetylcholine.* With examples of the sensitizing effect of destroying sympathetic and motor innervation, the question naturally arose as to whether depriving smooth muscle of its parasympathetic nerve supply would render it sensitive to acetylcholine. This question was answered by observations made by Shen and Cannon (1936) in studies on the iris after removal of the ciliary ganglion. In 1905 Anderson had proved that degeneration of the short ciliary nerves from that ganglion was accompanied by an increased responsiveness of the paralyzed pupilloconstrictor muscle to pilocarpine, and also that eserine, which in the normal eye induces strong contraction of that muscle, becomes quite ineffective. There was a high degree of probability, therefore, that positive results would be obtained in tests with acetylcholine.

Shen and Cannon found that instillation of a strong solution of acetylcholine (1 to 5 per cent) into the conjunctival sac, or intravenous injection of 1 to 5  $\gamma$ , had no influence on the normal iris. After excision of the ciliary ganglion, however, instillation of the strong solution caused a prompt and maximal contraction of the paralyzed sphincter. On this responsive sphincter a more dilute solution of acetylcholine (0.1 to 0.01 per cent) had no effect, but if protective eserine had been previously instilled, the dilute solution produced a marked contraction. The powerful myotic action of the drug could not have been due to eserine, for, as Anderson showed, eserine has no effect on a denervated sphincter.

Hampel's studies brought out the fact that about a week is required for smooth muscle, after losing its nerve supply, to reach approximately its maximal responsiveness to adrenine. When the parasympathetic fibers are cut, how-

ever, the pupillary sphincter muscle shows a slight degree of change within an hour, and maximal sensitization is attained within twenty-four hours. In Figure 41 the cat's paralyzed left sphincter, denervated 14 days before the test, allowed full dilation of the pupil. Denervation of the

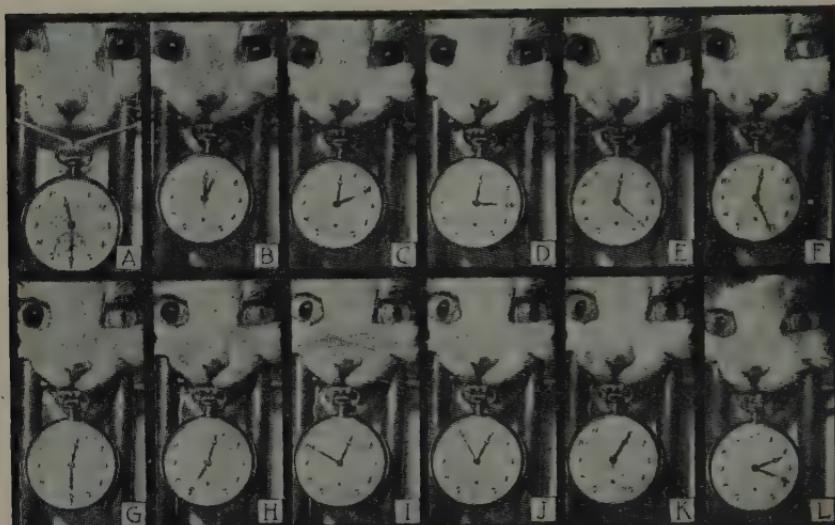


FIG. 41. Sensitization of the sphincter of the iris to acetylcholine by section of the parasympathetic nerve supply. A, cat with its left ciliary ganglion excised two weeks previously. B, thirty-five minutes after A, when the right ciliary ganglion and both superior cervical ganglia had been removed. Now two drops of eserine (1 per cent) were instilled into both of the conjunctival sacs. C, five minutes after the eserine instillation; no change in size of pupils. D, five minutes later; two drops of acetylcholine (0.01 per cent) instilled into each conjunctival sac. E-L, successive stages of pupillary change. (Shen and Cannon, 1936.)

right iris caused dilation of the right pupil. Eserine had no immediate effect. Now instillation into each eye of two drops of 0.01 per cent acetylcholine (which, though preceded by eserine, does not affect the normal iris) resulted in prompt contraction of the highly sensitized, chronically denervated left sphincter and a much slower contraction of the freshly denervated right. Since in this instance the sympathetic fibers were also severed both irises were free from nervous control. The constrictor response, therefore, could be due only to direct action of the instilled drug on the sphincter

muscle. There is no reason for supposing that any other tissue between the conjunctival membrane and the iris suffers a significant change as a result of excision of the ciliary ganglion. Differences in the rate and duration of the response are reasonably explained, therefore, by a change in the reacting tissue, the sphincter muscle itself. The nature of this change will be left to later discussion.

*The Sensitization of Denervated Neurones.* It is reasonable to regard the outlying neurones of the autonomic system—with cell bodies in sympathetic ganglia, for example—as being innervated by the preganglionic fibers. These fibers deliver impulses to the ganglion cells just as the postganglionic fibers deliver impulses to smooth muscle. Furthermore, as already presented (see p. 52), there is good evidence that in the transmission of nerve impulses from neurone to neurone in the superior cervical sympathetic ganglion a chemical agent is released which is like acetylcholine in its effects. What would result from denervation of the ganglion cells by severance of the preganglionic fibers—would they become specially sensitive to acetylcholine?

An answer to the question just raised was furnished by Cannon and Rosenblueth (1936). They employed the nictitating membrane as an indicator. After cutting the pre-ganglionic fibers of the cervical sympathetic strand on one side in the cat, they waited at least a week for the severed axons to degenerate. Then, with use of "dial" anesthesia, they cut acutely the preganglionic fibers of the still intact sympathetic strand on the other side of the neck. After attaching both nictitating membranes to levers so that the contractions would be recorded simultaneously, they performed two tests.

The first test involved injection of acetylcholine intravenously. In preparation the adrenal glands were removed, because acetylcholine stimulates them to secrete adrenine (see p. 54) and consequent contraction of the membrane would be confusing. Atropine was injected in order to lessen the depressive action of acetylcholine on blood pressure and

its stimulating action on the nictitating membranes (cf. 274), without influencing to an important degree the action on the ganglia. Also curare was injected to prevent movements due to the extrinsic eye muscles which, as shown by Duke-Elder (123), are stimulated by acetylcholine. And finally, eserine (prostigmin) was injected to protect the acetylcholine from being destroyed by the esterase. After these preliminary steps had been taken it was found that injection of 0.3 or 0.5 mgm. of acetylcholine caused the membrane on the chronically denervated side to undergo a quick contraction, followed by indications of an included slow contraction and thereupon a gradual relaxation, whereas the membrane on the freshly denervated side did not shorten. After both superior cervical ganglia had been removed and the same doses were repeated, the quick contraction wholly disappeared, and a slow contraction, which persisted, was much less than when the ganglia were present. The inferences were drawn that the quick, high response of the membrane supplied by the chronically denervated ganglion cells was due to a discharge from these cells; and since it did not occur on the side where the ganglion cells were acutely denervated, that the responsive cells had acquired an increased sensitiveness to acetylcholine.

The second method used by Cannon and Rosenblueth was simpler, for no preparatory drugs were required. Pledgets of cotton wet with acetylcholine (1 to 10 per cent) were applied directly to the exposed ganglia. After a brief latent period the nictitating membrane connected with the long denervated ganglion sharply contracted, but that connected with the newly denervated ganglion did not respond (see Figure 42A). As soon as the contraction was fully developed the pledgets were removed and the ganglia were washed with normal salt solution. The phenomenon could then be repeated. That the unresponsive membrane was capable of contracting was proved by injecting adrenalin (Figure 42B). If both ganglia were removed and the pledgets of cotton, wet with acetylcholine were placed in their former positions,

there was no effect. The contraction recorded in Figure 42, therefore, could not have been due to transport of acetylcholine from the region of the ganglion to the membrane, by the circulating blood. It must have resulted from direct stimulation of the ganglion cells sensitized by degeneration of the preganglionic fibers which had formerly innervated them. The possible influence of sensitization of the effector was definitely eliminated by the strikingly different ratio

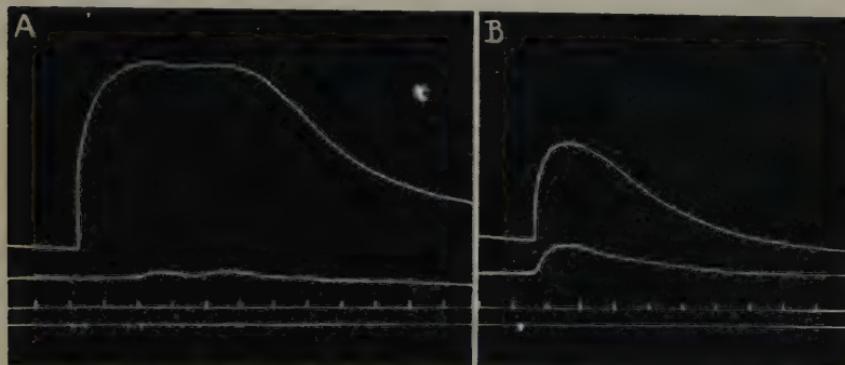


FIG. 42. Sensitization of the superior cervical sympathetic ganglion by severance of the preganglionic fibers. The right ganglion was denervated 15 days previously; the left acutely, just before the test. The upper record is from the right nictitating membrane; the lower, from the left. A, acetylcholine (1 per cent on cotton pledges) applied to the two ganglia: first pair of marks, pledges on; second pair, pledges removed. B, adrenalin (0.005 mgm.) injected intravenously. Time, half-minutes. (Cannon and Rosenblueth, 1936.)

of the responses of the two membranes to adrenine (Figure 42).

This demonstration of increased sensitiveness of denervated neurones supports the idea of a general rule that depriving structures of their nerve supply renders them more readily responsive to their natural stimuli. One might suppose that this result indicates that innervated structures require a certain amount of the natural stimulating agent to be supplied to them in order that their response may be normal. If this agent is not supplied one might suppose that they would become subject to smaller amounts than are necessary in usual circumstances. There are illustrations of such relationships in the organism; for example,

thyroxine is more efficacious in raising the metabolism of a patient with myxedema than the metabolism of a normal person; and insulin lowers the blood sugar more readily in a patient with diabetes than in a person not suffering from that disease. If this suggestion were the proper one, however, it would require the denervated organ to be specifically sensitized to the chemical agent which regularly operates, *i.e.*, to the natural chemical mediator. That proves not to be true.

Bacq (1933d) has offered a theory according to which heightened sensitiveness is ascribed to a reduced production of sympathin. Tonic sympathetic impulses, he states, would result in a continuous slight output of sympathin, and as a stimulating agent the sympathin would diminish the polarization of cell membranes. After the nerves were cut sympathin would not be produced, and therefore polarization would increase. The increased polarization, Bacq suggests, might reasonably be expected to favor the action of adrenine. On that, the crucial point, however, no evidence exists. Furthermore, Elliott (1905) reported that denervated pilomotors become sensitized to adrenine, although they do not manifestly belong to the class of muscles that regularly receive tonic impulses. And finally, in smooth muscles with nerves cut the electric potentials resulting from contraction are smaller than in normal smooth muscle (see Table 5, p. 144)—a fact more reasonably explained as due to lessened, rather than to increased, polarization of cell membranes. These appear to be pertinent objections to the theory which Bacq has proposed.

Another explanation for increased sensitiveness is that removal of the nerve supply results in an increased permeability of the surface membranes of the denervated cells. Certainly, in so far as capillaries are concerned, there is good evidence for this view. Gabbe (1926) showed in experiments on guinea pigs that section of sympathetic fibers made the capillaries in striped muscle more permeable, as revealed by the readier passage of colloidal dyes through

their walls, when compared with the undisturbed side. Should this be a routine effect on cell walls when nerve impulses are abolished, a given amount of a chemical agent would enter the cells more rapidly than under normal conditions, and therefore in a given time a larger concentration would appear within them than would appear inside normal cells. The denervated structures would then have a lower threshold than the structures properly innervated. Furthermore, if this suggestion is correct, the greater permeability should allow a demonstration of increased sensitiveness to other stimulating agents than those which are naturally acting. In fact, experimental tests have proved that increased sensitivity, resulting from denervation, is not specific.

Skeletal muscle, deprived of its nerve supply, becomes specially responsive not only to acetylcholine, but also, as Dale and Gasser (1926) have shown, to potassium chloride, nicotine and other substances belonging to the nicotine group. In smooth muscle, likewise, Rosenblueth (1932a) found that acetylcholine, pilocarpine and eserine, besides adrenaline, all caused a greater contraction of the chronically denervated than of the freshly denervated nictitating membrane. In accord with these observations is that of Anderson when he noted that pilocarpine is extra-effective on the sphincter of the iris which has lost its immediate nerve filaments. The lack of specificity, Cannon and Rosenblueth found, is true also of denervated ganglion cells. Making use of the testimony of Brown and Feldberg (1936a) that potassium ions stimulate ganglion cells, they applied pledges of cotton, wet with a 5 per cent solution of that salt, to a superior cervical sympathetic ganglion which had been isolated from preganglionic control thirty-two days before, and also to the opposite ganglion just isolated. The nictitating membrane connected with the ganglion sensitized by long denervation went into a strong contraction, the other underwent no obvious change. These data are all consistent with the view that severance of their nerves renders the denervated structures more permeable to stimulating agents.

In the small volume, *The Conduction of the Nervous Impulse*, which Lucas had nearly completed at the time of his tragic death, he raised two highly significant questions: "Are we to suppose that the central nervous system uses some process different from that which is the basis of conduction in peripheral nerves, or is it more probable that the apparent differences rest only on our ignorance of the elementary facts of the conduction process? If we had fuller knowledge of conduction as it occurs in peripheral nerve, should we not see Inhibition, Summation and After-discharge as the natural and inevitable consequences of that one conduction process working under conditions of varying complexity?" It is clear that Lucas had in mind not merely conduction in the nerve fiber, but also the transmission of impulses at neuromuscular junctions, because he regarded the two processes as qualitatively identical. After an analysis of the phenomena of summation and inhibition as exhibited in the nerve-muscle preparation, he ventured to suggest that the processes occurring at the synapse between nerve and muscle might be true also for junctional areas of the central nervous system. In 1922, Forbes in turn asked the questions which Lucas had asked, and elaborated a schema of conduction whereby branching and attenuation of terminal nerve filaments and an unsteady polarized membrane might be made to explain the typical events of spinal reflexes without involving other facts of peripheral nervous action than those then known.

As outlined in the foregoing pages, knowledge of the occurrences both at neuromuscular junctions and at neuronal synapses in sympathetic ganglia has been transformed since Lucas and Forbes offered their suggestions. The questions which Lucas raised, however, are still pertinent. Can the new knowledge of chemical mediation of nerve impulses, as revealed in peripheral transmission, be applied to the complex of processes occurring in the spinal cord and the brain? There is where the central problem of physiology lies. As Bowditch (1886) remarked, "the study of the

nervous system is the true field of battle for physiologists, all other investigations, however interesting and important, being of the nature of skirmishes, preparatory for and leading up to the final conflict in which we must engage before we can hope to gain a position from which nature's most mysterious processes are laid bare to our view." Already suggestions have been offered (see pp. 55-58) that the peculiar features of reflex response might be interpreted in terms afforded by action of chemical mediators. And signs are appearing that researches related to that possibility are on their way.



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NOTE. Sixty-one of the papers in the foregoing list of references were published by members of the Department of Physiology in the Harvard Medical School, as follows:—30, 48, 54, 77-92, 115, 125, 154, 155, 156, 161, 183, 208, 212, 229, 230, 250, 252, 254, 273-295, 303, 304, 313, 318 and 340.

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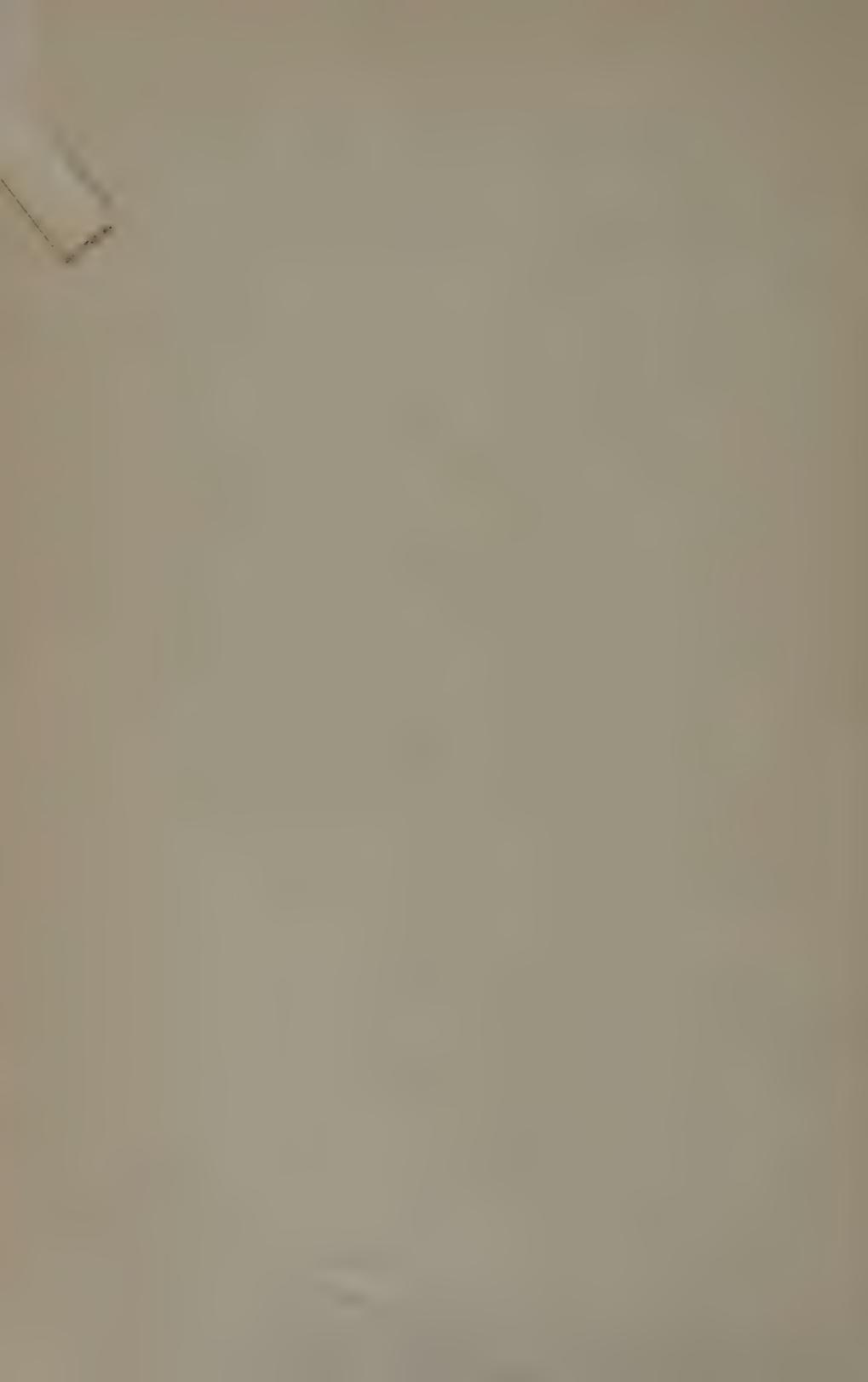
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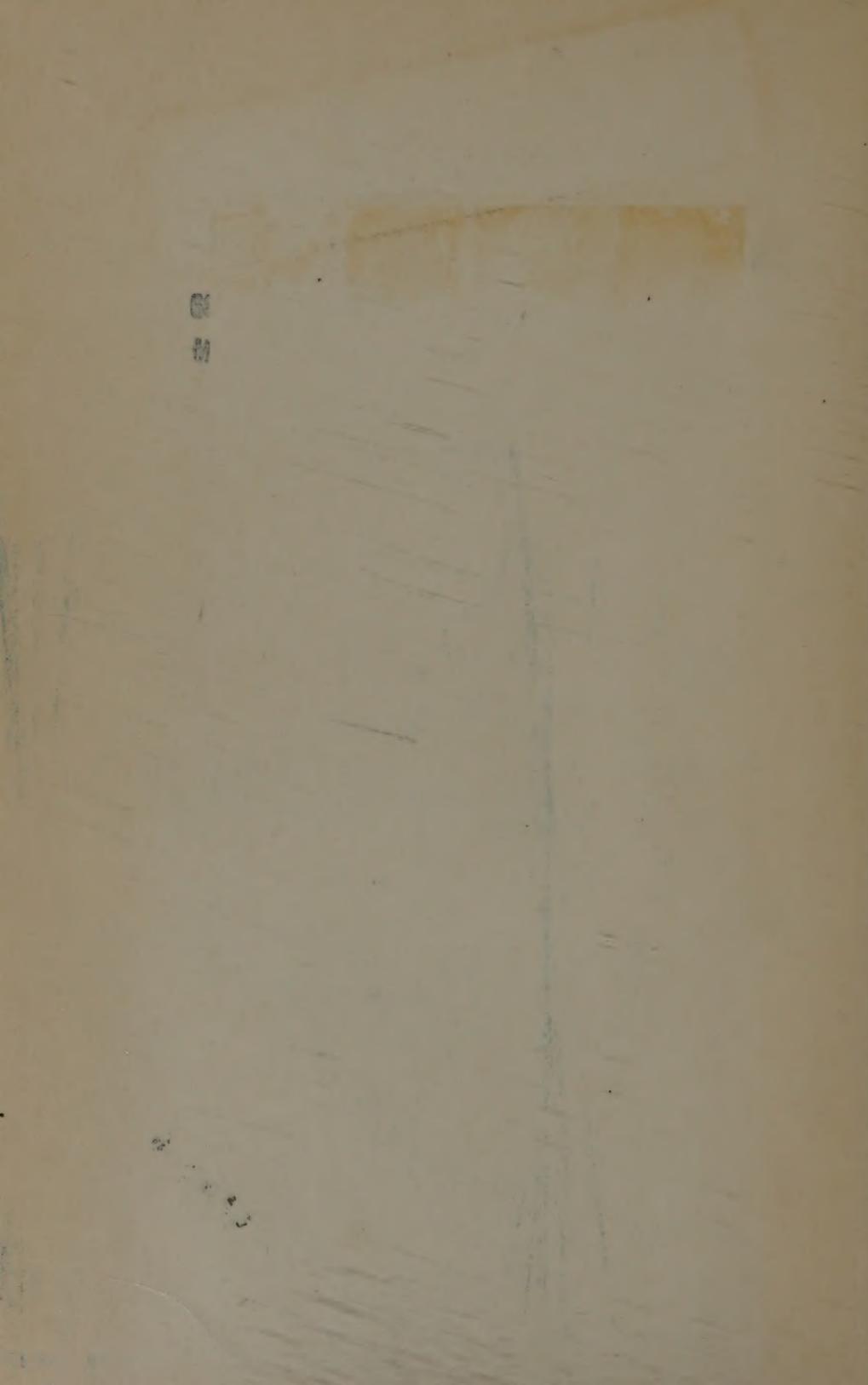
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