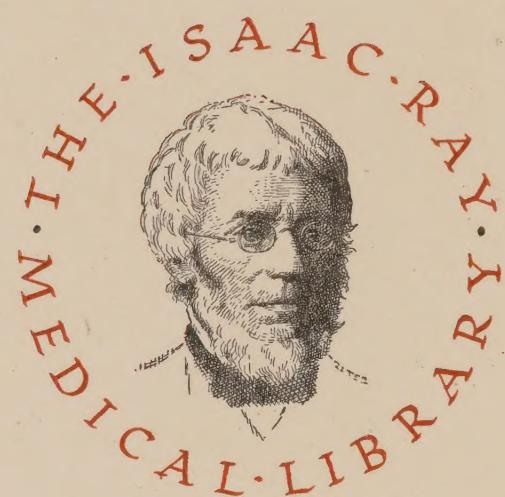


**EXPERIMENTAL  
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MONOGRAPHS**

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**THE SUPERSENSITIVITY  
OF DENERVATED STRUCTURES**

## EXPERIMENTAL BIOLOGY MONOGRAPHS

**Editors:** PHILIP BARD, *Johns Hopkins University*; L. R. BLINKS, *Stanford University*; W. J. CROZIER, *Harvard University*; J. B. COLLIP, *McGill University*; HALLOWELL DAVIS, *Washington University*; S. R. DETWILER, *Columbia University*; HUDSON HOAGLAND, *The Worcester Foundation for Experimental Biology*; J. H. NORTHROP, *Rockefeller Institute for Medical Research*; G. H. PARKER, *Harvard University*; GREGORY PINCUS, *The Worcester Foundation for Experimental Biology*; L. J. STADLER, *The University of Missouri*; SEWALL WRIGHT, *University of Chicago*.

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THE  
SUPERSENSITIVITY  
OF DENERVATED  
STRUCTURES

*A Law of Denervation*

BY  
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## PREFACE

The interest of Dr. Walter B. Cannon in the supersensitivity of denervated structures to the effects of chemical agents arose about fifteen years ago, when many pertinent observations were made in the Laboratories of Physiology at the Harvard Medical School in connection with studies on the chemical transmission of nerve impulses, which were an important concern of the Laboratories at that time. Witnesses to this interest are many of the studies analyzed in this monograph, studies which he carried out personally or which were performed at his suggestion and under his guidance. His scientific curiosity was not abated after he retired from academic duties in 1942, nor did his desire to investigate this problem decline. Prevented from doing further experiments by a heavy load of activities related to the war and by the lack of a laboratory, he began to write a monograph on the subject, hoping to complete it with experimental work at the first opportunity.

In 1945, the Instituto Nacional de Cardiología of Mexico had the privilege and honor of a visit from Dr. Cannon. He stayed here for ten weeks, in the course of which we did some of the experiments which he had planned. To me it was a great happiness to renew my interrupted collaboration with Dr. Cannon. Before his departure, he asked me whether I would finish the book he had started, in case he should not be able to complete it himself. This request was a premonition. Dr. Cannon died on October 1, 1945, without having finished the task which was the main scientific purpose of his last years.

When Mrs. Cannon asked me to prepare for publication Dr. Cannon's last contribution to physiology, we considered two possibilities, that of editing the manuscript as he left it, and that of completing it by the addition of other related

facts and theories without doing violence to his original plan. We adopted the second alternative, a decision which gives me the inestimable pleasure of seeing my name associated with that of Dr. Cannon in a scientific publication for the twenty-first and last time. Lest Dr. Cannon be blamed for my errors and lest I be given credit for contributions in which I had no part, it has seemed to me desirable to indicate the share that each of us had in the preparation of this monograph. Chapters 1, 3, 4, 5, 6, 7, 8, 9, 11, 12 and 13 appear practically as Dr. Cannon left them. Chapters 10, 14, 15, 20 and 21 were unfinished in the original manuscript; I have completed them and have rewritten some parts. Chapters 2, 16, 17, 18, 19 and 22 I have written in their entirety. I have tried to adhere to Dr. Cannon's clarity, simplicity and straightforwardness of presentation; I did not attempt to reach the elegance of his style, for that is inimitable.

I wish to express our thanks to the following persons, for permission to reproduce figures which appeared originally in publications under their control: the Editors of the *Journal of Physiology*, Figure 1; the Editors of the *Archivos del Instituto N. de Cardiología de México*, Figures 3, 50-55; the Editor of the *American Journal of Physiology*, Figures 5-14, 16-18, 20, 22-28, 30-40, 43-49, 56-58; the Editor of the *Archives of Neurology and Psychiatry* (Chicago), Figure 15; the Editor of the *Chinese Journal of Physiology*, Figure 19; the Secretary of the *Proceedings of the Society for Experimental Biology and Medicine*, Figure 21; the *Royal Society of Canada*, Figure 41; the Editor of the *American Journal of the Medical Sciences*, Figure 42; the Editor of the *Journal of Neurophysiology*, Figures 59-62.

ARTURO ROSENBLUETH

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## PART I. INTRODUCTION

### CHAPTER I

#### HISTORICAL DEVELOPMENT OF THE CONCEPT

Among the mysterious phenomena handed on from the physiologists of the nineteenth century to those of the twentieth century were the so-called "paradoxical pupillary dilation" and the "pseudomotor" or "Philipeaux-Vulpian phenomenon." In the development of new knowledge of physiology during the past few decades these mysteries have been explained. The explanation is significant not only as related to recent advances in physiological research but also as related to probable future physiological discoveries. They are, therefore, worthy of special consideration.

*The Paradoxical Pupillary Dilation.* In 1855, Budge noted in a young rabbit that severance of the left cervical sympathetic nerve (preganglionic fibers) and simultaneous severance of the sympathetic branches above the right superior ganglion (postganglionic fibers) was followed by a curious difference in the two eyes. The right pupil, after 48 hours, was larger than the left. In adult rabbits the same operation resulted, within 24 hours, in a similar difference. In both instances the iris was deprived of sympathetic connections with the central system, but the retractor muscle of the iris was shorter (i.e., the pupil was larger), when deprived of its ultimate sympathetic nerve supply than when deprived of the penultimate supply.

In 1868, Maurice Schiff published an account of what he called a "phénomène surprenant." He had cut the cervical sympathetic nerve on one side and had noted that when the animal was asphyxiated there was, after the first one or two breaths, an immediate withdrawal of the iris (i.e., a dilation of the pupil) on the normal side, and shortly thereafter there was also a withdrawal of the iris on the sympa-

thetically decentralized<sup>1</sup> side which developed to a greater degree and lasted longer, i.e., the pupil, at first narrower than on the normal side, remained after the asphyxiation much larger than on that side (see Figure 1).

Apparently Schiff was unaware of Budge's earlier observation. And Robert T. Edes, of Boston, seems not to have known of the work of either. In 1869, Edes reported that when, five days after removal of the right superior cervical ganglion from a kitten, he was taking stitches from the neck, the animal became much excited and the right pupil was dilated more than the normal; furthermore, ten days afterwards, when the animal was etherized, "the iris on the side which had been operated on was much more widely dilated than the healthy one."

In effect, Kowalewsky (1886) repeated Budge's operation. He cut the left cervical sympathetic trunk and excised the right superior cervical ganglion in a kitten three months old; later, when chloroform was administered, the right pupil became much larger than the left, the right eyelids were more widely separated, and the right nictitating membrane was more retracted than on the other side. Again, though sympathetic impulses were excluded from action on the smooth muscle effectors on both sides, abolition of postganglionic fibers had a greater influence than severance of preganglionic fibers.

The phenomenon was further investigated by Langendorff (1900). He removed the superior cervical ganglion, in some instances with and in others without section of the opposite cervical sympathetic trunk. Signs of paralysis of the retractor iridis were, of course, evident on the side without the ganglion, but subsequently a reversal of the effect was commonly observed. Administration of a volatile anesthetic evoked a pronounced paradoxical contrast between the effects on the two eyes—the pupil being enlarged though

<sup>1</sup> In order to avoid repetitive references to neuronal connections, "denervated" should be understood as referring to severed ultimate effector neurons and "decentralized" to severed penultimate neurons.

the pupillary dilator muscle was paralyzed, as had been seen by Edes and Kowalewsky. It was Langendorff who named the phenomenon "paradoxical pupillary dilation."

Many ingenious but highly theoretical explanations were offered to account for the greater retraction of the iris on the side deprived of the nervous influences which cause retraction than on the other side where these influences still persist. In the main, the earlier explanations were based on the assumption that somehow the *sphincter* muscle of the iris on the side deprived of the superior cervical ganglion became weakened.

The first physiologist to analyze the phenomenon with care and with discriminative experimental tests was Anderson (1904). He confirmed the appearance of the paradoxical effects in the iris after excitement, dyspnea and anesthetization. Also he observed a corresponding phenomenon in the denervated nictitating membrane, which is normally held in continuous contraction by sympathetic nerve impulses and which extends over the front of the eye if those impulses are shut off. The membrane, paralyzed by removal of the superior cervical ganglion, was uniquely retracted at the same time that the ipsilateral iris was retracted. The important forward step taken by Anderson was in furnishing evidence that the puzzling effect is due to local stimulation of denervated smooth muscle. His test was as follows: he applied eserine to the eyes of a kitten from which one superior cervical ganglion had been removed. Eserine causes a narrowing of the pupil by contraction of the circular (*sphincter*) muscle of the iris. In the circumstances the pupil on the affected side was smaller than on the other side. Only a slight dyspnea needed to occur, however, in order to render the pupil almost maximally dilated on the denervated side, while the normal control still remained quite narrow. This evidence definitely proved that the paradoxical pupillary dilation results from an actual contraction of the radial, dilator fibers on the side without a

ganglion, and not from a weakening or relaxation of the circular fibers. As just noted, a similar contraction occurs in the nictitating membrane, coincident with the withdrawal of the iris. Unlike the iris, the nictitating membrane has no parasympathetic nerve supply opposed to the sympathetic. The paradoxical effect, therefore, must be due to some sort of increased excitability of the contractile tissues on the side disconnected from the ganglion. This view had been expressed previously by Lewandowsky (1903), but he had not adduced definite proof in support of it. In relation to later observations it is pertinent to recall that Anderson found that the paradoxical effects appear after severance of the cervical sympathetic trunk, i.e., when the superior cervical ganglion is denervated, but that the effects seem to come later and are less pronounced than when the postganglionic fibers are cut or the ganglion itself is removed.

Although Anderson attributed the paradoxical pupillary phenomenon to an increased excitability of the denervated or decentralized iris and nictitating membrane, he had no suggestion as to the nature or source of the exciting agents. The next move in solving the riddle was made by Meltzer and Auer (1904). They noted that a given dose of adrenaline, administered subcutaneously to a rabbit 24 hours after the superior cervical ganglion had been removed from one side, caused a marked dilation of the pupil on that side and also caused a constriction of the denervated blood vessels of the ear, whereas on the normal side it produced no change. In the cat they confirmed the supersensitivity of the iris, but only after it had been denervated for 48 hours. The reports of the important observations by Meltzer and Auer, unfortunately, were marred by an elaborately erroneous explanation.

Another step forward was taken by Elliott, in his famous paper of 1905, wherein he established the law that adrenaline mimics the action of sympathetic nerve impulses. Like Meltzer and Auer he found that the blood vessels of the

ear and the dilator fibers of the iris, when deprived of sympathetic impulses, become especially sensitive to the action of adrenaline. Also he substantiated Langley's (1901) testimony that the pilomotor muscles of the head and neck are sensitized if the stellate ganglion has been excised.

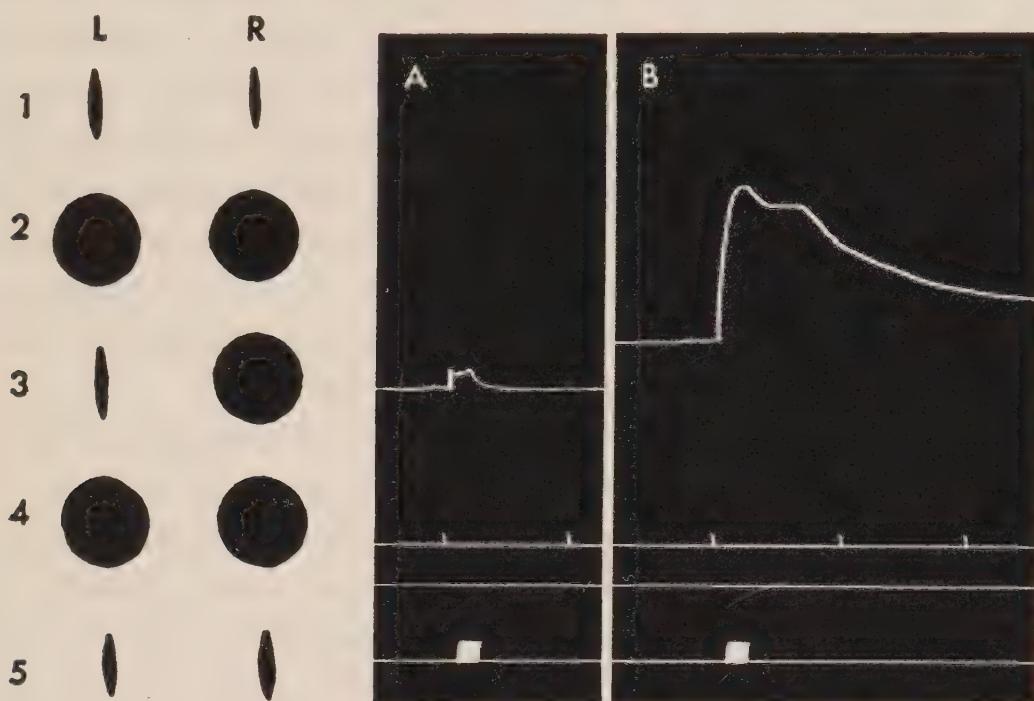


FIG. 1 (left). The paradoxical pupillary phenomenon. 1. Normal pupils (of cat) under bright illumination. 2. Immediately after 4 min. of asphyxia. 3. Thirty sec. after 2; the right iris sensitized by sympathetic denervation is much more withdrawn than the left which is still innervated by sympathetic nerves. 4 and 5. Same procedures as in 2 and 3, but after the adrenal glands were inactivated. (Kellaway, 1919.)

FIG. 2 (right). The Philipeaux-Vulpian phenomenon. Mechanogram of the tongue of a cat under dial. Right hypoglossal nerve cut ten days previously. Chorda tympani nerve stimulated at A before, and at B after, eserine (1 mgm. per kgm.); tetanic frequency at time indicated by the lower signal. Time signal: 30-sec. intervals.

Furthermore, he noted sensitization of the denervated retractor penis muscle and of the denervated nictitating membrane. Seven years later, in 1912, Elliott reported that slight excitement in a cat from which one superior cervical ganglion has been excised induces the paradoxical pupillary reaction on the denervated side and that after removal of the adrenal glands the phenomenon is absent. This observation supported the evidence announced the previous year by

Cannon and de la Paz (1911) that emotional excitement causes a discharge of adrenaline into the blood stream. It is also discharged by asphyxiation (Cannon and Hoskins, 1911). These observations were confirmed and extended by Hartman, McCordock, and Loder (1923) who reported that the completely denervated iris is largely retracted during etherization (asphyxia) and operative trauma, and retains the capacity to retract after adrenalectomy. Thus the paradoxical pupillary response, after remaining a mystery for many decades, was given a reasonable explanation as being due to increased secretion of adrenaline, resulting from emotional excitement and other conditions which discharge sympathetic impulses, and acting with special effectiveness on the denervated retractor muscle of the iris.

*The Philipeaux-Vulpian and Allied Phenomena.* This phenomenon, the second of the two mysteries mentioned above, consists of an anomalous response of striated muscle to stimulation of nerves distributed to blood vessels. It was first noticed in the tongue. The chorda tympani nerve contains sensory, vasodilator and secretory fibers, and the lingual branch of that nerve certainly has the last two of these components. Stimulation of either the chorda tympani or its lingual offshoot does not produce contraction of the normal muscles of the tongue. If, however, the hypoglossal nerve (i.e., the motor nerve of the tongue muscles) is severed and allowed to degenerate, and then the chorda tympani or its lingual branch is stimulated, the tongue muscles contract (see Figure 2). Here is a muscular shortening provoked by nerve impulses which normally have no motor influence. This remarkable phenomenon was first described by Philipeaux and Vulpian, in 1863, in experiments on the dog. Their testimony was confirmed by Cyon in 1871. Twelve years later, Heidenhain (1883) studied this puzzling fact in some detail. He noted that about four days after section of the hypoglossal nerve the muscle fibers were actively fibrillating; then the lingual nerve, which up to that time had been ineffective, began to be effective. The slow response of

the tongue muscles, which lingual stimulation first induced between the 4th and the 6th day after motor denervation, increased to the 8th or the 9th day and thereafter remained constant until the end of the 3rd week when it began to decrease. The contractile effect of lingual stimulation disappeared between 10 and 14 weeks after severance of the hypoglossal, a disappearance associated with regeneration of that nerve. Heidenhain showed, furthermore, that although the responses are due to the stimulation of nerve fibers which cause vasodilation of the tongue vessels, the mechanical effects are not a consequence of the vasodilation.

Another instance of "pseudomotor innervation" was discovered by Rogowicz in 1885. He noted in a dog that after the facial nerve was cut at its emergence from the skull and time was allowed for full degeneration of its distal fibers, stimulation of the cervical sympathetic trunk on the paralyzed side caused a slow contracture of the levator muscle of the upper lip and at times, also, of the orbicular muscle of the eyelids. This observation was confirmed by Van Rijnberk (1915).

A similar phenomenon was described by Sherrington (1894). He severed the anterior roots of a nerve supplying the muscles of the hind leg of a cat, and two or three weeks later, on applying a stimulus to the peripheral nerve trunk, which still contained normal sympathetic fibers and fibers of the dorsal roots, he observed a slow contracture of the paralyzed muscles. The contracture outlasted the stimulus for five seconds, whereupon the muscle slowly returned to its resting length. That the response was not the result of a spread of the electrical stimuli was proved by applying the exciting electrodes 20 cm. distant from the muscle and by noting that the phenomenon disappeared when the nerve was crushed between the muscle and the point of stimulation.

With much insight Langley, in 1921, offered the suggestion that the pseudomotor effects result from the liberation at

autonomic nerve endings of a substance which excites striated muscle only when the muscle is sensitized by degeneration of its nerve supply. Support for this view was indicated three years later by Bremer and Rylant (1924), who published evidence that the active fibers involved in the response are such as are found in the autonomic nerves distributed to blood vessels.

The pseudomotor phenomenon has had attached to it the names of various investigators who have studied it. It has been commonly called the "Vulpian" phenomenon, but also the "Heidenhain," "Rogowicz" or "Sherrington" phenomenon, or combinations of these names such as the "Vulpian-Heidenhain" or the "Vulpian-Sherrington" phenomenon. Other more recent investigators than these have repeated the earlier observations.

The first step in the solution of the mystery appeared in 1922, when Frank, Nothmann, and Hirsch-Kaufmann found that an injection of acetylcholine causes responses of skeletal muscles after their motor nerves have degenerated, just as does stimulation of vasodilator nerves. That acetylcholine is commonly produced at nerve endings when blood vessels are dilated by stimulating their nerve supply and that this acetylcholine diffuses to the denervated muscle was indicated by the observations of Dale and Gaddum (1930). They showed that the contracture of the tongue muscles is augmented by eserine (see Figure 2), thus pointing to the presence of acetylcholine, which eserine protects from destruction by cholinesterase. By extending these tests to the muscles of the face, in repeating the Rogowicz experiments, Euler and Gaddum (1931) demonstrated that a true anatomical sympathetic supply may contain cholinergic fibers, and Hinsey and Cutting (1933) extended this knowledge further when they proved that the Sherrington phenomenon disappears if the sympathetic supply to the blood vessels of the hind limb is abolished, i.e., the phenomenon results from cholinergic fibers in the sympathetic distribution.

Another addition to the evidence was made by Bain, who, in 1932, discovered that salt solution perfused through the blood vessels of the dog's tongue during stimulation of the lingual nerve acquires the ability to excite contraction of the rabbit intestine—a sensitive indicator of the presence of acetylcholine. Also Feldberg (1933 b) found that when the lingual nerve is excited in a dog dosed with eserine, blood from the tongue causes a fall of blood pressure in the cat and a contraction of eserinized leech muscle, effects reliably explained by the presence of acetylcholine. Moreover, Bülbbring and Burn (1934) showed that stimulation of the sympathetic fibers of the hind limb of the dog evokes into a perfusate of eserinized salt solution a substance which, when applied to proper indicators, elicits characteristic acetylcholine responses. Thus, in repetition of the experiments of Vulpian, of Rogowicz, and of Sherrington, evidence was obtained of intermediary action of acetylcholine from autonomic nerves—an action not effective in the normal state of striated muscle.

The two mysteries which we have been considering have their explanation in the increased sensitiveness of denervated structures. The radial fibers of the iris, when deprived of their sympathetic nerve supply, become sensitized to circulating adrenaline. Adrenaline mimics the action of sympathetic nerves, i.e., it causes retraction of the iris and enlargement of the pupil. Because denervation sensitizes the retractor of the iris, and also because the adrenal medulla discharges adrenaline into the blood stream in times of emotional excitement and asphyxia, the sensitized iris in these circumstances is retracted farther on the denervated side than on the normal side, a puzzling effect which, as we have seen, was so paradoxical as to have perplexed for many decades some of the most expert physiologists. The pseudomotor phenomenon is also accounted for by proof that skeletal muscle when denervated becomes sensitized to a chemical agent set free from the smooth muscle of

blood vessels within its structure, i.e., the acetylcholine liberated at the terminals of vasodilator nerves.

In chapters which follow, the phenomenon of sensitization to circulating chemical agents, which occurs in a wide variety of structures when they are deprived of their normal nerve supply, will be described in detail.

## CHAPTER II

### SUPERSENSITIVITY: DEFINITIONS AND EXAMPLES

At the outset it is well to recognize that there are various ways in which structures can be sensitized. For example, they are often quite easily rendered supersensitive to some agent, e.g., adrenaline, by preliminary treatment with one or another chemical substance, e.g., cocaine or thyroxine. Although in this monograph attention will be paid primarily to the sensitization brought about by depriving a structure of its natural nerve connections, an introductory consideration of the results of other experimental procedures is important, because this consideration clarifies the effects of denervation.

Broadly speaking, an agent or procedure *a* is said to sensitize a given structure or organ with regard to the effects of another agent or procedure *b* when the responses to *b* become greater than normal after *a* is applied. The sensitizing agent or procedure does not usually cause any permanent changes in the structure tested, although it may elicit a transient response; it merely enhances the reactions of the sensitized structure to the stimulatory or inhibitory second agent.

From this broad definition it is clear that the term supersensitivity covers several possibly different phenomena. Thus, the responses of the sensitized structure may become greater in amplitude or they may become longer lasting. Again, the increase of amplitude may be detectable only when the test doses of the stimulating agent evoke submaximal responses, but not when the responses are maximal, or else that increase may be seen with any test doses selected. Finally, the responses of a tissue may not be

importantly modified, but, if an agent exerts its stimulating action only when its concentration exceeds a critical threshold, this threshold may become lower as the main or only manifestation of supersensitivity. All these different modes of sensitization have been encountered experimentally.

As an example of the prolongation of the responses of an organ to a stimulating agent without any change in their amplitude may be cited the action of cocaine on the cardiac acceleration induced by adrenaline. Fröhlich and Loewi pointed out in 1910 that cocaine increases the amplitude of the rise of blood pressure and the dilation of the pupil elicited by small doses of adrenaline. A corresponding increase of cardiac acceleration was not seen by Rosenblueth and Schlossberg (1931). Peralta and Lizarralde (1946) confirmed this absence of sensitization of the heart, as revealed by the degree of acceleration, but showed that the duration of the chronotropic response to a given dose of adrenaline is significantly lengthened after the administration of cocaine (see Figure 3). The prolongation of response illustrated in Figure 3 may be attributed to a slower than normal rate of destruction of adrenaline by the heart after treatment with cocaine.

In the nictitating membrane of the cat cocaine augments not only the duration but also the amplitude of the contractions which take place in response to injections of small doses of adrenaline (Rosenblueth and Cannon, 1932) and to stimulation of the cervical sympathetic with slow frequencies (Rosenblueth and Rioch, 1933)—effects that have been confirmed by Clark and Raventós (1939). The larger the dose of adrenaline injected, or the higher the frequency of stimulation of the nerves, the smaller becomes the difference of amplitude between the normal and the sensitized responses. Indeed, the amplitude of the maximal responses of the membrane is not altered by cocaine. The phenomenon is illustrated graphically in Figure 4. In A the ordinates indicate the magnitude of the isotonic contractions of the

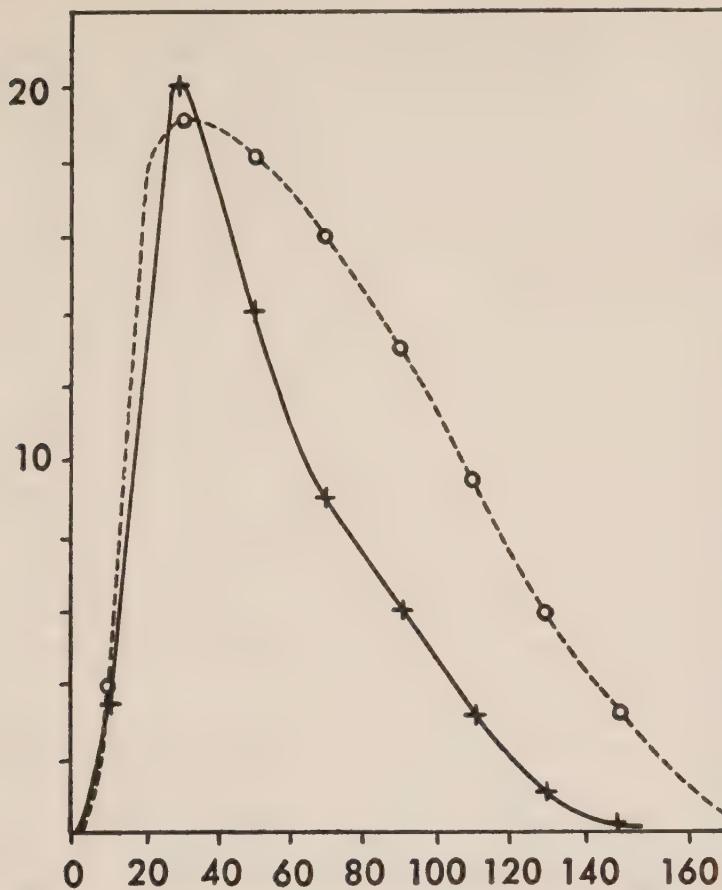


FIG. 3. Influence of cocaine on the time-course of the cardiac acceleration in response to adrenaline ( $60\gamma$ ). Cat under dial; heart decentralized acutely; adrenals ligated. Ordinates: heart rate per 10 sec., plotted at the time corresponding to the middle of the successive 10-sec. intervals measured. Abscissae: time (in sec.) after the injections of adrenaline. Solid line, before, and broken line, after, injecting cocaine (6 mgm. per kgm.). (Peralta and Lizarralde, 1946.)

nictitating membrane measured in millimeters in the kymograph records. The abscissae correspond to the doses (in  $\gamma$ ) of adrenaline injected intravenously. The lower curve (dots) represents the control responses obtained at the beginning of the experiment; the upper curve (circles) depicts the contractions registered after injecting cocaine. It is clear that while the responses to relatively weak stimuli—small doses of adrenaline—became considerably larger after administration of the drug, those elicited by strong stimuli were increased only slightly, and the maximal responses were not modified. This difference is apparent in the graph in B. The abscissae are again the amounts of adrenaline injected in the observations corresponding to A; the ordinates are

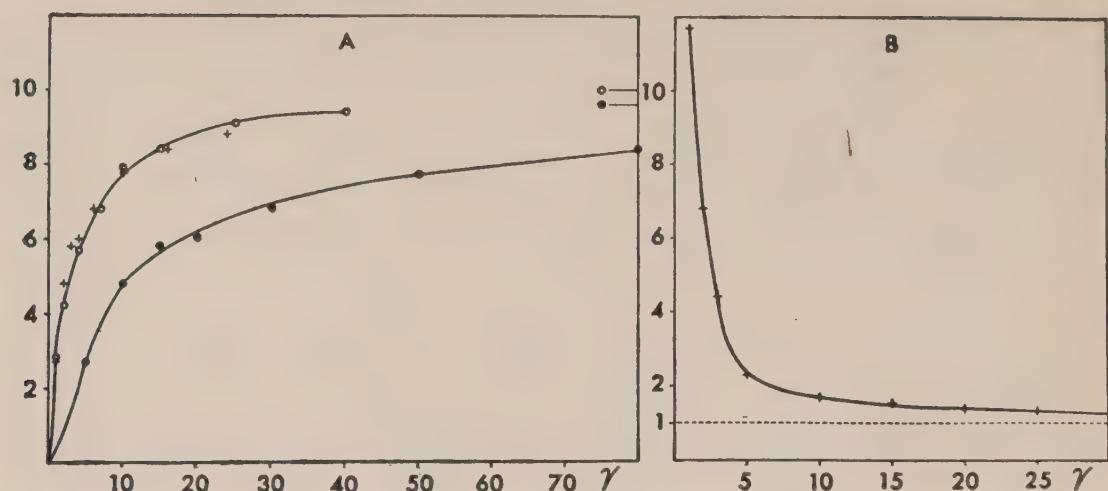


FIG. 4. Influence of cocaine on the amplitude of the responses of the nictitating membrane to various doses of adrenaline. Cat under dial; adrenals ligated; right superior cervical ganglion removed; right eyeball emptied and excised. Isotonic records of the contractions of the right nictitating membrane.

A. Dose-response curves for adrenaline. Ordinates: amplitude of the responses of the membrane in cm. in the original records. Abscissae: doses of adrenaline (in  $\gamma$ ), injected intravenously with constant volume and rate of injection. Dots (lower curve): responses before cocaine. Circles (upper curve): responses one hour after administration of cocaine (6 mgm. per kgm., intravenously). Crosses: ordinates of the responses obtained before cocaine (dots) plotted at the abscissae obtained by multiplying the doses injected originally by 0.2; the coincidence of these crosses with the curve obtained after cocaine proves that in this experiment the susceptibility of the membrane to adrenaline increased 5-fold upon the administration of cocaine.

B. Ratio of the amplitude of the responses obtained in A after cocaine to those registered before administration of the drug (ordinates) plotted at the abscissae corresponding to the doses of adrenaline (in  $\gamma$ ) which elicited the two series of responses. This ratio is high for small doses of adrenaline, and approaches 1 asymptotically as the doses increase, i.e., the amplitude of the maximal responses was approximately the same before and after cocaine. The two horizontal lines to the right in A show the asymptotes of the two curves, calculated theoretically on the basis of the fact that these curves are segments of hyperbolae. These asymptotes show the theoretical maximal responses and reveal that cocaine does not alter significantly the reactivity of the membrane.

the numerical ratios of the amplitude of the responses recorded after cocaine to the contractions registered in the control observations; these ratios are plotted at the abscissae of the corresponding doses of adrenaline.

The sensitizing action of thyroxine on the effects of adrenaline was proved for the heart by Sawyer and Brown (1935) and for the nictitating membrane by Lee (1942).

As Lee emphasized, the action of thyroxine on the smooth muscle of the membrane is quite different from that of cocaine. In contrast to the results obtained after injections of cocaine, the sensitization produced by thyroxine is the same for all frequencies of stimulation of the cervical sympathetic (Figure 5A) and for all doses of adrenaline (Figure 5B).

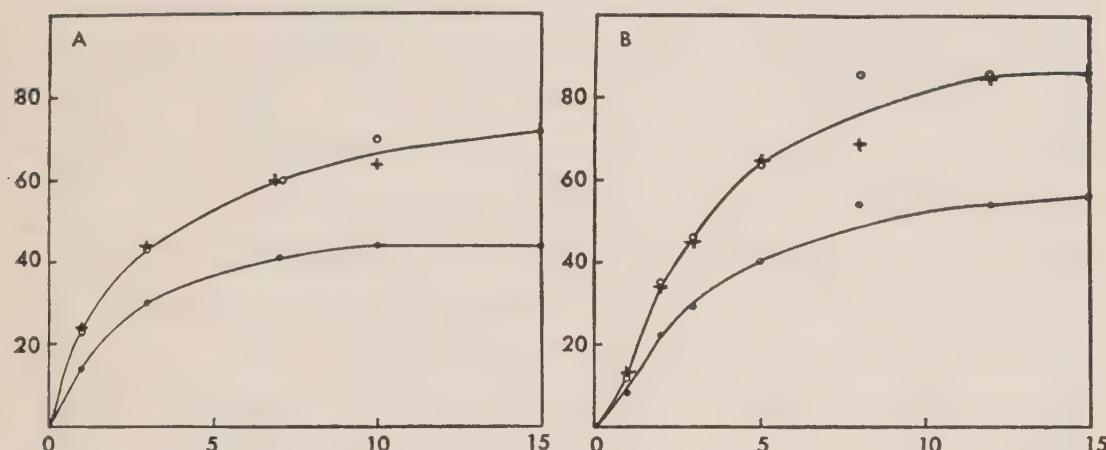


FIG. 5. Influence of thyroxine on the amplitude of the responses of the nictitating membrane to various frequencies of stimulation of the cervical sympathetic (A) and to various doses of adrenaline (B). Cats under nembutal. Isotonic records of the contractions of the nictitating membrane. A first series of determinations (lower curves in A and B) was made with aseptic precautions. Two days later thyroxine (20 mgm.) was injected. Six days later a second series of determinations was made (upper curves), under the same conditions as in the first observations.

A. Ordinates: amplitude of the responses. Abscissae: frequencies (per sec.) of stimulation of the cervical sympathetic for 15 sec. Lower curve (dots): control results. Upper curve (crosses): results after thyroxine. Circles: the dots of the lower curve, but with ordinates multiplied by the constant factor 1.43. The coincidence of the circles with the upper curve shows that the reactivity of the membrane to adrenaline was increased 43 per cent by thyroxine.

B. As in A; but abscissae: doses of adrenaline (in  $\gamma$ ) injected intravenously. Dots: controls. Crosses: after thyroxine. Circles: dots with ordinates multiplied by 1.6, i.e., the reactivity of the membrane to the action of sympathetic nerve impulses was increased 60 per cent by thyroxine in this animal. (Lee, 1942.)

The difference between the two sets of results plotted in Figures 4 and 5 is brought out by the tests illustrated by the crosses in 4 and by the circles in 5. The sensitizing action of cocaine becomes manifest as a decrease of the amount of stimulation necessary to elicit a given response. This decrease

is constant for all the responses. If the *abscissae* of the points in the control curve of Figure 4 are multiplied by a constant fraction (0.2), the points obtained thus (crosses) fall on the curve corresponding to the results recorded after cocaine was administered. Thus, while cocaine did not affect the contractile ability of the nictitating membrane, as revealed by the unmodified amplitude of the maximal responses, it reduced to one-fifth the amount of adrenaline necessary to elicit any given response. The sensitizing action of thyroxine, on the other hand, appears as a constant increase of the response to any given degree of stimulation. If the *ordinates* of the points in the normal curves in Figure 5 are multiplied by a constant factor greater than 1 (1.43 in A; 1.6 in B) the points thus obtained (circles) fall on the curves constructed from the observations made after thyroxine had been given. In other words, thyroxine increased by 43 and 60 per cent, respectively, the responses of the membranes to all the stimuli tested.

As shown by Brown (1937 b) and also by Rosenblueth and Luco (1937), acetylcholine elicits two types of responses from striated muscles (see Figure 29). One of these responses includes the initial appearance of propagated muscle impulses—it is a contraction; the other reaction is not attended by the development of spike potentials—it is a contracture (see Gasser, 1930). The muscle impulses revealed by the spike potentials in the contraction responses follow the all-or-nothing principle and hence require that the concentration of acetylcholine exceed a critical threshold. The concentration of acetylcholine that will initiate muscle impulses in denervated skeletal muscles is less than that necessary to stimulate normal muscles—i.e., denervation lowers the threshold of striated muscle to acetylcholine.

Excitability is often defined as the reciprocal of the threshold of a tissue to a stimulating agent, electrical or chemical. Since this definition is clear and precise it will be adopted in the present monograph. It should be noted, however, that this meaning of the term excitability is only

applicable to the stimulating action of agents on tissues when there is a critical threshold for stimulation. The present knowledge of the action of adrenaline and acetyl-choline on autonomic effectors indicates that there is no threshold for the stimulating, or inhibitory, influence of those substances on these effectors (see for references and discussion Cannon and Rosenblueth, 1937, and Rosenblueth, 1949). The precise and restricted concept of excitability adopted is consequently not relevant to autonomic effectors. As generic terms for the greater or lesser ease with which stimuli may evoke specific responses from neurons or effectors we propose and adopt here the classical term "irritability" and the more recent designation "sensitivity." Much as "excitability" is defined as the reciprocal of the threshold of a tissue, we suggest that the term "susceptibility" be employed to denote the reciprocal of the amount or degree of stimulation necessary to evoke any given response, whether the response be positive (contraction, secretion, nervous discharge, acceleration) or negative (relaxation, deceleration, inhibition). The influence of cocaine on the sensitivity of the nictitating membrane is then an increase of the susceptibility of this muscle to the action of adrenaline and of sympathetic nerve impulses. We propose, also, that the degree of response of a tissue, whether positive or negative, to a given degree of stimulation be considered the definition of the "reactivity" of that tissue. The action of thyroxine on the membrane is then adequately described by the statement that the hormone increases the reactivity of the muscle, without modifying its susceptibility.

From this argument the conclusions emerge that when an agent or procedure modifies the susceptibility of a structure to the influence of a substance, the appropriate method by which to measure this modification is to compare the stimuli necessary to cause equal responses, while if the agent or procedure changes the reactivity of the structure, the pertinent measurements are those of the responses to constant stimuli. Information as to the type of sensitization

which obtains in different experimental conditions can be derived by these measurements or by a different approach, as follows. When the law correlating the amplitude of responses with the doses of a stimulating chemical agent, e.g., adrenaline, approximates a rectangular hyperbola, as is the case for the curves in Figures 4 and 5 (see Rosenblueth, 1932 *b* and *c*), then the sensitization due to increased susceptibility can be readily distinguished from that due to increased reactivity by the characteristics of the hyperbolas. The equation which correlates the response (*R*) with the dose of the agent or the frequency of nerve stimulation (*S*) is

$$R = \frac{S}{k + k'S},$$

where *k* and *k'* are parameters easily determined from the experimental observations. The asymptotes of this hyperbola are  $1/k'$  (asymptote parallel to the *S* axis, that is, asymptote which represents the maximal response which the effector can yield), and  $-k/k'$  (asymptote parallel to the *R* axis). The slope of the hyperbola is given by

$$\frac{dR}{dS} = \frac{k}{(k - k'S)^2},$$

which becomes  $1/k$  when  $S = 0$ , i.e., at the origin. Increased susceptibility will be revealed by a steeper slope of the hyperbola at the origin, that is, by a decrease of *k*. Increased reactivity, on the other hand, will appear as an increase of the amplitude of the maximal responses, that is, as a decrease of *k'*.

The foregoing comments lead to the recognition of four different types of increased sensitivity. In the first, the amplitude of the responses is unchanged but their time-course is prolonged—there is superduration of response. In the second, the stimulating agent has to exceed a threshold and this threshold is lower than normal—there is hyper-

excitability. In the third, lessened stimuli which do not have to exceed a threshold bring forth responses of normal amplitude—there is increased susceptibility. Finally, in the fourth the capacity of the tissue to respond is augmented—there is superreactivity.

An important question for the topic which is the primary concern of this monograph is that of the type or types of supersensitivity consequent to the severance of nerves. The answer to this question can only be given after careful consideration of the data on the supersensitivity of various tissues. These data will be presented in detail in the chapters which follow. Suffice it to say now that with the exception of an increased reactivity all the other types of sensitization mentioned have been observed to develop after denervation. Thus, as stated above, the threshold of denervated striated muscles to acetylcholine is lower than that of the innervated controls—i.e., these muscles are hyperexcitable with regard to this agent.

The autonomic effector that has been studied most carefully after denervation or decentralization is the nictitating membrane. In this smooth muscle, supersensitivity becomes manifest by augmented susceptibility and by increased duration of response. Thus, Lee (1942) found that the maximum response of the membrane, i.e., the reactivity, is not modified after decentralization, while the susceptibility is markedly increased. Lee's observations are illustrated in Figure 6A. There is a striking similarity between the changes brought about by degenerative section of the cervical sympathetic, illustrated in this figure, and those produced by injections of cocaine, shown in Figure 4. The circles in Figure 6A were determined by the procedure used for the determination of the crosses in Figure 4A, i.e., by multiplying the abscissae of the control observations by a constant fraction (0.5 in this instance). Here, as in Figure 4A, these points coincide satisfactorily with the curve obtained after sensitization had developed. Figure 6B, like Figure 4B, shows that the ratio of the sensitized to the

control responses falls with increasing doses of adrenaline and approaches 1 as a limit. Lee's results are in agreement with the earlier observations of Hampel (1935; see

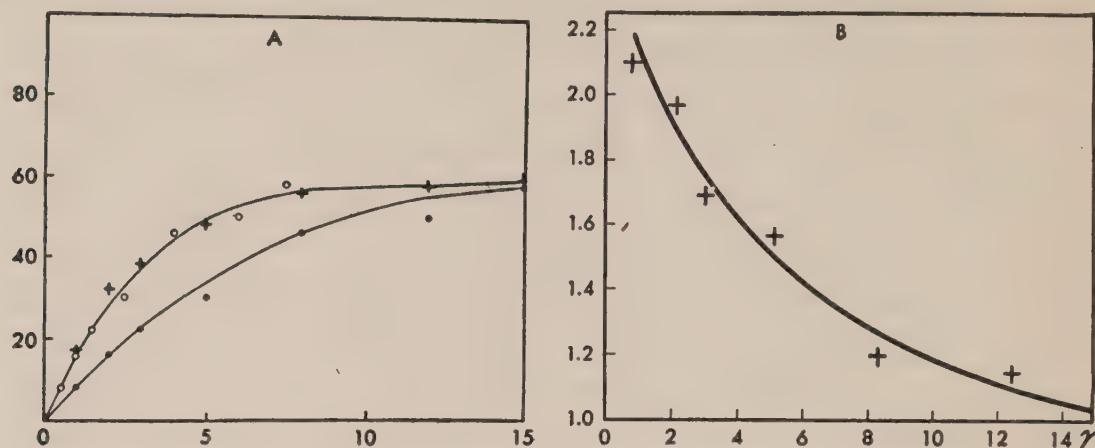


FIG. 6. Influence of chronic decentralization on the responses of the nictitating membrane to various doses of adrenaline. Cat under nembutal. Isotonic contractions of the membrane.

A. Ordinates: amplitude of the responses. Abscissae: doses of adrenaline (in  $\gamma$ ) injected intravenously. Dots (lower curve): control observations before decentralization. Crosses (upper curve): 15 days after section of the preganglionic fibers in the cervical sympathetic. Circles: the dots plotted at the abscissae obtained by multiplying the original doses injected by 0.5, i.e., decentralization caused a 2-fold increase of the susceptibility of the membrane to adrenaline. (Lee, 1942.)

B. Ratio of the amplitude of the responses obtained in A after decentralization to those registered in the control series (ordinates), plotted at the abscissae corresponding to the doses of adrenaline which elicited the two series of responses. As in Fig. 4 B, this ratio is high for small doses of adrenaline, but tends to approach 1 as the doses increase, i.e., the reactivity of the membrane is not modified by decentralization.

Figures 7 and 8) and of Klopp (1940; see Figures 11 and 12). That in addition to increased susceptibility there is also increased duration of responses after denervation was pointed out by Elliott in 1905. In many personal observations we have noted the longer duration of the responses of the nictitating membrane and of other structures when previously denervated, as compared to those of acutely decentralized controls, whether those responses were submaximal or maximal. Although accurate studies of other autonomic effectors than the membrane have not been made, it will be assumed that the section of nervous pathways does not change the reactivity of the elements distal in

the tract, and that the main change in the sensitivity of these effectors is an enhanced susceptibility.

A corollary of the concepts developed in this chapter deserves emphasis, namely that the measurement of the degree of supersensitivity of a structure after an appropriate experimental procedure requires different methods, depending on the type of supersensitivity developed. Increased reactivity and prolonged duration of response can be detected even if intense test stimuli are employed, but hyper-excitability and augmented susceptibility require the use of test stimuli which yield *submaximal* reactions. The *maximal* responses of a denervated structure may be practically equal to those of the same structure before denervation or to those of its opposite normally innervated partner, used as a control. In many of the observations to be mentioned in the following chapters the degree of sensitization was evaluated numerically. The criteria for this evaluation have not always been the same nor have they often been appropriate for the measurement of the type of supersensitivity that took place. These considerations explain discrepancies in the reports of different observers; they also indicate that the numbers which will be quoted as measuring the degree of supersensitivity represent often only gross orders of magnitude.



## PART II. SUPERSENSITIVITY TO CHEMICAL AGENTS

### CHAPTER III

#### SMOOTH MUSCLE: SYMPATHETIC STIMULATION

##### NICTITATING MEMBRANE AND IRIS RETRACTOR

In 1905 Elliott asserted that all muscles thrown into contraction by adrenaline respond in the presence of the hormone with "greater irritability and persistence" after decentralization (i.e., degenerative section of the preganglionic sympathetic nerves), and still more clearly after denervation. The data in this and in following chapters corroborate Elliott's assertion.

*The Nictitating Membrane.* An inquiry into the gradual development of sensitization of smooth muscle after it has been deprived of connection with the central nervous system was undertaken by Hampel and reported in 1935. He arranged apparatus for registering the contractions of the nictitating membrane repeatedly, at intervals of days, of the same animal (cat) and under the same conditions. Two writing levers were set up in such manner that the animal, after being temporarily anesthetized with nembutal, could be replaced in exactly the same relation to these levers time after time. Thus records were made of the contraction of the two nictitating membranes, one normally innervated and the other either denervated or decentralized. The stimulus in every instance was adrenaline, injected into the femoral vein in standard doses of different strengths. The volume was the same in all the tests (1 cc.) and was administered at a uniform rate over a period of 10 seconds.

During the first 6 to 8 days after removal of the superior cervical ganglion the magnitude of the response of the corresponding nictitating membrane to the same dose of adrenaline rapidly increases. Thereafter a more gradual

increase follows until a maximal degree of sensitization is reached after 6 to 8 days more, i.e., approximately two weeks after the denervation the sensitized state is maximal. The gradual change of sensitivity is illustrated in the lower records of Figure 7 and the upper graph of Figure 8.

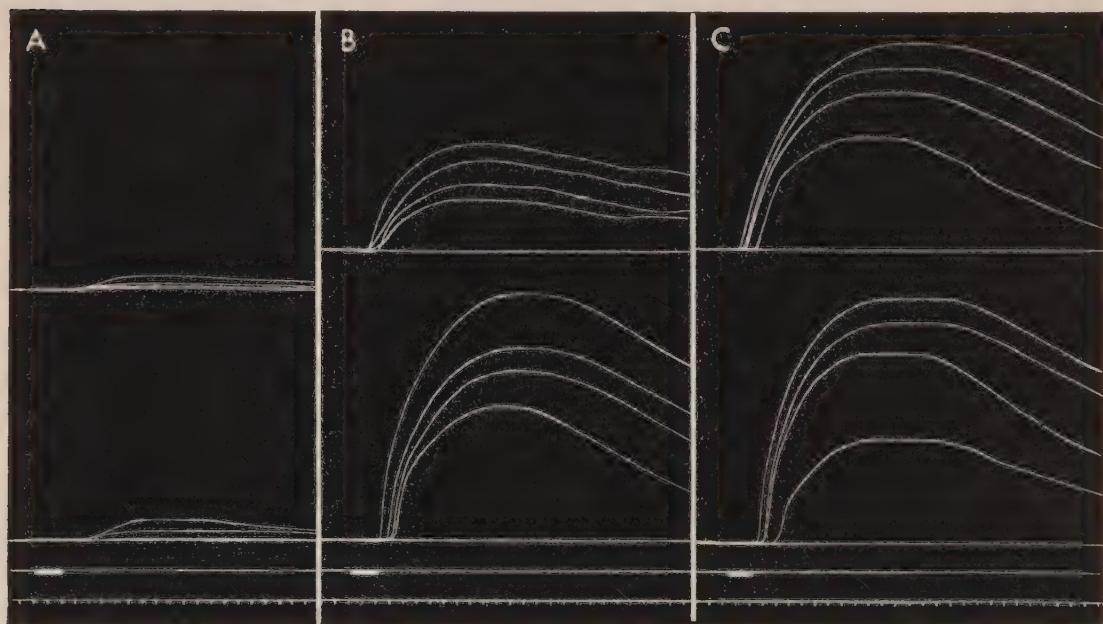


FIG. 7. Isotonic responses of the right nictitating membrane (above) and the left nictitating membrane (below) of a cat to intravenous injections of 2.5, 5.0, 7.5, and 10 $\gamma$  of adrenaline. Injections during 10 sec., at the signal. Time in 5-sec. intervals.

- A. Immediately after section of the right cervical sympathetic nerve and removal of the left superior cervical ganglion.
- B. Responses recorded 14 days after the first operation and immediately after removal of the right superior cervical ganglion.
- C. Responses recorded 28 days after the initial operation, i.e., 14 days after removal of right ganglion. (Hampel, 1935.)

The increase of response as a consequence of decentralizing the membrane resembles that resulting from denervation; but, as shown in the upper records of Figure 7B and in the lower graph of Figure 8, the increase is only about half as great. After the denervated ganglion has been removed, however, the now denervated membrane undergoes increased sensitization; the responses to adrenaline are augmented until the final contractions elicited by standard doses are about equal to those recorded by the membrane after it has been subjected to a primary denervation.

In 1938 Brücke reported that in his experiments, although the responses to adrenaline of the nictitating membrane whose preganglionic sympathetic fibers had undergone degenerative section were usually greater than those of the control freshly decentralized membrane of the other side, removal of the eyeballs abolished the difference. He concluded, therefore, that the smooth muscle of the nictitating membrane is not significantly sensitized by decentralization and that the greater responses after degeneration of the cut nerves are to be attributed, when the eyeballs are intact, to the sensitization of the smooth muscle of the orbit. When that muscle contracts, it protrudes the eyeball and thereby causes the extended membrane to be withdrawn. Since in Brücke's experiments the effect

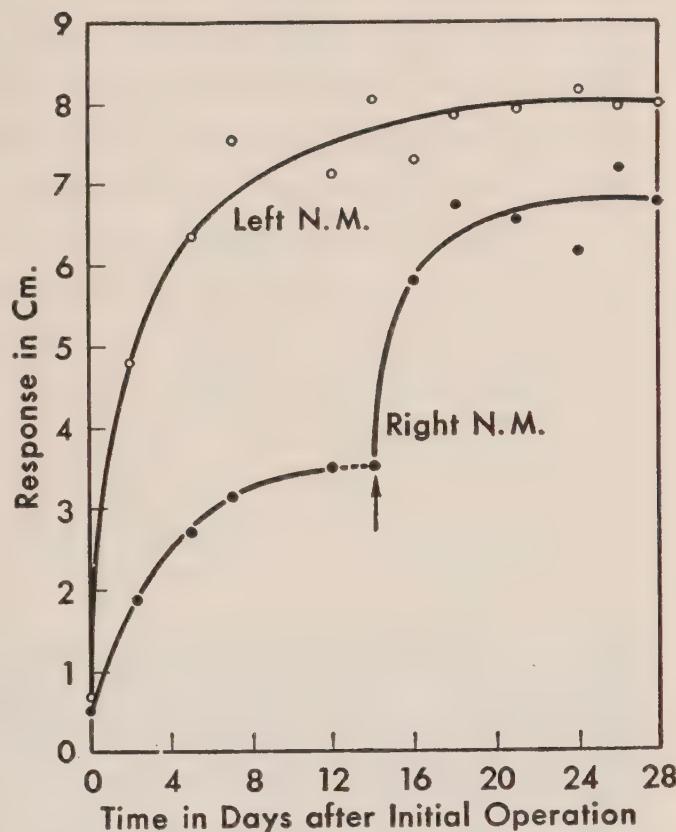


FIG. 8. Amplitude of the isotonic responses of the nictitating membranes of a cat to  $10\gamma$  of adrenaline, at different times after removal of the left superior cervical ganglion and section of the right cervical sympathetic nerve. The right superior cervical ganglion was removed 14 days after section of the right nerve, as indicated by the arrow. Magnification, 15-fold. (Hampel, 1935.)

of adrenaline was admittedly greater on the long-decentralized side if the eyeballs were in place, his view would lead to the inconsistent conclusion that the orbital smooth muscle of the nictitating membrane remains unaffected. In 1939 Rosenblueth and Cannon recorded contractions of the nictitating membrane in response to small doses of adrenaline (less than  $20\gamma$ ) before and after emptying the eyeballs and also after removing them completely. Emptying the

eyeballs, thereby preventing protrusion, does not modify the response to adrenaline, whether the membrane is freshly or chronically decentralized. Passive effects, therefore, may be discarded. When, in addition, the eyeballs are removed, however, some of the smooth muscle elements which withdraw the membrane may be destroyed. It is noteworthy that in the observations of Rosenblueth and Cannon, unlike those reported by Brücke, only 3 out of 18 animals, after the drastic operation of removing the eyeballs, gave approximately equal responses from the two sides. In the other 15 instances the contractions of the decentralized membrane were markedly greater than those of the normal control. Besides probably injuring the muscle of the membrane, Brücke was not careful to use submaximal doses of adrenaline; he commonly injected 30 to 70γ—large doses produce equal contractions of sensitized and normal muscle.

The observations of Hampel on the course of development of supersensitivity were confirmed by Simeone (1937), who carried the inquiry still further by testing whether with passage of time there could be a decrease of sensitization of

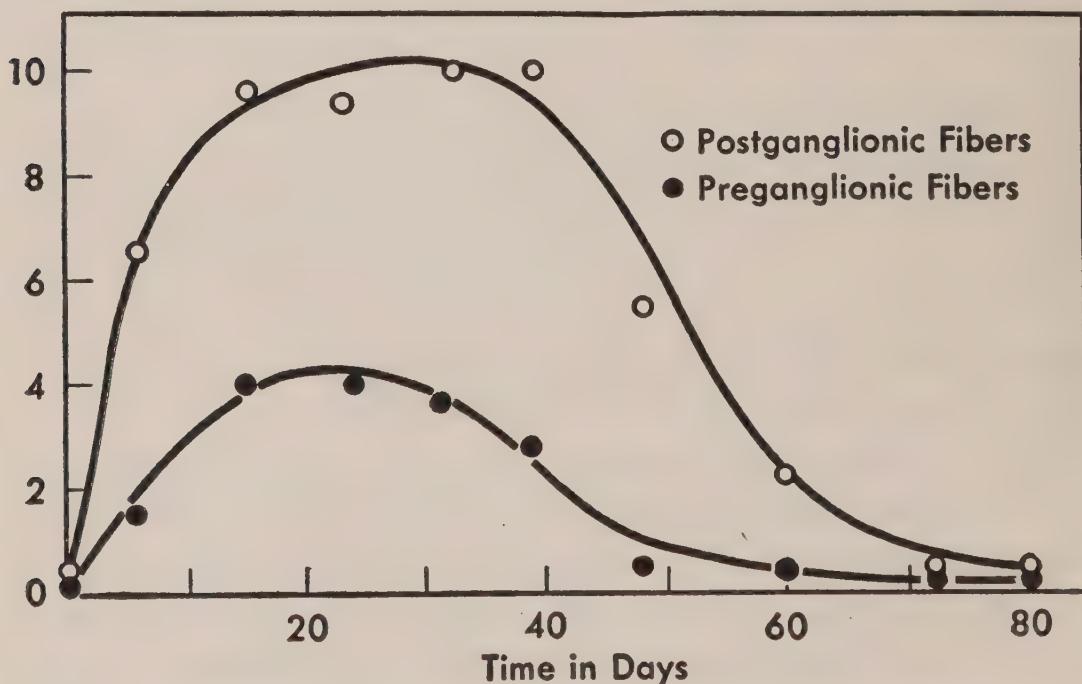


FIG. 9. Amplitude of the responses of the nictitating membranes of a cat to  $2.5\gamma$  of adrenaline, at different time intervals after postganglionic denervation of the right nictitating membrane and preganglionic denervation of the left. Responses in cm., magnification, 19-fold. (Simeone, 1937.)

the membrane. Elliott (1905) had observed supersensitivity of the sympathectomized iris as long as ten months after removal of the superior cervical ganglion. Simeone found that if the sympathetic fibers which supply the nictitating membrane, either preganglionic or postganglionic, are crushed, instead of being cut, the increased sensitivity begins to decline after some time (see Figures 9 and 10). The first sign of a decreased responsiveness was taken as an indication that there might be a regrowth of nerve filaments through the crushed region and onward to the natural terminus. This indication was tested, and was proved to be correct, by stimulating electrically the nerves proximal to the crushed region and noting that there was dilation of the pupil and retraction of the nictitating membrane. The average length of time required for the first definite decrease of sensitivity of the membrane when preganglionic fibers were crushed was two and a half weeks. The fibers were interrupted from

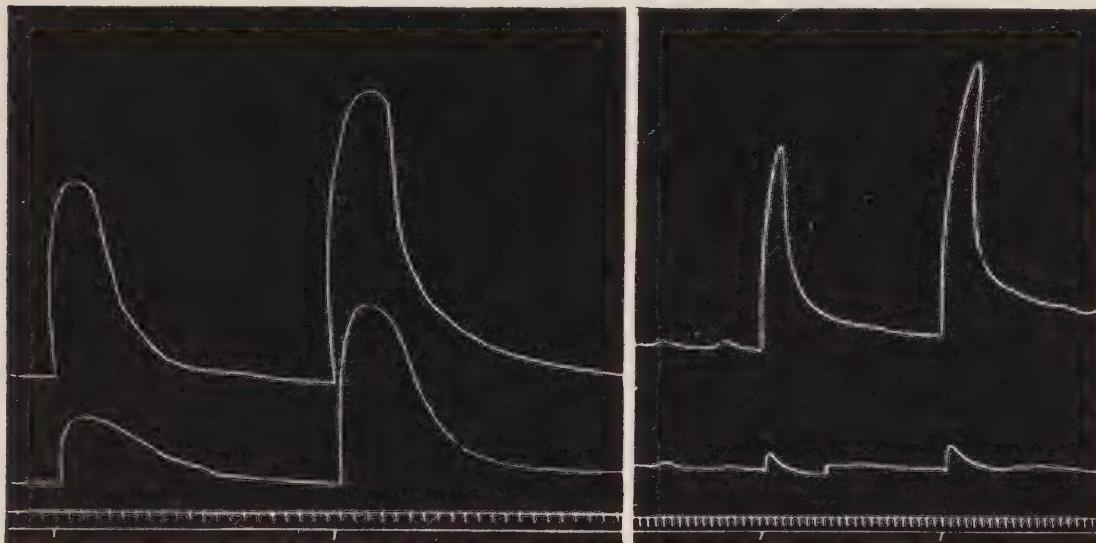


FIG. 10. Responses of the nictitating membranes of a cat to adrenaline (successively 2.5 and 5.0 $\gamma$ ). The first tracings show the contractions obtained two weeks after section of the postganglionic fibers to the right membrane (upper record), and after preganglionic section on the left side (lower record). The second tracings show the contractions obtained 32 days after the initial operations. (Simeone, 1937.)

1.0 to 1.5 cm. proximal to the ganglion. The average length of time required for the first definite decrease of sensitivity when postganglionic fibers were crushed was longer, a result

reasonably associated with the much longer distance (5 to 6 cm.) through which the fibers must grow from the point of interruption, near the ganglion, to the smooth muscle of the membrane. The average was seven weeks, but in one animal it was only five and a half weeks.

In general the disappearance of the greater sensitivity was more rapid and more complete with preganglionic regeneration than with postganglionic. Indeed, complete disappearance of the supersensitivity resulting from a crush of postganglionic fibers was not attained even after months allowed for regeneration. Simeone attributed this difference to a difference in innervation of the ganglion and the nictitating membrane. In the ganglion, as shown by Eccles (1935), there is a considerable overlap in the distribution of preganglionic filaments to the cell bodies of the ultimate neurons. Complete functional innervation of the cells, therefore, might not depend on complete anatomical regeneration of the preganglionic fibers. In the nictitating membrane, on the other hand, there is probably no such overlap. Indeed, according to histological evidence (see for references Cannon and Rosenblueth, 1937) only some of the smooth muscle cells are supplied with nerves. Failure of postganglionic fibers to regrow, therefore, might leave many cells lacking their nerve supply, so that there would be a continuance of supersensitivity for a long period. In any case, the evidence clearly indicates that the increased responsiveness to adrenaline of the denervated smooth muscle in the nictitating membrane is not an irreversible change, but disappears when the nerves have regenerated.

In relation to the evidence that at the terminals of the ultimate sympathetic nerve fibers there is a discharge of adrenaline as an intermediary between the nerve impulses and the effector organs (see Loewi, 1921; also Cannon and Lissák, 1939) was the interesting observation by Simeone that as the nerve fibers regenerated there was an enlargement of the ipsilateral pupil beyond the normal size and a corresponding greater retraction of the nictitating mem-

brane, a condition which continued unchanged for several days and then slowly became less pronounced until a normal state was restored. The probable explanation of this phenomenon is that the membrane, sensitized by denervation, responds at first to a greater degree to the adrenaline liberated at the regenerated nerve terminals and only as regeneration proceeds is the greater sensitivity decreased until it disappears.

A precaution which early should have been regarded as a possibility, but which in fact was not considered, was disclosed by observations reported by Simeone in 1938 (*b*). He discovered that adrenaline itself has effects on the responsiveness to later test doses of that agent. For example, if a single injection of adrenaline (100 $\gamma$ ) is given, it usually potentiates the responses of the nictitating membrane to subsequent test doses (2 $\gamma$ , intravenously) on the sensitized side but not on the normal side. This potentiation suggests that there is a slight error in favor of results which indicate increased sensitiveness of the denervated smooth muscle. Yet this error is not likely to be important, for the potentiating dose must be large relative to the test doses, and the test doses must be given shortly after the potentiating dose has acted. The conclusion is not altered, therefore, that the differences between the effects on the normal and on the denervated membrane constitute reliable proof of a sensitizing influence which results from removal of the nerve supply.

An instructive inquiry was that of Klopp (1940) as to the effect of partial denervation of the sheet of smooth muscle in the cat's third eyelid. In performing the operation some of the postganglionic fibers were severed. On the other side, which served as a control, the innervation was treated in different ways; the superior cervical ganglion was removed, or it was preganglionically denervated, or it was left intact. In accordance with Hampel's evidence, a period varying from 7 to 14 days was allowed to elapse before observations were made. Adrenaline was injected in either gradually in-

creasing or gradually decreasing amounts. When the maximal height of the responses was plotted against the corresponding doses of adrenaline, the control membrane yielded a continuous curve. The graph from the partially denervated membrane when similarly plotted, invariably showed a discontinuity or break (see Figures 11 and 12). The partially denervated membrane obviously responded to the relatively large doses of adrenaline as did the normal control (see upper segment, Figure 11), whereas it reacted to small doses as did the totally denervated control (see lower segment, Figure 12). The break in the curves can readily be

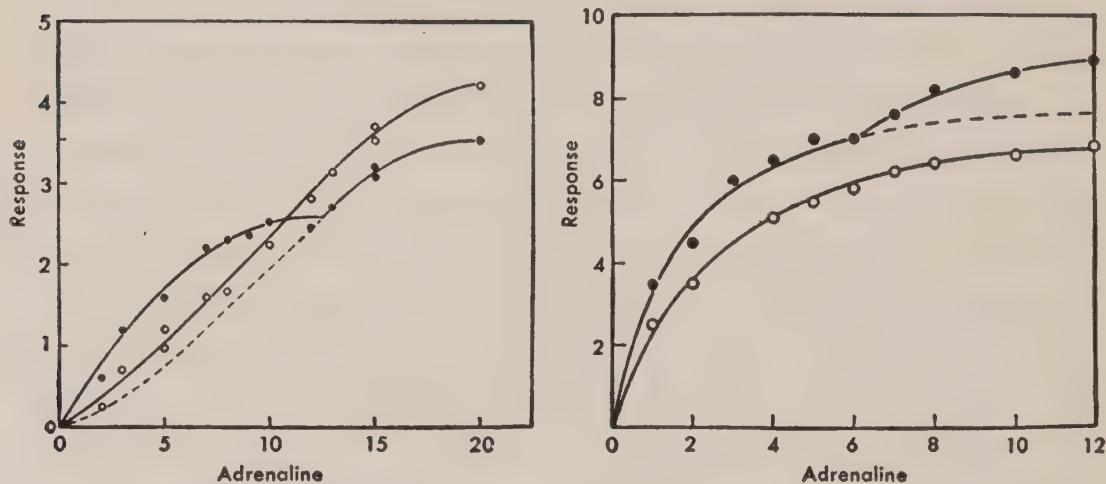


FIG. 11 (left). Dose-response curves of the nictitating membranes of a cat, for adrenaline. Ordinates: amplitude of isotonic responses in cm., magnification 20-fold. Abscissae: doses of adrenaline injected (in  $\gamma$ ). Dots: membrane partially denervated by the section, several days before, of some of the postganglionic fibers of the corresponding superior cervical ganglion; the broken line is the inferred extrapolation of the upper segment of the curve. Circles: normal control membrane of the opposite side. (Klopp, 1940.)

FIG. 12 (right). Dose-response curves for adrenaline. Ordinates and abscissae as in Fig. 11. Dots: partially denervated membrane; the broken line is the inferred extrapolation of the lower segment of the curve. Circles: totally denervated control membrane of the opposite side. (Klopp, 1940.)

interpreted if it is assumed that partial denervation leads to the sensitization of a fraction of the total number of cells in the muscle. The lower segment of the curve would then be the result almost exclusively of a contraction of the sensitized elements. Large doses of adrenaline on the other hand would activate not only the sensitized but also the normal elements, so that the response would correspond to that

of the normal side. These results, obviously, are not in accord with the concept that smooth muscle forms a single unit, syncytial in structure.

In 1921, Cannon and Uridil observed that when sympathetic impulses affect the liver there passes off from it into the circulating blood a substance which acts like adrenaline in causing a rise of blood pressure and an acceleration of the completely denervated heart. Ten years later Cannon and Bacq (1931) found that a similar adrenaline-like substance (sympathin) could be evoked by stimulating sympathetic fibers in other parts of the body. That denervation sensitizes the nictitating membrane to the action of sympathin was shown by Rosenblueth and Cannon in 1932. As in the case of adrenaline, decentralization causes a lesser degree of supersensitivity to sympathin than does denervation. Thus, in 1936 Partington compared the influence of sympathin on the two nictitating membranes, one denervated, the other decentralized. In order to exclude any action of adrenaline, the right adrenal gland was removed and the left denervated. Various methods were used to stimulate sympathetic nerves and thereby cause an appearance of sympathin in the blood stream—emotional excitement, vigorous activity, hypoglycemia, and exposure to cold. Except during periods of stimulation both membranes protruded equally over the eyes. During stimulation the denervated membrane was markedly contracted but at no time was there any retraction of the membrane which was decentralized. Complete sympathectomy abolished the response.

The supersensitivity of the denervated membrane is not restricted to adrenaline or sympathin, as shown by Rosenblueth in 1932 (*a*). Both acetylcholine and pilocarpine can cause contractions of normally innervated membranes. These contractions are not due to a discharge of nerve impulses from the superior cervical ganglion, for they may be registered after excision of that ganglion. They are not caused by adrenaline secreted by the adrenal medullae,

for adrenalectomy does not suppress them. They indicate direct effects of the substances mentioned on the smooth muscle of the orbit. The responses are markedly increased after denervation of the membrane. Again, Bacq and Rosenblueth (1934) found that calcium and potassium ions cause contractions of the membrane and that the responses are greater on the denervated than on the control side. And in 1936 Bacq reported that some days after removal of the superior cervical ganglion in dogs and cats, various substances—including arterenol, tyramine and other mono-phenolic amines, as well as aromatic amines which are not phenolic—had a greater effect on the denervated than on the normal membrane when the two registered their contractions simultaneously.

*The Iris Retractor.* Already, in the review of the historical development of knowledge on sensitization to adrenaline, the “paradoxical response of the pupil” has been recognized as due to an increased susceptibility of the iris when it has been deprived of its immediate nerve supply. Little more need be mentioned than reference of recent testimony. In accord with the observations of Meltzer and Auer (1904) were the results obtained on cats in experiments by Heinbecker (1937). His report on the time required for the full development of supersensitivity to adrenaline of the sympathetically denervated iris shows a gradual increase to a maximum which is attained between eight and ten days after the operation. This is in fair accord with the observations of Hampel on the nictitating membrane. The increased sensitivity of the iris retractor, when once attained, remains constant, according to Heinbecker, for a period of more than a year; beyond that time observations were not continued. Severance of preganglionic fibers resulted in a similar development of sensitivity, but the final degree which was reached in the decentralized iris was only 10 or 20 per cent of that developed after denervation. Heinbecker estimated that the increase of sensitivity of the denervated nictitating membrane is at least twice as great as that of the denervated

iris retractor. He reported that the smooth muscle of the sensitized iris became further sensitized by hypophysectomy and after thyroid feeding; the sensitivity was decreased after removal of the thyroid gland or the adrenals.

In 1940, Bender and Weinstein observed that in the cat the normal iris or the iris which had been recently decentralized was retracted only when the intravenous injections of adrenaline were as much as  $5\gamma$ ; on the other hand, the iris deprived of its immediate sympathetic supply was dilated with much smaller doses. The lapse of time was important. Whereas  $5\gamma$  was the threshold dose shortly after the operation, the minimal dose of adrenaline for detectable dilation that was required later, as the sensitivity increased, was as little as  $0.08\gamma$ . If the iris was completely denervated by the removal of both sympathetic and parasympathetic nerve supplies, the threshold dose was only  $0.02\gamma$ , an extraordinary exhibit of sensitization. And when, instead of being injected intravenously, the adrenaline was introduced into the common carotid artery on the denervated side, the amount required for minimal dilation was only  $0.0005\gamma$ !

The denervated iris of the monkey, compared with that of the cat, according to Bender and Weinstein, is much less supersensitive to adrenaline. The pupillary enlargement is never conspicuous or maximal on the denervated side, though the denervated iris does respond to relatively large doses ( $50$  to  $200\gamma$ ), while the normally innervated iris remains unaltered.

Meltzer and Auer reported definite withdrawal of the denervated iris of the rabbit when adrenaline was applied locally by instillation into the conjunctival sac; a similar effect was observed in the cat. In accord with the observations of Meltzer and Auer are the experiments of Sachs and Heath (1940), in which tests were made with isolated strips of the iris submerged in various concentrations of adrenaline, even as low as  $1:200,000,000$ . The records from these strips, when contracted by adrenaline, revealed that the retractor muscle 16 or 18 days after denervation is much more sensitive than

that which has not been denervated. Unfortunately these various types of experiments are not reliably comparable because different ways of administering adrenaline were employed—by intravenous or intra-arterial or subcutaneous injection, or by immersion. They are all consistent, however, in displaying supersensitivity after sympathectomy.

Like the nictitating membrane, the iris retractor, when denervated, is sensitized to other chemical agents than adrenaline. In a study of responses to the synephrines, Drake, Renshaw and Thienes (1939) found that in the rabbit this muscle was supersensitive also to epinine, cobe-frine, neosynephrine, and others. Similar results were obtained in the cat.

## CHAPTER IV

### SMOOTH MUSCLE: SYMPATHETIC STIMULATION (Continued)

#### BLOOD VESSELS AND PILOMOTORS

*Blood Vessels.* Meltzer and Meltzer (1903 *a*) called attention to a remarkable phenomenon observed in the ears of adult rabbits from which the superior cervical ganglion had been removed on one side. They gave what we now recognize as a huge dose of adrenaline (as much as 1 mgm. intravenously). The effects in the two ears were equal, but on the operated side the contraction of the vessels lasted "incomparably longer" than on the normal side. The experiments were more decisive when the injection was given subcutaneously (Meltzer and Meltzer, 1903 *b*). They still gave large doses (10–30 minims, 1:1000, i.e., up to nearly 2 mgm.); but in these conditions the adrenaline passed slowly into the blood stream. They noted that "medium" doses (i.e., about 1 mgm.) induced a distinct constriction of the vessels on the operated side and a dilation on the unoperated side.

The sensitization of the blood vessels of the ear after section of the sympathetic nerves was also noted by Elliott in 1905. In both cats and rabbits he found a "greater delicacy of reaction" and longer lasting constriction than normal after removal of the superior cervical ganglion or as a sequence to simple decentralization by section of the pre-ganglionic axons. He also reported that other blood vessels show the same change as the result of denervation. Thus, 7 days after removal of both stellates, the inferior mesenteric and the semilunar ganglia in a cat, the injection of adrenaline (6γ) evoked an abnormally prolonged reaction

from the intestinal blood vessels. The sensitization of the denervated ear vessels of the rabbit was confirmed by Lichtwitz and Hirsch in 1910. In the dog also, sympathectomy of the blood vessels of the hind leg results, after time for nerve degeneration, in more intense action of adrenaline than on the control vessels of the opposite side (Dániélopolu, Aslan and Marcou, 1932).

The supersensitivity of denervated blood vessels is not limited to the reactions to adrenaline. Thus, Dale and Richards reported in 1918 that the sympathectomized vessels of the cat respond to abnormally small amounts not only of adrenaline but also of histamine and acetylcholine. In accord with this report Grant (1935) found that not only adrenaline but also pituitrin, histamine and ergotoxine, in suitable doses, can produce considerable constriction of the denervated blood vessels of the rabbit's ear without altering the size of the vessels on the normal side. Similar observations were made by LeCompte (1941), who quantified the effects by measuring the drop of temperature due to vasoconstriction and also by measuring the diameter of the blood vessels seen directly under the microscope.

That the sensitized vessels of the ear of the rabbit can be affected by circulating adrenal secretion was shown by White, Okelberry, and Whitelaw in 1936. They observed that a struggle of the animals caused the denervated vessels to be markedly constricted and that after elimination of the adrenals a similar struggle produced a barely detectable change in the vascular state.

Another mode of revealing sensitiveness of denervated blood vessels was demonstrated in experiments by McCloskey, Co Tui, Mulholland and Wright (1937). They deprived of its vasomotor nerves a skin area in a dog's leg. The vessels became so sensitized that when adrenaline ( $250\gamma$  in 1 cc.) was injected subcutaneously, the vasoconstriction was intense and prolonged enough to produce necrosis of the skin. The dose which caused necrosis where blood vessels were supersensitive had no permanent effect when injected

similarly under the normal skin. Interestingly, the skin areas distal in the leg were relatively more sensitive than the proximal areas.

White, Okelberry, and Whitelaw used the cooling effect on surface temperature as an indicator of vasoconstriction. They noted in the sympathectomized fingers of a monkey, which was given an injection of adrenaline, that a drop of temperature occurred from 86° to 74.5° F. (30° to 23.6° C.), without any change on the normal side. The dose of adrenaline was large, but with half that amount an identical response was obtained. Excitement of the animal resulted in a drop from 86° to 81° F. (30° to 27.2° C.), a response observed whenever the animal became irritated or frightened. The next year Ascroft (1937), using the same method as was employed by White and his collaborators, confirmed their results in monkeys. He estimated that the denervated vessels become approximately 10 times as sensitive to adrenaline as the vessels on the normal side, and about 3 times as sensitive as decentralized vessels.

The removal of the superior thoracic ganglia in the treatment of Raynaud's disease, when the operation is done first on one side, allows observations to be made on the effect of denervation on the blood vessels of human beings. In 1934, Freeman, Smithwick, and White made use of this opportunity. Into an arm vein a Y-shaped cannula was fastened in such manner that either warm normal salt solution or a weak solution of adrenaline could be injected. The amount of the hormone injected varied from 0.1 to 0.3 γ per kilo per minute. The rate of the injection was governed by the degree of reaction, as estimated from the pulse rate, from the blood pressure, and from subjective symptoms. No untoward reactions were encountered. There was a drop of nearly 5.0° C. in surface temperature in the extremity where the blood vessels had been rendered sensitive by sympathectomy, a change associated with cyanosis of the fingers. In the other extremity, where nervous control of the blood vessels had been freshly blocked by injection of novocaine around

the ulnar nerve, the temperature did not drop. Observations made on seven other patients who were similarly studied revealed the same supersensitivity of the denervated vessels as compared with those on the normal side.

The sensitized vessels in man are not only affected by injected adrenaline; they are responsive also to adrenaline secreted from the adrenal medulla. In previous pages there has been reference to adrenal secretion induced by struggle and emotional excitement. Observations carried on for many years in the Harvard Physiological Laboratory have demonstrated that adrenaline is discharged in extra amount from the adrenal glands in emotional excitement, in pain, in hypoglycemia, in vigorous muscular activity and on exposure to cold. The record in Figure 13 was obtained from a patient studied by Freeman, Smithwick and White (1934). Hypoglycemia resulting from an injection of insulin, known to provoke an output of adrenaline, was accompanied by a drop in the surface temperature on the sensitized side while the temperature on the other, normal side actually rose. After orange juice and sugar were swallowed, the low temperature of the sensitized side was soon restored to its initial level. Similar responses to injections of insulin were noted in five other subjects. That the adrenal secretion due to cold may produce vasoconstriction of the sensitized vessels in man was proved by Freeman (1935). He found that chilling of the body, such as induces gooseflesh by sympathetic stimulation of the pilomotor muscles, causes a constriction of denervated vessels. This effect is not due to direct action of cold on the sensitized vascular muscles, for it appears when the hands are maintained at a constant temperature while icebags are applied to both supra-clavicular regions. Similarly Grant and Pearson (1938) found that when one of their patients struggled against resistance for a minute there was contraction of his denervated blood vessels, as indicated by a drop of 3° C. in the temperature of the foot. And White, Okelberry, and Whitelaw report seeing the hands of an unusually sensitive woman,

that had been sympathectomized for treatment of Raynaud's disease, become cyanotic when her condition was demonstrated before a group of medical students—a consequence of an emotional outflow of adrenaline.

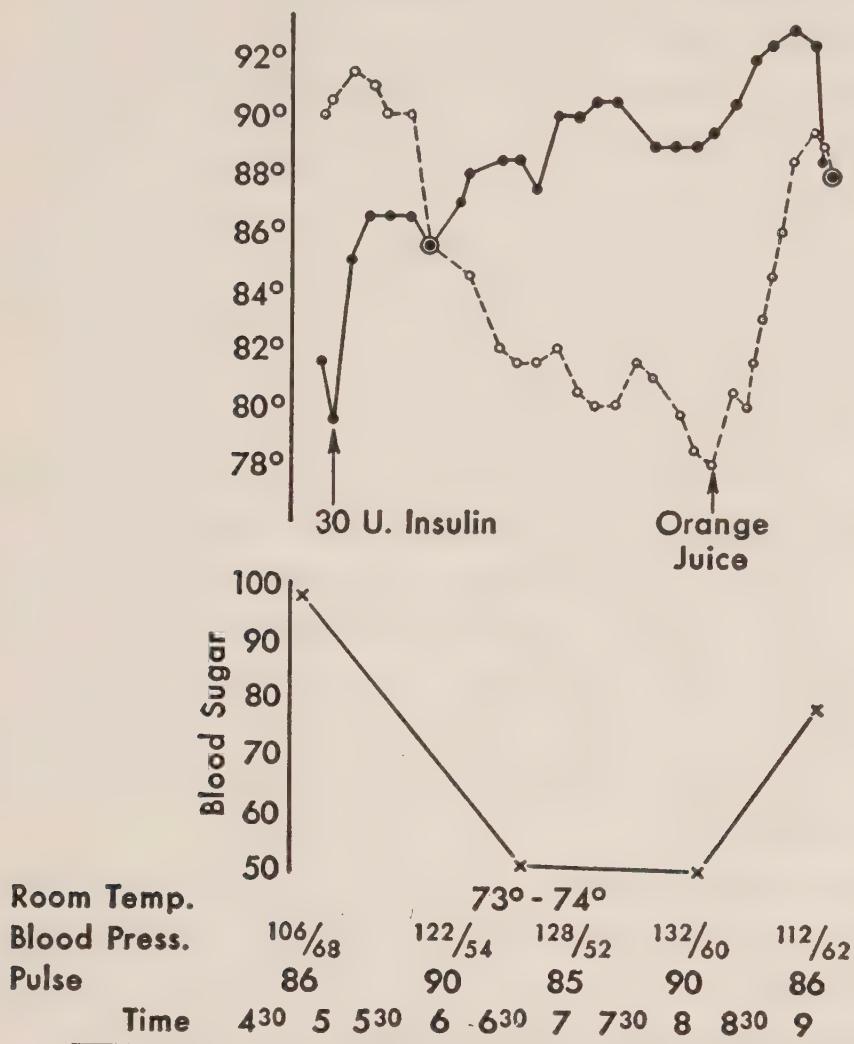


FIG. 13. Vasomotor responses in the hands of a patient when insulin induced secretion from the adrenal medulla by lowering blood sugar. Vasoconstriction (dotted line) is revealed by a fall in temperature of the sympathectomized finger, and vasodilation (solid line) by a warming of the corresponding normal finger. (Freeman, Smithwick, and White, 1934.)

In 1938 Hinsey and Phillips reported observations in which sympathetic resections did not result in supersensitivity of the denervated blood vessels to the action of adrenaline. This negative testimony was amended, however, the following year by Phillips, Hinsey and Hardy, who found an increased sensitivity of the blood vessels of the

sympathectomized foot pad of the cat, as compared with the normal.

The only objection which has been raised against the evidence that sympathectomy sensitizes blood vessels to adrenaline has come from Fatherree and Allen (1938). They used temperature changes in human fingers as an indicator of vasoconstriction. Like Hinsey and Phillips, they emphasized the importance of initial dilation of vessels and an external temperature equal to that of the skin. Their results were very variable and consequently they expressed doubt as to the reliability of temperature changes as evidence of vasoconstriction. The doses of adrenaline ranged from 5 to 11 cc. per minute, in a dilution of 1:250,000. This is to be compared with injections of 2 to 6 cc. per minute of the same dilution in the case reported by Freeman, Smithwick, and White. Unfortunately Fatherree and Allen did not state the rate of flow of adrenaline into the blood stream in terms of kilo body weight. From the information given it is not clear that proper attention was paid to the use of *submaximal doses*. Unless that condition is scrupulously regarded, as already emphasized, discriminative results cannot be obtained.

Observations on warm-blooded animals have been confirmed by experiments on frogs. Okuyama (1926), after severing on one side the sciatic nerve (and, of course, its contained sympathetic fibers), perfused the blood vessels of the two hind legs with Ringer solution and recorded the outflow from a vein on each side. Tests were made up to seven weeks after the denervation. When a small amount of adrenaline was added to the Ringer solution he found that in some experiments the effect was greater on the denervated than on the normal side after one week. If the interval was longer—two, three or four weeks—there were no exceptions; there was always indication of greater sensitiveness of the denervated vessels compared with the normal. The degree of sensitization was from four to eight times that of the normal side. These observations are in harmony with results

which were reported by Schneider (1926) in a similar study of the denervated blood vessels of the frog.

In the examples of contraction of denervated blood vessels that occurs as a consequence of hypoglycemia, cold, struggle and excitement, the effect was attributed solely to augmented adrenaline secretion. As revealed by Partington's observations on the sensitized nictitating membrane (see p. 31), sympathin, which circulates in the blood in these same conditions, must be taken into consideration. It is probable that other structures, rendered supersensitive by sympathectomy, become specially responsive to sympathin. In 1935, Grant reported that after removal of the adrenal glands or the pituitary body (because pituitrin contracts blood vessels), the previous reactions of the normal and the sensitized denervated ear of the rabbit, when the animal struggled for example, were unaltered. Grant admitted the possibility that the sensitive vessels might be affected by circulating sympathin. Since sympathin is derived from effector organs when they are excited by sympathetic impulses, it diffuses into the blood stream at approximately the same time as adrenaline is discharged from the adrenal medulla. In most instances sympathin and adrenaline collaborate in producing effects in structures which are under sympathetic control. That sympathin alone, coming from various parts of the body, can cause contraction of sensitized blood vessels was shown by LeCompte (1941) in experiments on the denervated vessels of the ear of the cat and the rabbit. By recording the changes of surface temperature of the ear under standard conditions, he learned that struggle induces constriction of the vessels. This constriction occurred in the absence of the adrenal glands, and in two instances it failed to occur when, in cats, the sympathetic nervous system had been completely removed. These studies indicate that there are conditions which augment the secretion from the adrenal medulla and also liberate sympathin into the blood stream and thus can explain the anomalies which have been reported regarding the occasional and temporary return

of contractions of denervated blood vessels long after they have been deprived of their sympathetic nerves.

It will be recalled that Hampel demonstrated that sensitization of the smooth muscle of the nictitating membrane after sympathectomy develops faster at first and later more slowly. LeCompte (1941) measured the extent of constriction (i.e., decrease in diameter) of the rabbit's ear vessels, on successive days after they were denervated, in response to struggle and also in response to a standard dose of adrenaline (Figure 14). Again it was demonstrated that there is a rapid increase of sensitivity during the first week after the operation and thereafter a more gradual development.

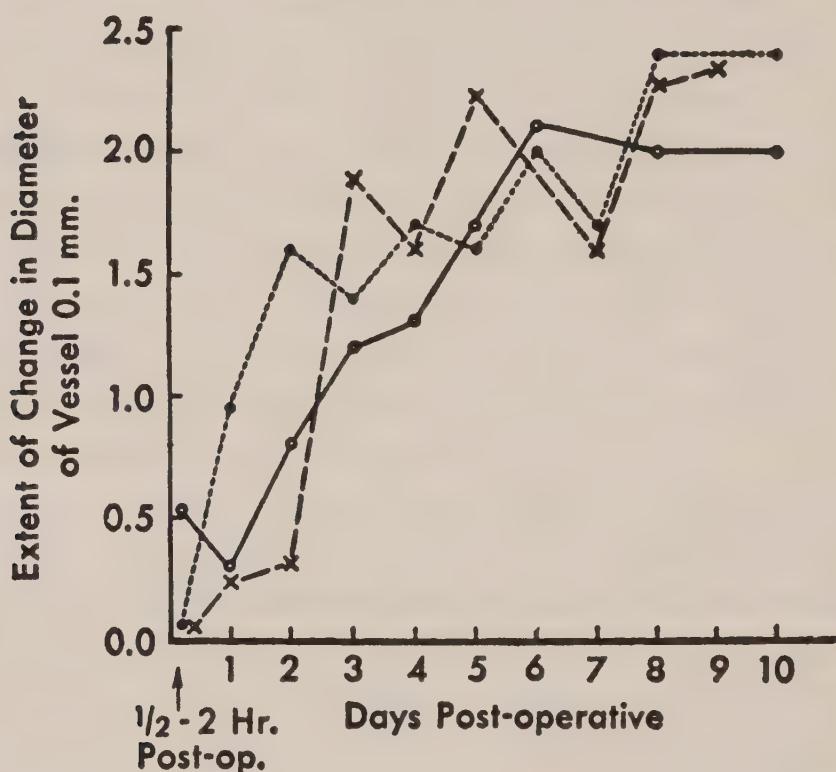


FIG. 14. Graphs of the increasing degree of constriction of the rabbit's ear vessels in response to struggle (dotted line) and to a standard dose of adrenaline (dash line) as days pass after denervation. The solid line represents the recovery of tone of a vessel after denervation. (LeCompte, 1941.)

In relation to the time-course of increasing sensitivity the duration of the state is of interest. Fatherree, Adson, and Allen (1940) have declared that supersensitivity to adrenaline due to denervation will gradually disappear as time elapses. This testimony is quite out of accord with that of

other investigators. For example, McCloskey, Co Tui, Mulholland, and Wright (1937) found that after sympathectomy there was an increased sensitivity to adrenaline that lasted at least two years in the dog on which they had operated. And in a remarkable investigation reported by Essex, Herrick, Baldes, and Mann (1943) evidence was obtained showing that sensitization persists in this species for more than ten years. Blood flow was measured in both femoral arteries simultaneously, under local infiltration anesthesia, before sympathetic ganglionectomy and at various times after the operation, from fifteen days to ten years and two months. Whereas the flow was twice as great in the denervated vessel as in the opposite control vessel fifteen days and as long as ten months and twenty-five days after the denervation, it was almost the same in the two vessels at the end of nine and ten years. Nevertheless the denervated femoral artery remained strikingly supersensitive at the end of the decade. A small injection of adrenaline, which had only a slight effect on the innervated artery, caused so extreme a constriction on the denervated side that the blood flow was reduced to zero for at least two minutes. This result was repeatedly observed by the indirect method which was routinely employed; it was directly confirmed by opening a small vessel of each leg and noting that when adrenaline was given, the bleeding from the sympathectomized side was stopped while that from the control side appeared to be actually augmented. The effects on the flow were confirmed also by simultaneous plethysmographic records of the two hind feet. There was a transient decrease of the volume of the innervated foot in response to small doses of adrenaline, in contrast to a very prolonged decrease on the denervated side.

In the treatment of Raynaud's disease the earlier practice of the surgeons was to remove the first lumbar ganglia in order to disconnect the blood vessels of the toes from the central nervous system, and to remove the upper thoracic and lower cervical ganglia in order to deprive the smooth

muscle of the fingers of its sympathetic supply. Thus the toes were decentralized and the fingers were denervated. As already stated (p. 23), Elliott (1905) emphasized that although decentralization, like denervation, causes supersensitivity of smooth muscles to adrenaline, the degree of sensitization attained by section of the preganglionic fibers is less than that which results from the degeneration of the postganglionic axons. Hampel's observations on the nictitating membrane confirmed Elliott's inference (see Figures 7 and 8). The dramatic case of the unusually nervous woman, mentioned above, who was operated upon for Raynaud's disease and who had a cyanosis of the denervated fingers when emotionally upset during a clinical demonstration (see p. 38), proved to be critical; simultaneously in such circumstances the decentralized toes remained warm and normal in color. Inquiry among surgeons disclosed that this difference between decentralized toes and denervated fingers was not uncommon in cases of Raynaud's disease. The difference in sensitivity to circulatory vasoconstrictor agents provided a reasonable explanation for the unsatisfactory results of surgical treatment of Raynaud's disease in the upper extremities and the excellent results obtained in the lower extremities (see Figure 15). A modification of the technique for sympathectomy of the upper extremities, so that only the preganglionic portion of the vasoconstrictor path to the vessels of the fingers was interrupted, was suggested and applied by Smithwick (1936). A year earlier Telford had made the same proposal. It resulted in a striking clinical improvement, i.e., in only a moderate sensitization of the fingers such as is seen in the decentralized vessels of the lower extremities.

The evidence and testimony of White and Smithwick (cf. 1941) has been questioned by Fatherree, Adson, and Allen (1940). Into patients treated for Raynaud's disease they report injecting adrenaline intravenously; in some cases with a dilution of 1:10,000 and with an amount varying from 0.17 to 1.0 cc., without stating the rate of injection;

and in other cases, without mentioning the dilution, varying the rate from 2.7 to 10.8 cc. per minute. From their results they concluded that both preganglionic and postganglionic sections caused increased sensitivity of digital arteries, but

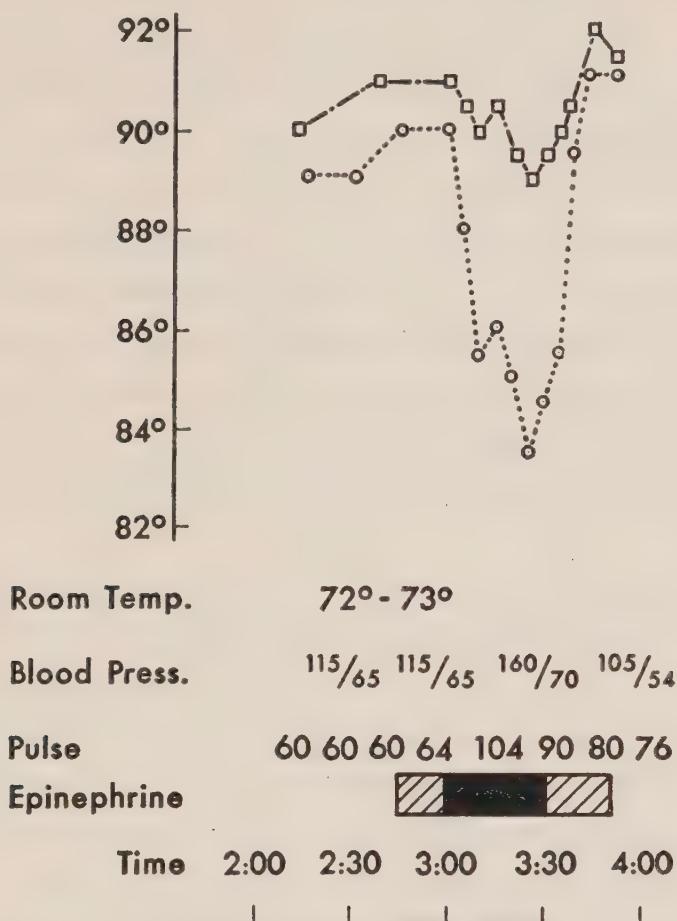


FIG. 15. Effect of an injection of adrenaline on skin temperature in a patient with decentralized vascular muscles of the toes (preganglionic fibers cut 16 months previously) and denervated muscles of the fingers (sympathetic ganglionectomy 6 months previously). Note the markedly greater sensitiveness of the denervated (dotted line) as compared with the decentralized (dot-dash line) smooth muscle. (White, Okelberry, and Whitelaw, 1936.)

to about the same degree under the two conditions. It is clear that this testimony is not in accord with other clinical evidence and with numerous experiments on lower animals. Again the question may be raised as to whether there was due concern for use of *submaximal doses*.

A nice comparison in the fingers of a man, showing the difference between preganglionic and postganglionic sympa-

thectomy, has been made by Atlas (1941). His patient was suffering from a paralysis of the median nerve, which contains vasoconstrictor postganglionic fibers to all but the little finger—i.e., the other fingers were in effect denervated. In an effort to abolish a painful condition resulting from the nerve lesion, Atlas severed preganglionic fibers normally distributed to the hand, thus decentralizing the blood vessels of the little finger. Ten months later an intravenous injection of adrenaline caused the surface temperature of the denervated fingers to drop  $2.3^{\circ}$  C., a change to be compared with a drop of only  $0.1^{\circ}$  C. in the decentralized little finger. The difference in effect on fingers of the same hand, registered simultaneously, offers striking confirmation of the claims of White and Smithwick that decentralization is much preferable to denervation as a means of interrupting sympathetic paths.

*Pilomotors.* As early as 1901, Langley reported that after excision of the superior cervical ganglion an extract of the adrenal gland produced a slight erection of hairs in the face area. Taking this observation as a hint, Elliott (1905) decentralized the smooth muscle of the head region on one side in the cat by excising the left stellate ganglion. About two weeks later he proved that there had been complete degeneration of the preganglionic fibers in the cervical sympathetic trunk. Now when adrenaline was injected the left iris was retracted to a greater degree and for a longer time than the right; and also the hairs on the left side of the neck were well erected, whereas those on the right side could not be seen to move at all. From these results it is apparent that not only do sympathectomized pilomotor muscles manifest supersensitivity to adrenaline but decentralization has a similar effect.

In accord with these inferences, we found in some unpublished observations (Cannon and Rosenblueth, 1934) that unilateral removal of the lumbar sympathetic chain in cats renders the pilomotors of the tail supersensitive to adrenaline and to acetylcholine.

## CHAPTER V

### SMOOTH MUSCLE: SYMPATHETIC INHIBITION

#### INTESTINE, UTERUS, AND BRONCHIOLES

There has been question as to whether removal of nervous influences which cause *relaxation* of smooth muscle induces an increased degree of sensitiveness. Elliott (1905) reported that degenerative section of preganglionic sympathetic fibers results in a more prolonged inhibition of the smooth muscle of the cat's bladder by adrenaline, "but perhaps not any greater irritability." Indeed, he testified, "greater ease of inhibition as a sequel to denervation I have not observed." The same year, Langley and Magnus published the statement that after denervation the intestine yields normal responses to adrenaline. Mitsuda (1924) declared that adrenaline has a *stimulant* action on the denervated intestine; on the other hand, Shimidzu (1924) denied such action and claimed that there is an inhibitory sensitization, a claim stated incidentally by Modern and Thienes (1936) in a brief report on vagal and sympathetic endings in the intestine. These observers used as controls for the denervated loops of intestine a normally innervated intestinal strip from the same animal. As Alvarez (1914) has shown, however, adrenaline has quantitatively different effects on different segments along the length of the gut, less in the upper and more in the lower portions. A comparison of a denervated segment with only one control, unless from a neighboring part of the tract, may not be satisfactory: only double controls, taken above and below the denervated segment, can wholly obviate this objection.

*Intestine.* Experiments to test further the sensitization of smooth muscle which is inhibited by sympathetic impulses

were undertaken by Luco (1937). In rabbits, under ether anesthesia, a segment 6 to 8 cm. long, about midway in the length of the small intestine, was denervated by aseptic section of the nerve fibers accompanying the related branch of the mesenteric artery. To insure complete destruction of the nerve filaments, concentrated phenol was painted on the branch vessel at the point of section; excess of the phenol was removed with alcohol and the area then washed with normal salt solution. The rabbits were killed 2, 5, 6, 8 and 9 days after denervation. As rapidly as possible the middle portion of the denervated strips (3 to 4 cm.) was excised. Two other segments of normal intestine, each 3 to 4 cm. in length, also were removed, one from above, the other from below the denervated portion. The three segments were placed in the same bath, 250 cc. of Ringer-Locke solution, with a temperature maintained at 38° C. One end of each segment was attached to an L-shaped glass rod near the bottom of the container; the other end was attached to a writing lever. After a preliminary record was written, adrenaline (1 to 10 γ) was added to the bath by means of a pipette and was diffused uniformly by stirring.

The spontaneous contractions of the denervated segment appeared later than those of the normal segments—often the delay was twice as long. The effect of adrenaline was consistently greater on the denervated segment than on the two controls, as shown in Figure 16. The greater sensitivity was evidenced by a total inhibition of the rhythmic contractions, a marked loss of tone, and a longer duration of the response. Incidentally, it is significant that there was no sensitization of the denervated segment when acetyl-choline was added to the bath.

The observations of Luco were confirmed by Youmans (1938) who in dogs made Thiry and Thiry-Vella fistulae from loops of the jejunum, and recorded the intestinal activity by means of a balloon inserted into the loop and connected with a mercury manometer. In various ways some of these loops were deprived of their sympathetic connec-

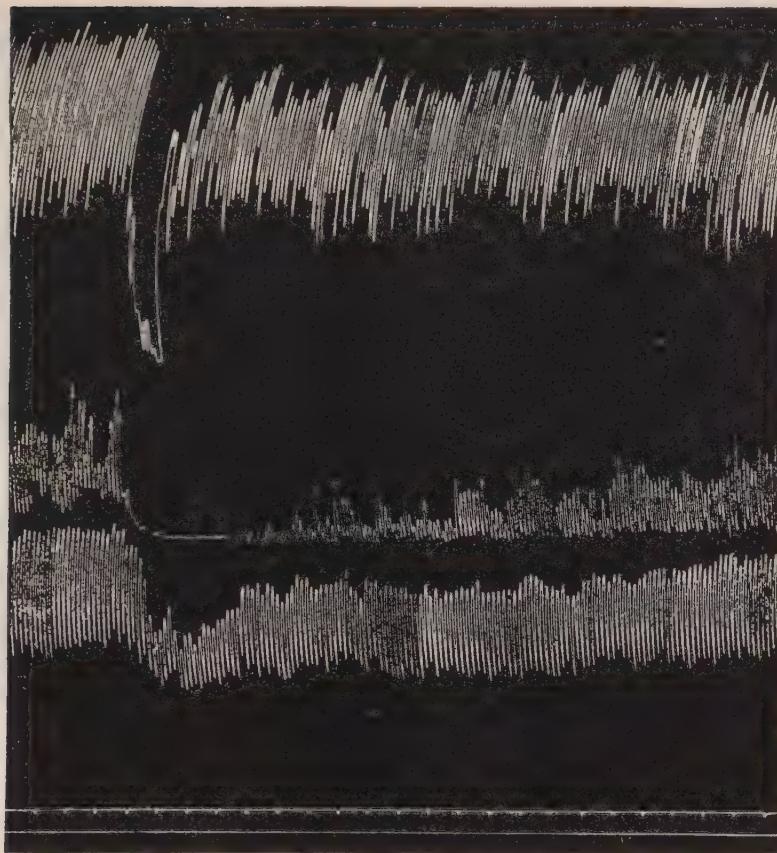


FIG. 16. Records of three segments of rabbit intestine. Top record, upper control segment; bottom record, lower control segment; middle record, middle segment denervated seven days previously. Effects of adding at lower signal  $1\gamma$  of adrenaline to the salt-solution bath (250 cc.) which contained all three segments. Time signal: 30-sec. intervals. (Luco, 1937.)

tions. In some animals there were two such preparations of the intestine, one properly innervated, the other denervated. Thus, side by side, from adjacent sections of the intestine, there were a test strip and a control. In both there was a normal blood supply and an absence of the disturbing influences which modify conditions when the abdomen is opened. Youmans found that the innervated loop was quite unaffected by the injection of adrenaline at a rate which just produced complete inhibition of the rhythmic contractions of the denervated intestinal loop. Indeed, the minimal rate of injection of adrenaline capable of producing a short period of complete inhibition in the denervated loop could be doubled and in one animal trebled without affecting the innervated control (see Figure 17).

Injection of adrenaline at a constant rate of  $0.14 \gamma$  per

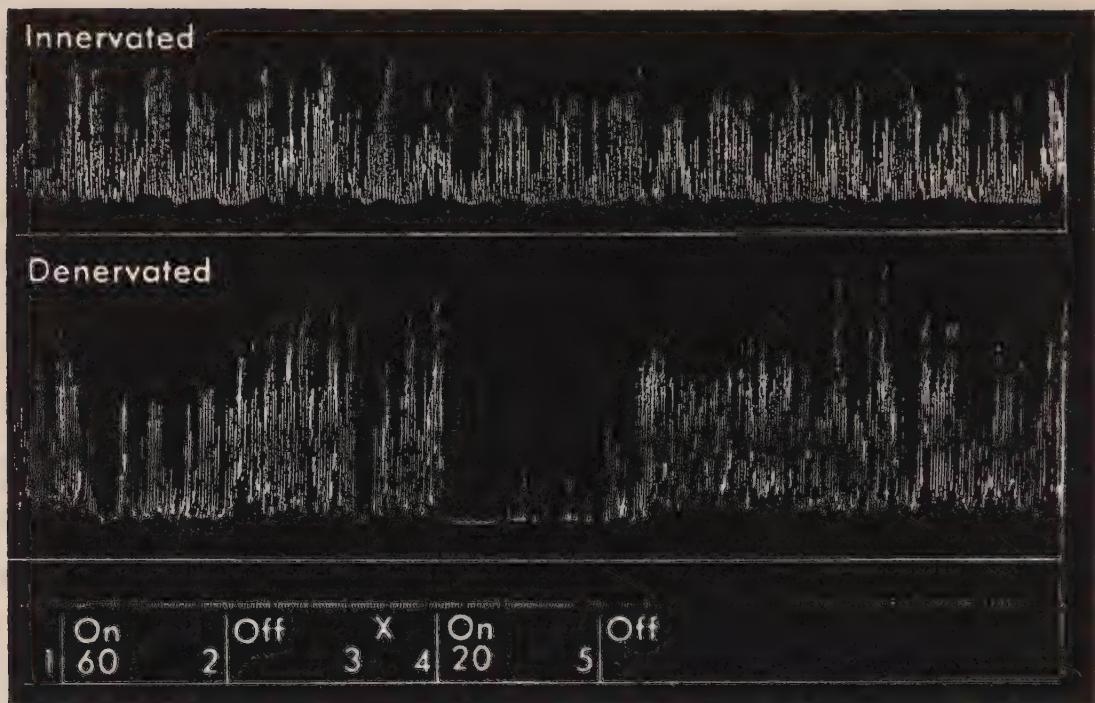


FIG. 17. Contractions of an innervated and of a denervated jejunal Thiry fistula of a dog. Between 1 and 2 a solution of adrenaline was injected intravenously at the rate of  $2\gamma$  per min. Between 4 and 5 the same solution was injected three times as fast. At 3 a few drops of the solution were introduced while washing the needle. Weight of dog: 14.5 kgm. Time signal: 5-sec. and 1-min. intervals. (Youmans, 1938.)

kilo per minute was sufficient to produce in one dog (weighing 14.5 kg.) a 40-second period of complete inhibition of the motility of the denervated Thiry fistula. This amount, if a minute-volume of blood flow of 1500 cc. is assumed for the dog, would result in a further dilution of the adrenaline to 1 part in 750,000,000 parts of blood. In other animals an injection rate of 0.20 or  $0.28\gamma$  per kilo per minute was necessary to produce complete inhibition. The increase of sensitivity varied from three- to seven-fold. Wada and Kanowoka (1935) estimated that the minimal effective rate of injecting adrenaline for increasing the pulsations of the denervated heart and for raising blood pressure in non-anesthetized dogs (with adrenal glands demedullated) varied from 0.20 to  $0.30\gamma$  per kilo per minute. Youmans did not abolish possible secretion from the adrenal medulla in his tests and therefore did not demonstrate whether the rate of injecting adrenaline required to inhibit the denervated

intestine would be affected by adrenal secretion. He concluded that the denervated intestine of the dog is one of the most sensitive of indicators for the presence of adrenaline in the blood. Quite possibly, observations on the sensitized iris might have shown that it is a more delicate indicator than either the sensitized blood vessels or the sensitized intestine. In a comparative study on the rabbit, Shimidzu (1924) found that the denervated ear vessels were from 2 to 8 times more sensitive than the innervated, the smooth muscle of the sympathectomized intestine 2 to 4 times more sensitive than the innervated controls he used, and the iris, after superior ganglionectomy, 16 to 40 times more sensitive than the normal iris of the other eye.

Youmans noted that the sensitized smooth muscle of the intestine becomes less sensitive as an adrenaline injection is continued. This is in accord with the testimony of Simeone (1938 *b*) that there is "a slight but definite decrease in the magnitude of the contractions of the sensitized nictitating membrane to test doses of adrenaline after its prolonged administration." The membrane is perhaps less susceptible to this desensitizing action of adrenaline than the intestine, for, according to Youmans, an injection rate several times as fast as the rate required to produce a short period of initial complete inhibition of the intestine is not sufficient to maintain it longer than a few minutes. There occurs a gradual escape from the inhibitory influence. Another way of showing that the sensitized muscle becomes less sensitive with continued injection was to increase gradually the rate of the injection. Thus, in one animal the denervated intestine was completely inhibited when the injection rate was suddenly changed from 0 to 0.2 γ per kilo per minute. But when the injection rate was increased by small increments, during a period of several minutes, from 0 to 0.4 γ per kilo per minute, complete inhibition did not occur. This phenomenon has theoretical importance which will be considered later.

Youmans performed experiments which led him to the

conclusion that denervated smooth muscle of the intestine is sensitized to inhibitory sympathin as well as to adrenaline. In these experiments he stimulated mechanically the rectum through the anal canal in order to elicit activity of the anal sphincter. In an animal with both vagus nerves severed above the diaphragm, one adrenal gland removed and the other demedullated, and a Thiry-Vella fistula denervated by severing the nerves in the mesentery and along the blood vessels, there was typical inhibition of the intestine during rectal stimulation. Rhythmic contractions were almost completely eliminated and the intestinal tone was decreased, as indicated by a drop in the pressure on the balloon 11 mm. of mercury below any level before or afterwards. The onset of the inhibition and the recovery from it were gradual. As when adrenaline was injected, there was a gradual escape of the intestine from the inhibition if stimulation was continued. After stimulation was stopped the amplitude of the intestinal rhythmic contractions steadily increased until they were greater than before, and with the greater amplitude there was a development of greater tone in the denervated muscle. All these effects can be duplicated by means of a continued injection of adrenaline at a proper rate. That the denervated intestinal loop is inhibited by rectal stimulation to a greater degree than the innervated loop affords evidence that a supersensitivity to inhibitory sympathin results from the denervation.

In a research on the relation of the various groups of the adrenaline molecule to its inhibitory effects on the intestine in unanesthetized dogs, Youmans, Aumann and Haney (1939) found that arterenol, cobefrin, kephrine, neosy-nephrin and synephrin all inhibit the contractions of intestinal loops arranged with a Thiry fistula. The records were taken simultaneously from innervated and denervated loops in the same animal. An injection rate was determined which produced complete inhibition of the denervated segment without significantly affecting the innervated one. They reported that within one or two weeks after post-

ganglionic denervation the intestine becomes two to eight times more sensitive to each of the compounds than it was before denervation. Sensitization of the intestines, these investigators declare, develops quite as rapidly to the inhibitory action of adrenaline and these related compounds as does the sensitization of the nictitating membrane reported by Hampel. The results obtained by Youmans, Aumann, and Haney are in accord with those reported by Drake, Renshaw, and Thienes (1939). These observers denervated loops of rabbit intestine by doubly ligating one or more branches of the superior mesenteric artery together with accompanying nerves, veins and lymphatics. After two weeks, segments of the denervated intestine and of adjacent normal intestine were excised and suspended in a warm bath of oxygenated salt solution. The addition of epinephrine, cobefrin, neosynephrin, and other synephrins to the solution resulted in a greater inhibitory effect on the denervated segments than on the nondenervated.

In 1943 Youmans, Karstens and Aumann reported that atropine has an inhibitory effect on denervated intestinal segments. The inhibitory action is not reduced by section of the vagus nerves and it is actually increased by sympathectomy. The mechanism of the sensitization was not determined; the investigators expressed the opinion, however, that it is due to the action of atropine on the neural or muscular elements of the intestinal wall, rendering the intestinal muscle unresponsive to a stimulatory action of acetylcholine being produced by the local nerve net, independent of extrinsic nerves.

An important inquiry is that of learning to what degree intestinal muscle is sensitized by decentralization as compared with denervation. According to Modern and Thienes (1936), section of the splanchnic nerves in the rabbit (i.e., decentralization) does not alter the response to adrenaline. Using the Thiry-fistula method, Youmans, Karstens, and Aumann (1942) registered from a decentralized loop of jejunum simultaneously with a denervated loop in the

same animal. The denervated loop exhibited the usual sensitiveness to adrenaline; it was from 3 to 6 times more sensitive than the loop connected with sympathetic ganglia which had been isolated by severance of the splanchnics and removal of the lumbar sympathetic chains. In other words, decentralization of a loop either had no effect or at most produced an increase of sensitivity to adrenaline that was less than 2-fold. This result indicates that disconnecting the celiac ganglia from the centers in man would probably produce less sensitization of the intestine to the inhibitory action of adrenaline than would be produced by removal of the ganglia. Either operation, however, would involve the abolition of most or all of the nerve supply of the adrenal glands. In that part of the intestinal tract which is under control of the inferior mesenteric ganglion, however, decentralization would be preferable to denervation. Little or no sensitization would result, and adrenal secretion would not be affected. It is noteworthy that, like Luco, Youmans and his collaborators found that section of the vagus nerves has a slight or no effect on adrenaline sensitivity of the smooth muscle of the jejunum.

*Uterus.* The nonpregnant uterus of the cat is inhibited by sympathetic impulses delivered through the hypogastric nerves. Observations were made by Luco (1937) on this organ and records were obtained with the uterine muscle *in situ* and disconnected from the central nervous system.

Because the hypogastric nerve of one side can produce relaxation of both uterine horns in the cat, complete unilateral denervation of one of the horns (the right) was obtained only by severing it at its vaginal end. The ovarian vessels proved to be adequate for maintaining a proper blood supply. Later, with a needle passed through the vaginal end of each horn, to serve as a fixed point, and with the ovarian attached to a lever, the contractions of the two horns could be recorded simultaneously under dial anesthesia. Both showed rhythmic contractions (see Figure 18). Stimulation of either hypogastric nerve produced relaxation only on

the left side, i.e., there was total isolation of the right horn from the nervous system. If adrenaline was injected intravenously immediately after such isolation of the right horn there was equal relaxation on the two sides. When, however, the right horn had been isolated by aseptic operation under ether and eight or nine days had elapsed before the record was taken, injections of adrenaline (0.5 to 10 $\gamma$ ) into a femoral vein elicited a greater relaxation on the denervated than on the normally innervated side (see Figure 18).

The observations on uterine smooth muscle *in situ*, after it had been sensitized by denervation, were confirmed by tests applied to the excised horns. The right horn was denervated in the manner described above. From 6 to 8 days later the two horns were excised and placed in a bath of warm salt solution and arranged to record their activities by the method employed for intestinal segments (see p. 48). The spontaneous rhythmic contractions of the two were similar. When adrenaline was added to the bath it produced a greater inhibition of the horn which had been denervated than of the other. This evidence of greater sensitivity resulting from denervation appeared either as greater relaxation or as more

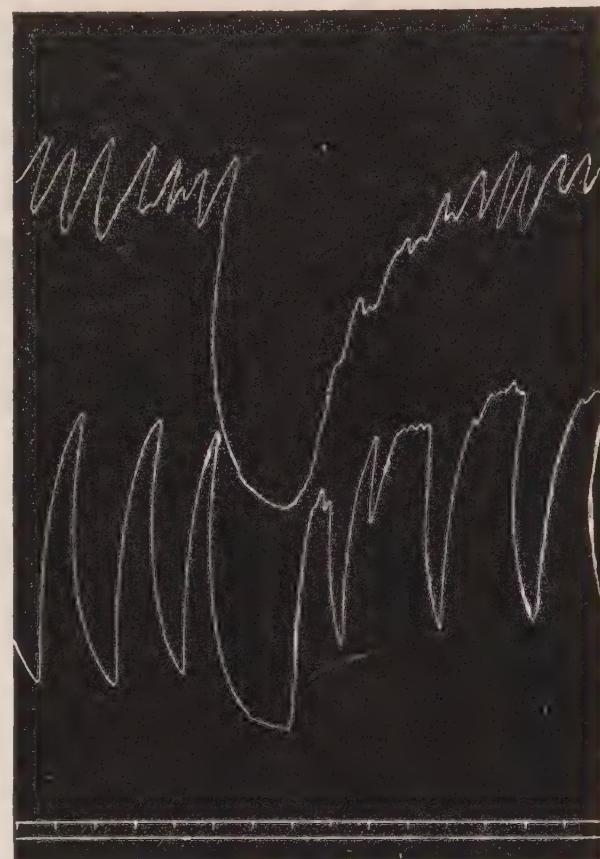


FIG. 18. Records of the two uterine horns *in situ* in a cat under dial anesthesia. Upper record, right uterine horn, denervated seven days previously. Lower record, left uterine horn, normally innervated. The lever weights and the amplification were the same for the two sides. At signal, adrenaline (2 $\gamma$ ) injected intravenously. Time signal: 30-sec. intervals. (Luco, 1937.)

marked diminution of the spontaneous contractions or both.

*Bronchioles.* The bronchioles are dilated by sympathetic impulses, and also by adrenaline as shown by its common use in the treatment of asthma. The question as to whether the bronchiolar smooth muscle, which is relaxed or inhibited by adrenaline, is rendered more sensitive by denervation was given an answer in experiments on cats by Co Tui, Burstein, and Wright (1936). They destroyed on one side the sympathetic nerve supply to the lungs, mainly from the stellate ganglia. After a lapse of time, 10 to 13 days, which permitted degeneration of the severed nerves and the development of supersensitivity, they removed the lungs with the trachea. Through the trachea, the bronchi, the bronchioles and alveoli they perfused Locke's solution at 40° C. The rate of perfusion was assumed to be related directly to the pressure of the perfusing fluid (which was kept constant) and inversely to the caliber of the conducting system, and to the resistance of the lung tissue where the fluid escaped. Since that resistance was probably not affected by adrenaline, a change in the rate of flow would be dependent upon variations in the caliber of the very small tubes through which the fluid must pass.

Control conditions were regarded as satisfactory when successive perfusions, during an arbitrary time set at 25 minutes, yielded a fairly constant value. After such controls had been established adrenaline was injected into the perfusate through the wall of the connecting rubber tube, near the trachea. Care was taken to avoid change of perfusion pressure as a consequence of the injection.

With a dose of adrenaline of about 60γ per gram of lung there was always an increased rate of flow through the denervated lung as compared with that which had not been denervated previously. This might amount to more than 125 cc. in 25 minutes. In these tests during one experiment the average increase of flow on the normal side, after introduction of adrenaline, was from 222 cc. to 231 cc.—an

increase of 4 per cent. On the sensitized side the flow was simultaneously increased from 236 cc. to 327 cc.—an increase of 38 per cent.

All these experiments, on intestine, uterus, and bronchioles, are consistent in showing that smooth muscle which is relaxed by sympathetic impulses becomes more responsive to sympathomimetic agents after it has been denervated.

## CHAPTER VI

### SMOOTH MUSCLE: PARASYMPATHETIC STIMULATION AND INHIBITION

#### IRIS AND BLOOD VESSELS

The sensitizing effect on smooth muscle of destroying its sympathetic innervation naturally raises the question as to whether depriving it of its parasympathetic connections would render it sensitive to the natural chemical mediator of parasympathetic nerves, acetylcholine.

*Iris.* Anderson (1905 *b*) proved that after section of the oculomotor nerve within the skull the paralyzed iris sphincter is sensitized to both pilocarpine and eserine, and that after degenerative section of the short ciliary nerves by removal of the ciliary ganglion the iris remains supersensitive to pilocarpine but loses its response to eserine. We now know that pilocarpine acts in some respects like acetylcholine and that eserine protects acetylcholine from destruction by cholinesterase. Engelhart's (1931) observations showed that after degeneration of the oculomotor nerve, acetylcholine disappears from the iris and the ciliary body. The failure of eserine to act in these circumstances can thus be accounted for—there is no acetylcholine to protect. From all these observations there was a high degree of probability that tests would show that the iris, disconnected from the ciliary ganglion, would prove to be sensitized to acetylcholine.

As a means of testing the effects of parasympathetic denervation on smooth muscle, the iris sphincter of the cat is ideal. The peripheral ganglion of the parasympathetic supply to the iris is located at a considerable distance from the muscle which it innervates, and not within the muscular

structure as is true of the outlying ganglia of the parasympathetic almost everywhere else in the body. Consequently the postganglionic fibers can be severed, or the ganglion can be removed, without much difficulty and without damage to the muscle itself. Furthermore, the response of the sphincter can readily be seen and measured, and control observations are easily performed by noting simultaneously the reaction of the other, normal eye.

In order to test whether the denervated iris sphincter is sensitized to acetylcholine Shen and Cannon (1936) excised the ciliary ganglion on one side in cats. All operations were performed under ether anesthesia and with strict asepsis. During the operation the cornea was protected from injury by closing the eyelids with adhesive tape. A straight incision about 3 cm. long, beginning in front of the auditory meatus and running immediately below and along the malar bone, revealed the approach to the orbit. A portion of the zygoma and of the external wall of the orbit was removed. The sheath of the orbit was then opened and its lower edge, together with the temporal muscle, was retracted with a weighted hook. The external rectus muscle was sought and drawn upward by a retractor. No ocular muscle was cut. The ciliary ganglion was readily found by tracing centrally the branch of the third nerve supplying the inferior oblique muscle. Special care was taken not to injure any of the intra-orbital vessels, for they might be important for the absorption of the drug when it was instilled and for its effect on the iris.

When a strong solution of acetylcholine (1 to 5 per cent) was instilled into the conjunctival sac, or when 1 to 5γ was injected intravenously, there was no effect on the normal iris. If the ciliary ganglion had been excised, however, or the short ciliary nerves had been severed, instillation of the strong solution caused a prompt and maximal contraction of the paralyzed sphincter. On this delicately responsive sheet of smooth muscle 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 of the sphincter. This powerful myotic action of acetylcholine could not be due to eserine, for, as Anderson showed, eserine has no effect after degeneration of the nerves—a fact explained, as noted above, by the absence of acetylcholine in the iris in these circumstances.

It will be recalled that Hampel's studies (p. 23) revealed that about two weeks are required for smooth muscle, after losing its sympathetic nerve supply, to reach its maximal degree of sensitization to adrenaline. When the parasympathetic fibers are cut, however, the sphincter of the iris shows a slight degree of change within an hour and a marked sensitization within 24 hours. This rapid change was confirmed by Keil and Root (1941), who reported that a maximal degree of supersensitivity was reached in about 4 days. This period corresponds in length to the period required for a disappearance of the action of eserine (Shen and Cannon, 1936).

The sensitivity of the denervated iris sphincter to acetylcholine is illustrated in Figure 19. In this instance the short ciliary nerve on the cat's left side had been cut 14 days before the test. The operation was followed by full retraction of the iris and by absence of a light reflex. In an acute operation under ether anesthesia the cat's right ciliary ganglion and both superior cervical ganglia were removed. Of course there was no immediate sensitization. Both pupils were maximally dilated. Now the animal's head was held in a fixed position on a horizontal bar between the upper and the lower jaws. With care to assure the instillation of the same amount of eserine into the conjunctival sac of each of the two eyes, two drops of eserine (1.0 per cent) were let fall from a fine pipette, each drop amounting to about 0.04 cc. After a delay of 5 minutes there was no change in the size of the pupils (see C, Figure 19). Thereupon two drops of acetylcholine chloride (0.01 per cent) were, with similar care, instilled into each conjunctival

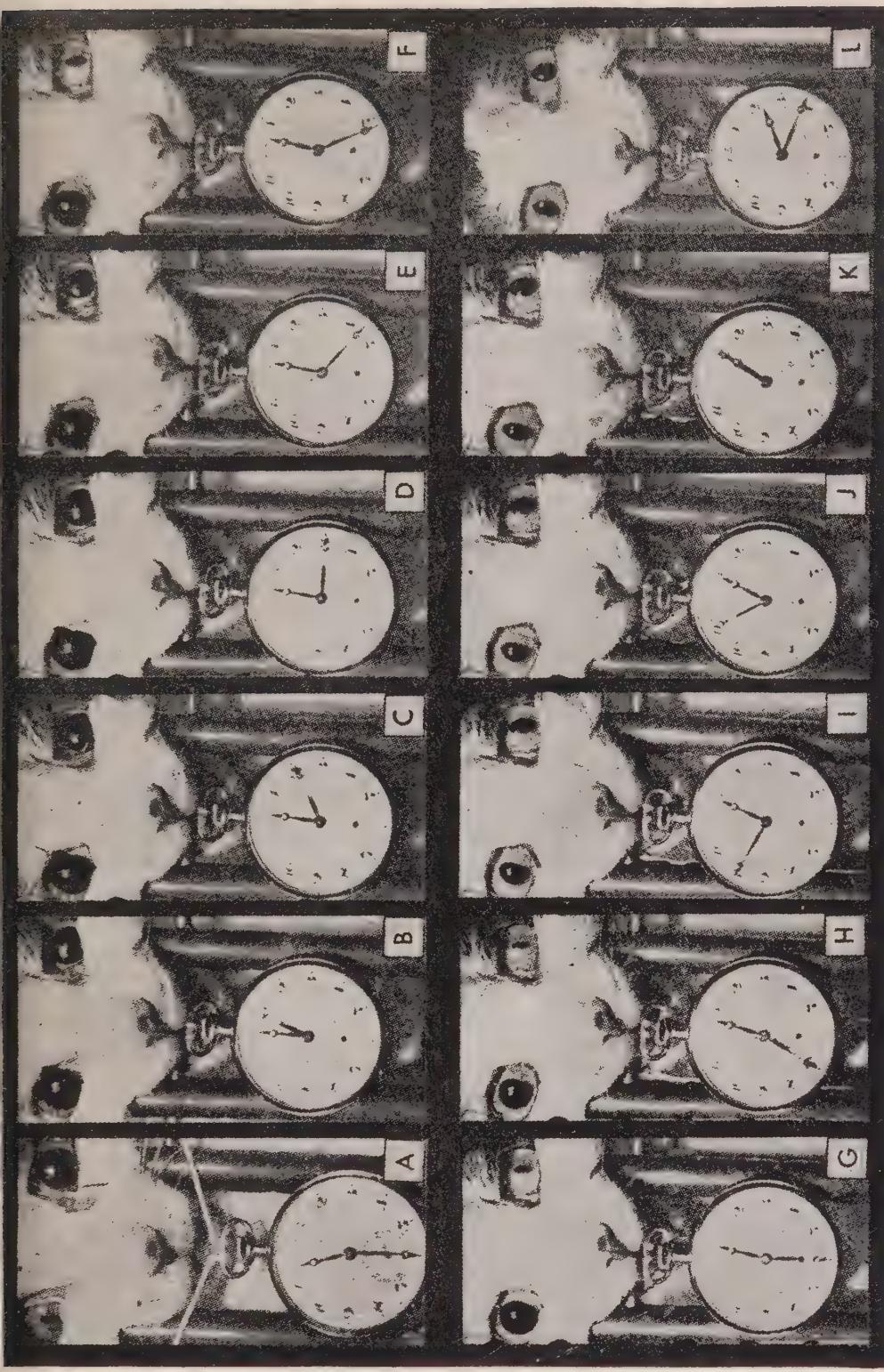


Fig. 19. Sensitization of the iris sphincter to acetylcholine by section of the parasympathetic nerve supply. A, cat with its left ciliary ganglion excised two weeks previously. B, 35 min. after A, when the right ciliary ganglion and both superior cervical ganglia had been removed. Now two drops of eserine (1 per cent) were instilled into each conjunctival sac. C, 5 min. after the eserine instillation; no change in pupillary size. D, 5 min. later; two drops of acetylcholine (0.01 per cent) instilled into each conjunctival sac. E to L, successive stages of pupillary changes. (Shen and Cannon, 1936.)

sac. This concentration of acetylcholine, though preceded by eserine, does not affect the normally innervated iris. As shown in Figure 19F, there was a marked and fairly prompt contraction of the sensitized left iris sphincter—it started to constrict within five minutes and continued to narrow the pupil until a maximal myosis was reached in about fifteen minutes (Figure 19G). Meanwhile the freshly denervated right sphincter underwent no noteworthy change and only thereafter became constricted. Since in this instance sympathetic fibers supplying the iris had been severed, the two pupils were utterly free from nervous control. The constrictor response, therefore, could be due only to direct action of the drug on the sphincter muscle. There is no basis for surmising that any other tissue between the conjunctival membrane and the iris suffers a significant change as a result of excision of the ciliary ganglion. Tests on the rate and duration of the response are reasonably explained by some change in the smooth muscle of the iris itself.

The observations of Shen and Cannon were repeated by Lissák and Martin (1940). They extirpated the ciliary ganglion on one side in cats; they confirmed Anderson's report that thus denervating the sphincter renders eserine ineffective; and they found that there resulted a marked supersensitivity to acetylcholine on the side of the operation—an effect which was blocked by atropine. Another method of testing sensitization was used by Sachs and Heath (1941). The iris, deprived of its parasympathetic innervation for 16 to 18 days, was removed, cut into strips and submerged in different concentrations of acetylcholine and eserine. When compared with normal strips, similarly tested, the denervated strips were found to average more than twice the sensitiveness of the normal.

Keil and Root (1941) also substantiated the results described above. In addition, they discovered that with respect to acetylcholine the sensitization of the iris is of temporary duration. The intravenous injection of 5 mgm.

of acetylcholine per kilo of body weight—an amount which in the cat causes only a slight effect on the normal iris—results in a marked narrowing of the pupil when the iris has been deprived of its immediate parasympathetic nerve supply. The sensitization becomes maximal in about 5 days and continues at that degree until about the 18th day after the operation. Thereafter the responses to the standard dose of acetylcholine decrease until they reach a steady minimum in approximately 35 days. These facts are illustrated in Figure 20. A denervated iris sphincter, which in

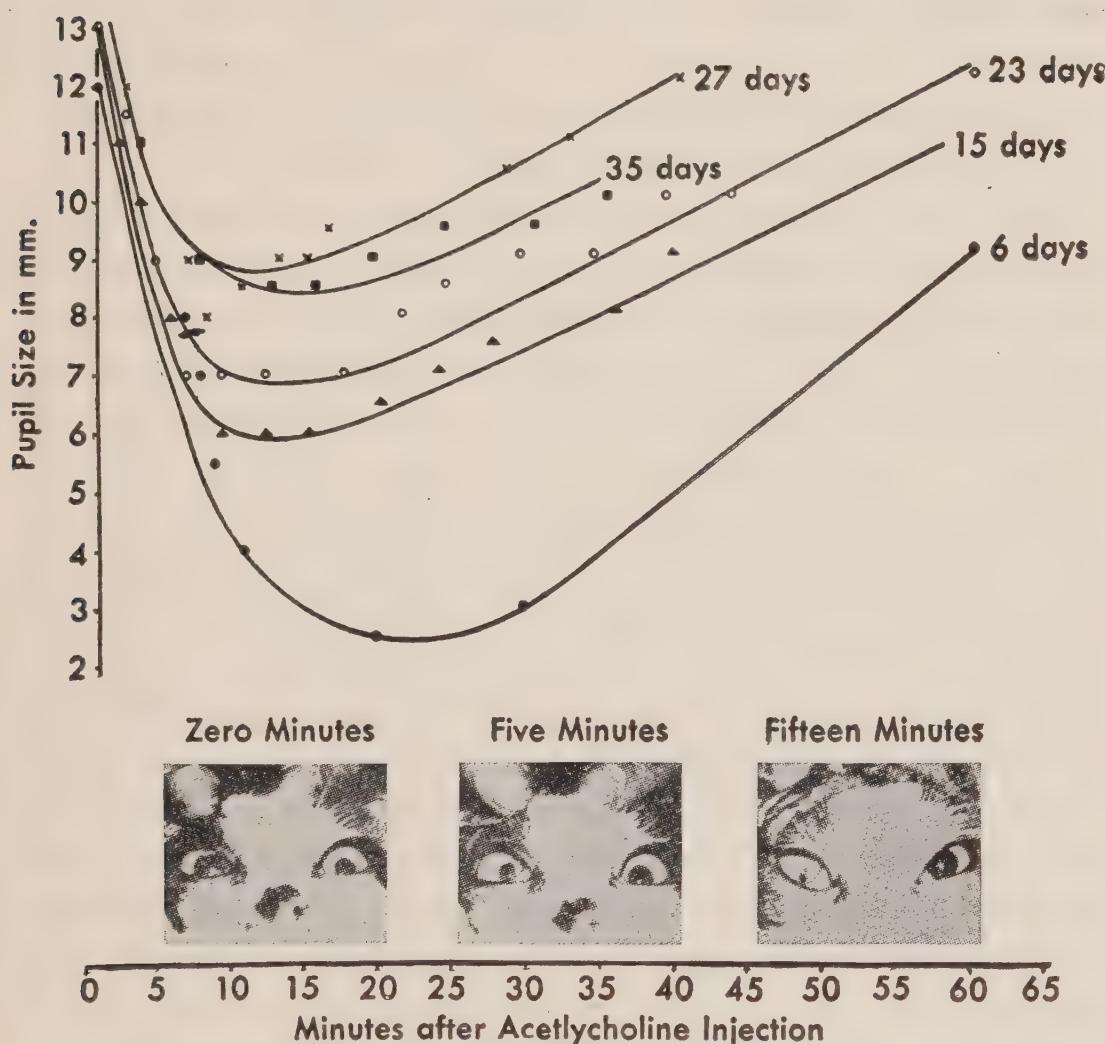


FIG. 20. Gradual disappearance of the sensitization of the iris sphincter to acetylcholine. The graphs show the effect of intravenously injected acetylcholine (5 mgm. per kgm.) on the size of the right pupil, 6, 15, 23, and 35 days after denervation by section of the cat's right oculomotor nerve. The photographs are related to the 6-day graph. (Keil and Root, 1941.)

the course of five weeks has apparently lost much of its early sensitiveness to acetylcholine, is revealed again as being sensitive to that agent if eserine is instilled into the corresponding conjunctival sac—an instillation which by itself is without effect. Interruption of cholinergic fibers is known to decrease the concentration of the destructive cholinesterase at their terminals (see Brücke, 1937; Couteaux and Nachmansohn, 1940). Acetylcholine might become more effective, therefore, because it is not destroyed. Recovery of the destructive action of cholinesterase with the passage of time would result in lessened potency of the acetylcholine. That this suggestion might be correct is indicated by the efficacy of eserine in nullifying the power of the esterase and thereby allowing the sensitiveness of the iris to become again manifest.

Does the gradual loss of sensitiveness to acetylcholine in about five weeks result from restoration of the esterase in the tissues? An answer to that question was sought by Keil and Root (1942) by comparing the effect of removal of the ciliary ganglion on the action of acetylcholine and on the action of the urethane of beta methylcholine chloride (abbreviated by them as Ubm), a substance which has 10 times the stimulatory effect of acetylcholine and is stable in the presence of cholinesterase. They found that intravenous injections of 100 to 200  $\gamma$  of Ubm per kilogram of body weight of the cat causes a constriction of the parasympathetically denervated iris and has little effect on the normally innervated side. The results of tests made on the same animal at any time between 2 and 6 weeks after removal of the ciliary ganglion do not vary significantly in the degree of contraction of the sphincter or in the rate at which the contraction occurs. Furthermore, the responses to the intravenous injection of a proper dose of Ubm continue to occur with undiminished intensity when the responses of the same iris to the standard injection of acetylcholine have markedly decreased. Thus tests on the same animal under identical conditions indicate that the decrease of influence of

acetylcholine, which begins approximately three weeks after ciliary ganglionectomy, is due not to a lessened sensitiveness of the sphincter but to a lessened efficacy of acetylcholine, an alteration which might be explained by renewed or increased activity of the cholinesterase system. According to Couteaux and Nachmansohn the decrease in the cholinesterase of the superior cervical ganglion (about 60 per cent) after preganglionic fibers have been cut reaches a maximum in about 2 weeks and thereafter the amount does not increase, at least through a period of 5 weeks. This observation, if generally true of cholinergic nerve endings, would clearly require reconciliation with the inference drawn by Keil and Root.

From the observation of Anderson that destruction of the parasympathetic supply of the iris sensitizes the sphincter muscle to pilocarpine, and also from the demonstration of Keil and Root that there is a similar sensitization to Ubm, it is evident that, quite apart from probable interference with the cholinesterase system, denervation sensitizes the sphincter in other ways to circulating or instilled chemical agents.

As shown earlier, the nictitating membrane is rendered supersensitive to adrenaline to a much greater degree when the postganglionic fibers are severed than when there is section of preganglionic fibers. The experiments of Anderson and of Keil and Root proved that the iris is sensitized when decentralized by severance of the oculomotor nerve inside the skull. The interesting fact reported by Keil and Root, that this severance of preganglionic fibers leading to the ciliary ganglion has a sensitizing effect approximately the same as that induced by severance of the postganglionic fibers, calls for explanation.

Bender and Weinstein (1940) studied cats and monkeys with the iris completely denervated, i.e., sensitized to both adrenaline and acetylcholine. The two animals differ: in the monkey intravenous injection of acetylcholine routinely results in myosis of the isolated iris; in the cat this substance

produces variable effects, sometimes a smaller, sometimes a larger pupil. A reasonable explanation of a pupillodilator instead of a pupilloconstrictor response in the cat, when acetylcholine is injected, is that it stimulates adrenal secretion, which is subject to cholinergic nerves, and that the adrenaline put forth is more effective on the totally sensitized iris than is the injected acetylcholine. Indeed, because all preganglionic fibers of the autonomic system are cholinergic, acetylcholine is universally a stimulant of the outlying neurons. Since Keil and Root did not sensitize the iris by severing ultimate sympathetic fibers, they avoided involvement of the iris retractor in their experiments.

An interesting observation on the sensitized iris was Anderson's (1905 *a*) discovery that there is a "paradoxical pupillary constriction." A few days after removal of the ciliary ganglion or section of the oculomotor nerve, partial asphyxiation or a small intravenous injection of lactic acid can cause the pupil to become much smaller on the denervated than on the normal side. After death the phenomenon is especially striking. The effect is local and appears to be due to a change in the acid-base relation in the blood or bathing fluids.

A phenomenon which has not been explained and to which Bender and Weinstein have called attention is the myotic effect of acetylcholine on the iris long after the agent must have disappeared from the circulating blood. No suggestion has been offered for this puzzling phenomenon.

*Blood Vessels.* The Sherrington phenomenon, previously described (p. 7) was explained by liberation of acetylcholine from cholinergic fibers ending on the blood vessels of the limb. Hinsey and Cutting (1933) proved that the Sherrington phenomenon disappears if the sympathetic supply to the blood vessels of the hind limb is abolished; it results, therefore, from cholinergic fibers in the sympathetic distribution. In 1936, Bülbbring and Burn offered evidence that stimulation of the sympathetic fibers of a hind limb of

the dog causes to appear in a perfusate of eserized Locke's solution a substance which pharmacologically acts like acetylcholine. Although the foregoing observations clearly point to cholinergic vasodilators in the sympathetic distribution to the leg they do not exclude the existence of adrenergic vasodilators. Rosenblueth and Cannon (1935), using pharmacological methods, were led to conclude that the sympathetic trunk contains some cholinergic and some adrenergic vasodilators in dogs and cats, but no vasodilators in rabbits.

The question arose as to whether the smooth muscle of blood vessels, made to relax by the liberation of acetylcholine at nerve terminals, could be sensitized to that agent by denervation. The answer to this question was sought by Hoagland (1941). He removed the abdominal sympathetic chain on one side down through the first and second sacral ganglia. Scrupulous care was taken to avoid injury to the contralateral chain. Time was allowed for degeneration of the cholinergic nerve supply before testing possible sensitization.

For the test the hind limbs were each inserted into a glass plethysmograph, to record simultaneously the leg volumes. A rubber cuff made a tight junction between the leg and the glass tube. Air space around the legs was displaced by water at a temperature of approximately 37° C. Accompanying the records of leg volume was a record of blood pressure. The registered response of the leg chronically deprived of its vasodilators was compared with that on the normal side; in some animals, after this preliminary record had been obtained the remaining abdominal sympathetic chain was immediately removed and the tests were repeated. When compared with the previous record, acute sympathectomy had no consistent effect on the responses of the two limbs.

After each experiment the plethysmographs were calibrated by injecting from a hypodermic syringe measured volumes of water into the rubber tube connecting the limb

vessels with the recording tambour. Each plethysmograph gave displacements on the record of approximately 16 mm. per 0.5 cc., and they seldom deviated from each other more than 1 mm. per 0.5 cc. When experiments showed deviations of one or more millimeters per 0.5 cc. between the two plethysmographs the records were rendered comparable by corrective factors.

In Figure 21 are shown the increases of leg volume and the changes of blood pressure of a cat in which the sympathetic

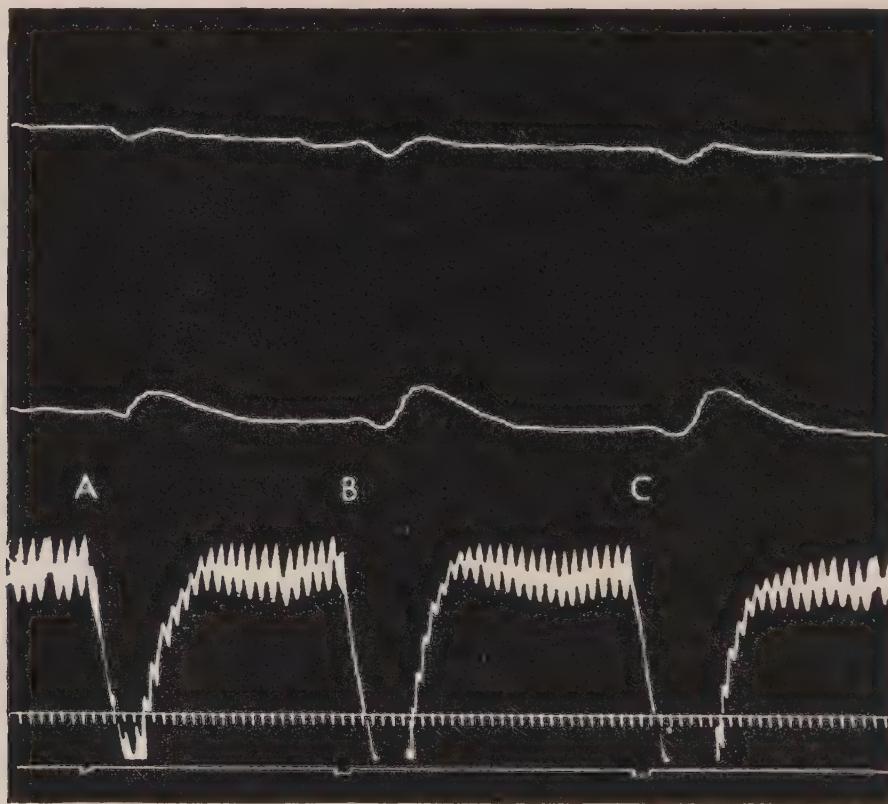


FIG. 21. Sensitization of vascular smooth muscle to acetylcholine after severance of cholinergic nerves. Upper two tracings are plethysmographic records of the two hind legs. The left lumbar and sacral sympathetic ganglia had been removed twenty days previously. A, B, and C correspond to the injection of 0.3, 0.8, and 2.0 $\gamma$  of acetylcholine, respectively. The denervated leg (lower tracing) shows a marked dilation in response to acetylcholine as compared with the normal leg. The lowest tracing is a record of blood pressure. (Hoagland, 1941.)

ganglia, from the sixth and seventh lumbar through the first and second sacral had been removed on one side several days previously. Acetylcholine, in doses of 0.3, 0.8 and 2.0  $\gamma$ , was injected intravenously. The lower tracing from the

chronically denervated leg clearly reveals a larger rise, i.e., a greater dilation, than on the normal side and therefore a greater sensitiveness. From 18 intravenous injections of acetylcholine the mean ratio of the responses on the chronically denervated side showed a sensitization, thus measured, of 230 per cent, the extremes of the ratio ranging from 1.4- to 5-fold.

From the foregoing evidence it is clear that whether smooth muscle is stimulated by cholinergic fibers or inhibited by them, the response to acetylcholine, and in some instances the response to other chemical agents which act like acetylcholine, is accentuated by depriving the muscle of its normal cholinergic nerve supply. And there is indication that when the penultimate neurons are cut, the smooth muscle thus decentralized becomes quite as sensitive to the circulating chemical agents as when the ultimate neurons are severed.

## CHAPTER VII

### MELANOPHORES

Investigators working on the color changes of bony fishes have long been familiar with the rapid and complete concentration of the black pigment in melanophores when sympathetic nerves are stimulated. As is true of other structures in the vertebrate body that are subject to sympathetic control, adrenaline mimics the effects of sympathetic impulses in its action on the pigment; when it is injected the dispersed melanin is quickly and fully concentrated in the center of the color cell.

In 1931, Smith noted a puzzling reaction in the minnow, *Phoxinus*. An area on the head of this fish was denervated by sectioning the ophthalmic nerve and allowing time for degeneration. Now, when the animal was placed in an aquarium with a black background, the skin became uniformly dark—an adaptive response resulting from dispersal of the pigment in the chromatophores. A striking change occurred when a sudden frightening stimulus, such as a sharp tap on the side of the aquarium, startled the animal. The denervated area became pale, although the rest of the body remained dark. At the time it seemed probable that release of adrenaline, which alone is capable of producing so rapid and thorough a response, was the explanation of this change, but Smith did not see why the entire body was not affected. It is reasonable to assume that this was another instance of a "paradoxical" adrenaline effect, similar to that observed in the "paradoxical" pupillary phenomenon, i.e., that it was a consequence of increased sensitivity to adrenaline of melanophores which had been deprived of their sympathetic nerve supply, and that the normally innervated cells, less sensitive, did not respond.

Evidence that melanophores when sympathectomized actually do become sensitized to adrenaline was reported by Smith in 1941, in tests on the common tautog (*Tautoga onitis*). With forceps he carefully pulled out trunk scales from their pockets, thus obviously severing all connections with the nervous system. The scales were immediately slipped back into the pockets and left there undisturbed. In the great majority of cases the scales healed in place, soon had new circulatory connections, and were maintained in a healthy condition. Regeneration of the nerves was characteristically slower than reestablishment of connections with the blood system, as indicated by the failure of the scales to participate for some time in the normal color changes shown by the animal. When placed on a light background the body of the fish would turn pale but there would be dark splotches on it due to patches of denervated scales in which the melanophore pigment was not concentrated.

The nerve filaments on the scale are approximately only a millimeter in length; about a day is required for their complete degeneration. Evidence indicates that in about two weeks there is a regrowth of filaments into the scales which have been isolated; after that period the melanophores again participate in the normal color responses. The pigment is concentrated when the fish is placed on a light background or when the pigment motor center in the medulla is electrically stimulated.

In tests of the effects of denervation on the response to adrenaline during the period of degeneration the scales which had been withdrawn and replaced were compared with normal scales and with scales which had recovered from the denervated state. The method of recording the responses was ingenious. The scale to be tested was removed and placed in a suitable quantity of a balanced salt solution consisting of the chlorides of sodium, potassium, and calcium. To this solution enough adrenaline hydrochloride was added to make the adrenaline strength of the solution 1:100,000. This concentration was sufficient to produce a

typical adrenaline response, a rapid concentration of the melanophore pigment, whether the scale was normal or denervated. The speed of the response was measured photo-electrically by the effects on the amount of light transmitted through the isolated scale, the amount being a function, in the main, of the degree of black pigment dispersed within the melanophores. As the pigment in the melanophores became concentrated more light passed through the scale and there was a consequent deflection of the galvanometer which was attached to the photocell. Thus the galvanometer deflection was used as a sign of the movement of the pigment.

Because of variations in the size, thickness, and number of melanophores on a scale, the galvanometer deflections were averaged from a number of tests. For example, the deflections of 10 normal scales were recorded and averaged and plotted (see Figure 22). Similarly the concentration of the melanophores in 6 scales denervated for 4 days, and in 3 scales denervated for 6 days was averaged and plotted. Also the results obtained from 6 scales 13 days after the denervation were treated in the same manner. As shown in Figure 22, where all four graphs are plotted, the melano-

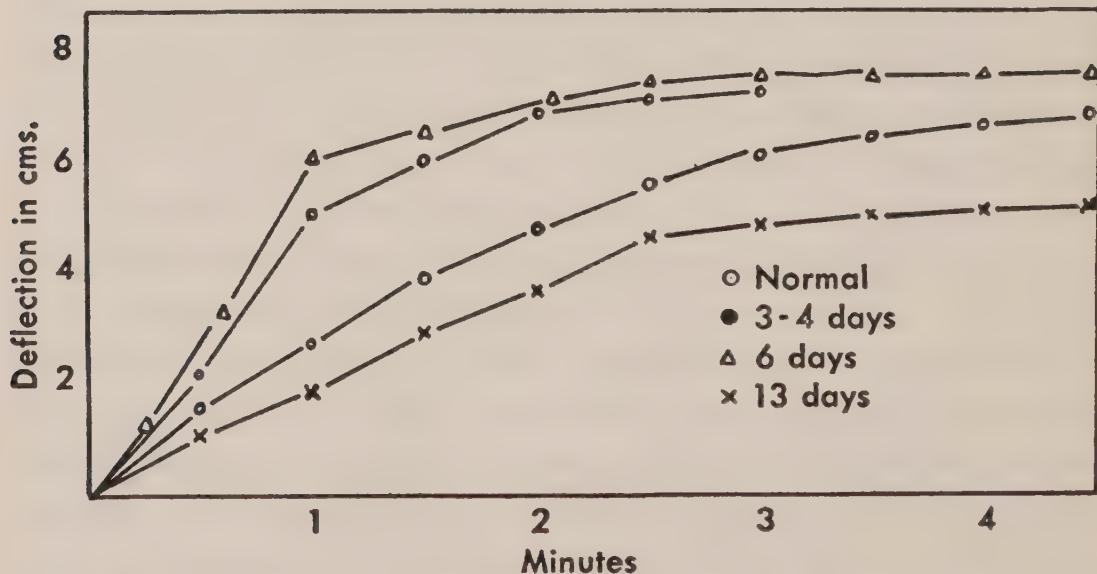


FIG. 22. Responses to adrenaline (1:100,000) of melanophores, both normal and denervated several days previously, in isolated scales of the Tautog. Photo-electric recording. Increase of galvanometer deflection indicates concentration of the pigment. (Smith, 1941.)

phores of scales which had been deprived of their sympathetic connections 4 to 6 days manifested a concentration of their pigment at a rate approximately twice that of the rate in the normal scales. Thirteen days after the operation, however, the response was again about the same as in the normal scales, thus indicating a regrowth of the sympathetic fibers and a reconnection with the melanophore effectors. These observations not only illustrate the sensitization of melanophores to adrenaline by denervation; they also indicate that melanophores are affected by the liberation of adrenaline at nerve terminals.

The disappearance of supersensitivity from the denervated melanophores when they again come under control of the nervous system is analogous to the change which occurs in smooth muscle. As Simeone demonstrated (p. 26), when the sympathetic fibers which have been severed grow forth and renew their connections between the central nervous system and the nictitating membrane, the sensitization which followed severance of the nerves disappears. The phenomenon in the two responding structures is quite the same.

The sensitization of melanophores by degenerative nerve cutting was used by Chang, Hsieh and Lu (1939) in studies on the snake fish (*Ophiocephalus argus*). They removed the sympathetic system between the region of the pectoral fin and the cloaca. The denervated region turned black from dispersion of melanin in the pigment cells. When the denervated area had become sufficiently sensitized, stimulation of the myelencephalic sympathetic center turned the black area pale. This change they attributed to diffusion into the denervated area of an adrenaline-like substance liberated at the nerve endings of the neighboring normal regions when the sympathetic system was stimulated.

The observations by Smith were extended by Parker (1942) in experiments on the scaleless fresh-water catfish (*Ameiurus nebulosus*). He studied especially the macro-melanophores situated in the derma. The pigment of these

cells is concentrated by adrenaline and is dispersed normally either by intermedine from the pituitary gland or by acetylcholine.

When a single fin ray in the tail of *Ameiurus* is cut, a band of tissue which accompanies the rays is deprived of nervous influences. The cut nerves contain two kinds of fibers, which can produce either concentration or dispersion of the black pigment (see Parker and Rosenblueth, 1941). About two weeks are required for the severed fibers to undergo complete degeneration; and about a month must elapse before they grow back again. The best period in which to make tests of the fully denervated melanophores is during the third week after the nerves have been severed.

Strong concentrations of adrenaline (1:5,000 and 1:20,000) blanched the fishes quickly, the tails and the bands becoming pale at the same time. Weak concentrations (1:1,000,000 to 1:50,000,000) had no appreciable effect within the first five minutes, though at 1:1,000,000 there was commonly a blanching of the whole fish in the course of an hour. Critical concentrations were those of medium strength (1:50,000 and 1:100,000). The stronger of these solutions caused the tail to assume an intermediate tint while the denervated band became decidedly blanched, i.e., the pigment in the denervated melanophores was concentrated while that in neighboring innervated cells remained dispersed. With the weaker concentration the tail, during the first five minutes, remained intermediate in tint but the band was noticeably paler. These tests on *Ameiurus* are in accord with those reported by Smith on the melanophores of the tautog in revealing a heightened sensitivity to adrenaline after cutting the sympathetic nerve connections.

Cutting a fin ray deprives the corresponding caudal band of both concentrating and dispersing fibers. Severance of parasympathetic nerves which supply smooth muscle results, as previously noted, in the muscle cells becoming sensitized

to acetylcholine and other substances. It was possible that severance of the dispersing fibers would likewise render the melanophores more sensitive to the natural dispersing chemical agents, acetylcholine and intermedine. This possibility was examined by Parker. In the catfish acetylcholine has a limited action; it can convert the pale band due to concentration of the pigment into an intermediate state by partial dispersal but cannot carry the process to the completely dark conditions. That requires the action of intermedine.

When acetylcholine was injected subcutaneously into a previously eserized catfish it was found that weak solutions were without effect and that strong solutions proved poisonous, as evidenced by the death of many of the fishes. Only an intermediate dose (0.004 mgm. of acetylcholine per 100 grams of fish) caused the fish to darken without endangering its life. At this concentration there was no noticeable difference of response between the denervated band and the rest of the tail. This result was so regularly obtained that Parker drew the conclusion that either denervation did not sensitize to acetylcholine or the technique employed was not appropriate for demonstrating supersensitivity. It seems probable that the second suggestion was correct for he succeeded in demonstrating by use of intermedine that the melanophores were indeed sensitized to that agent. When a pituitary extract, containing intermedine, obtained from the glands of the catfish itself, was injected into three catfish which were intermediate in tint, all began to darken within five minutes. At the end of ten minutes the denervated bands were noticeably darker than the rest of the tail, a condition which remained constant for three to four hours, whereupon both the bands and tails were indistinguishably dark and of the same tint as the rest of the fish. Varying the amount of the injection brought out confirmatory evidence. From numerous observations it became clear that denervation of the melanophores of the catfish renders them more sensitive to intermedine than they were when innervated,

an effect comparable to the sensitization to adrenaline after severance of sympathetic fibers.

Supersensitivity induced by denervation brings melanophores into the same category with smooth muscle when it is deprived of its appropriate nerves.

## CHAPTER VIII

### GLANDS

#### SUBMAXILLARY, LACHRYMAL, SUDORIFIC

Little attention has been paid to the sensitization of glands by degenerative section of their nerve supply. To be sure, Fröhlich and Loewi (1910) compared the responses to adrenaline of the normally innervated submaxillary gland with those of the opposite gland after long-standing sympathetic denervation, and they found no difference. It was a single experiment, however, and perhaps proper care was not given to administering submaximal doses of the hormone. Because of the effects on other structures it seemed desirable to determine whether glands obey the general law that the deprivation of nervous control induces a supersensitive state.

*Submaxillary.* In 1937, Pierce and Gregersen reported on changes of the submaxillary secretory response to pilocarpine after section of the chorda tympani. They used dogs as subjects. Bilateral submaxillary fistulae were established, and at the time of the test glass funnels were fixed to the skin around the fistulous openings. The saliva was collected in graduated tubes held in place by an elastic harness. The dogs were trained to recline in a comfortable position on a laboratory table so that no anesthetic was used and no restraining apparatus was necessary. In all experiments salivary secretion was elicited by intravenous injection of normal salt solution containing 0.02 mgm. pilocarpine hydrochloride per cc. The solution was introduced into the short saphenous vein by a small automatic pump which permitted accurate duplication of the amount and the rate of the injections in repeated tests on the same dog. The injection

was invariably continued for 20 minutes. The start of the secretion was recorded, as well as the amount of saliva in the collecting tube at the end of every minute throughout the period of injection and in most experiments during 10 minutes after the injection stopped.

In control experiments, performed before interruption of the nerve supply, the response to pilocarpine was practically the same from the right and the left submaxillary glands. Thereafter, under ether anesthesia, one or the other of the glands was decentralized by severance of the chorda tympani and chorda-lingual nerves.

A difference of effect from injecting pilocarpine did not appear immediately after the decentralization. Even 2 days after the operation there was still no noteworthy difference between the plotted records of the salivary flow from the two sources. By the 6th day, however, the denervated glands began definitely to secrete before the normally innervated glands and also to deliver more saliva. One of these experiments is shown in Figure 23B. On the 21st day the difference in the responses was more pronounced (Figure 23C). The effect of decentralization in augmenting the efficacy of pilocarpine (i.e., in producing supersensitivity) obviously undergoes a gradual development.

Pierce and Gregersen considered the possibility that the greater flow of saliva from the decentralized gland might result from a greater vasodilation of the local blood vessels. They argued that a maximal dilation of the vessels resulting from amyl nitrite or nitroglycerine ought to make the response more nearly alike from the two glands. When these vasodilator drugs were administered, before and during the course of the usual injection of pilocarpine, no significant changes occurred in the differential rates of secretion; both glands, however, began to secrete more quickly. A further argument against a vascular cause of the difference of response from the decentralized and the normal glands was that no measurable difference between the two occurred

for several days after the chorda tympani had been severed, i.e., after the removal of vascular dilator control.

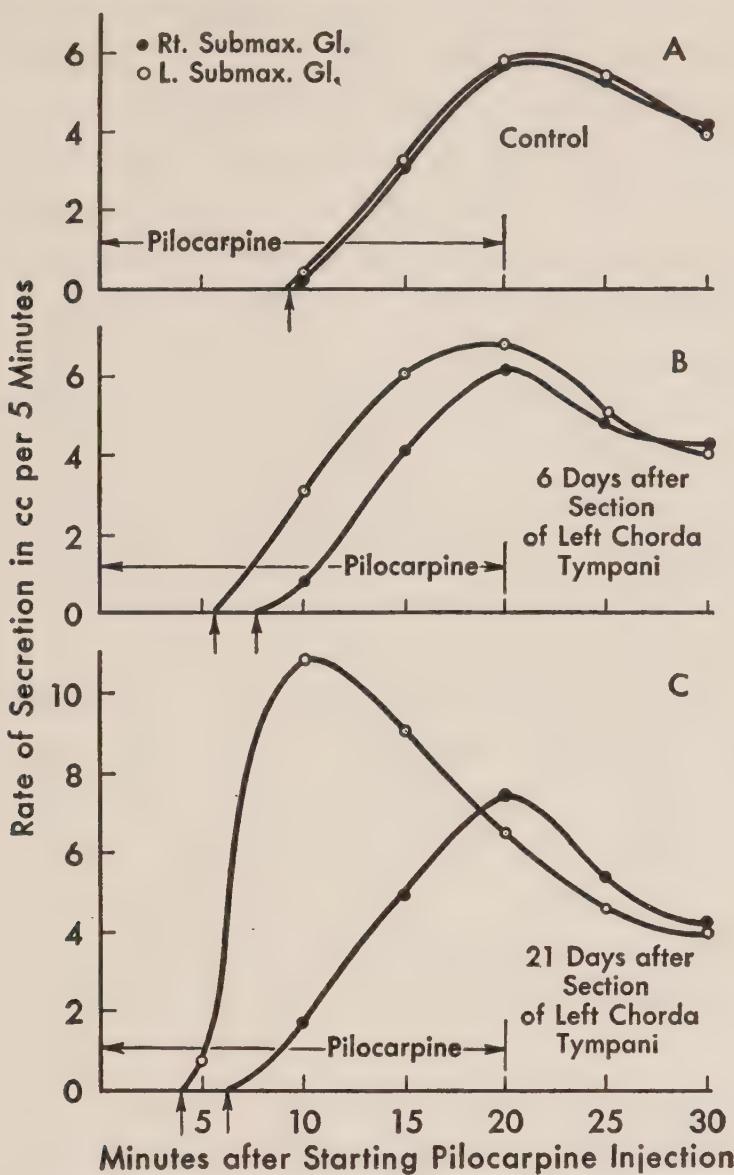


FIG. 23. Responses of the right and the left submaxillary gland to continued intravenous injection of pilocarpine (2 mgm. per 100 cc. in salt solution, at the rate of 37 cc. per hour). A, control experiment; B and C, 6 and 21 days respectively after parasympathetic decentralization of the left gland. (Pierce and Gregersen, 1937.)

Since acetylcholine is the natural chemical mediator of parasympathetic nerve impulses it was to be expected that the decentralized submaxillary gland would be more responsive to that agent than the normal contralateral gland. When Pierce and Gregersen tested sensitivity by injecting

acetylcholine they found that the decentralized gland yielded a smaller response than did the normal, an observation that confirms a previous report by Fleming and MacIntosh (1935). The experiments of Pierce and Gregersen were performed, however, two months after the chorda tympani had been severed. It will be recalled that Keil and Root discovered that the sensitization of the iris, after its parasympathetic supply had been destroyed, gradually disappeared until at the end of a month and a half it was contracted by acetylcholine little differently from the normal iris. As noted previously (see p. 64), the loss of sensitivity is possibly attributable to a restored activity of cholinesterase. Perhaps the failure of Pierce and Gregersen to find supersensitivity of the submaxillary two months after the gland was decentralized was due to destructive activity of an esterase. Thus, in 1942 Wills reported a clear sensitization of the submaxillary gland of the cat to the secretory action of acetylcholine; this supersensitivity was apparent after severance of the chorda tympani and lasted at least through the 27th day after the operation.

Observations made by Maeovsky in 1923 and reported by Babkin (1928) show that the degenerative section of the chorda tympani leads to a supersensitivity of the submaxillary gland to other substances than pilocarpine. Maeovsky found that in the course of time the gland became more sensitive than normally to the secretory effects of injected adrenaline, as well as to the corresponding action of this hormone when released into the bloodstream by the adrenal medullae in animals during asphyxia. His observations were confirmed by Fleming and MacIntosh (1935).

The submaxillary gland receives nerve impulses from the sympathetic as well as from the parasympathetic cranial division of the autonomic system. Furthermore, stimulation of the cervical sympathetic trunk in the cat commonly evokes an abundant flow of saliva. Experiments to test the effect of denervating the submaxillary gland by removal of either the right or the left superior cervical ganglion were

undertaken by Simeone and Maes (1939). Tests were made from 47 to 90 days after the operation. Under urethane anesthesia the still intact superior cervical ganglion was excised or decentralized, and the chorda tympani nerves were excluded from action. Cannulae were introduced into the submaxillary ducts and were connected through small flexible rubber tubing to a pipette. The tube systems were filled with salt solution and the response of the glands was recorded as drops of the solution fell from the pipette on a drop-recorder. Tests made on normal animals revealed that secretion from the right and the left submaxillary glands, elicited by injections of adrenaline, were not significantly different. There was, however, a noteworthy variation in the sensitiveness of the glands of different animals, some responding to as little as 5  $\gamma$  injected intravenously, others requiring at least 100  $\gamma$ .

The degree of sensitization that resulted from removal of the ultimate sympathetic supply was far from being uniform. In six of ten animals the salivary flow of the chronically denervated glands when adrenaline was injected was 50 per cent greater than that from the contralateral, freshly denervated, control gland. In one case the flow was 9 times that of the normal; in two it was about 20 per cent greater, though in the first few tests it was as high as 50 to 75 per cent more than on the normal side. In the remaining two of the ten animals no difference was detected in the output of the two sides.

The effects of injecting acetylcholine were also examined, for, as already shown, denervated structures are sensitized not only to the natural chemical mediator of the degenerated nerve fibers but also to other chemical agents. Again considerable variability in the responses was disclosed. In three of ten animals the secretion of saliva from the denervated gland was more than 50 per cent greater than that from the normal control. In four others it was likewise greater but only by 15 to 30 per cent. In the remaining three of the ten the responses were equal on the two sides.

Pilocarpine also was tried in order to reveal whether at a late stage in the sympathetically denervated submaxillary there is sensitization to that agent. In six of seven animals the salivary secretion was greater by 100 per cent or more on the denervated than on the normal side. The one animal in which pilocarpine had equal effects on the two sides failed to show any sensitization also to adrenaline and to acetylcholine. In Figures 24, 25, and 26 typical instances of

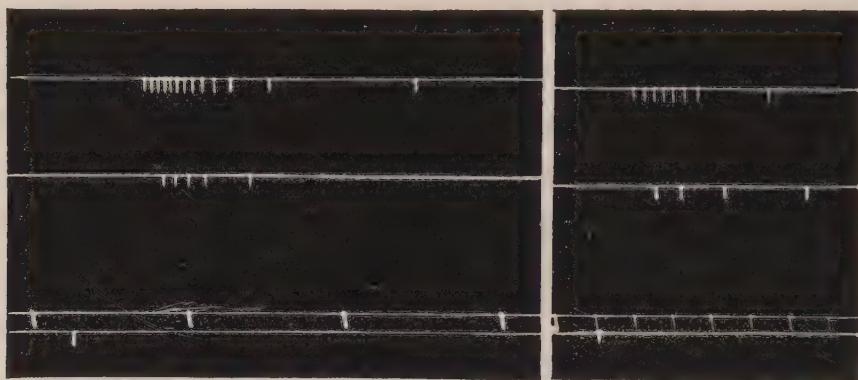


FIG. 24 (left). Simultaneous records of drops of saliva from a chronically denervated submaxillary gland, i.e., deprived of ultimate sympathetic fibers (upper record), and from the contralateral control gland with the superior cervical ganglion removed acutely (lower record). Cat under urethane; adrenals ligated. At lower signal adrenaline (10 $\gamma$ ) injected intravenously. Time signal: 5-sec. intervals. (Simeone and Maes, 1939.)

FIG. 25 (right). As in Fig. 24, but at lower signal acetylcholine (1.2 mgm.) injected intravenously. (Simeone and Maes, 1939.)

sensitization of the submaxillary gland, deprived of its ultimate sympathetic nerve supply, are illustrated by comparison with responses of the normal gland when adrenaline, acetylcholine, and pilocarpine were used as test agents. Cocaine, by potentiating the action of pilocarpine on the normal gland and affecting less the denervated gland, rendered less striking the difference between the salivary outflow from the two. The effects of pilocarpine on secretion, after sympathetic denervation of salivary glands, are in accord with results reported by Takakusu (1922). He removed the superior cervical ganglion on one side in rabbits and after different intervals, from 5 to 57 days, injected pilocarpine (0.6 to 0.7 mgm.). Whereas, previous to the operation, the drug had the same effect on the two sides,

the secretion induced by it after the glands were sensitized invariably appeared earlier, lasted longer, and was more abundant on the denervated than on the normal side.

Simeone and Maes suggest that the variability in the responses of the denervated submaxillary gland, as compared with the reliable and uniform responses of the nictitating membrane, may be due to the more complicated cellular structure of the secreting organ and also to differences in the distribution of nerves to the alveolar and the demilune cells. This suggestion, however, would be difficult to prove.

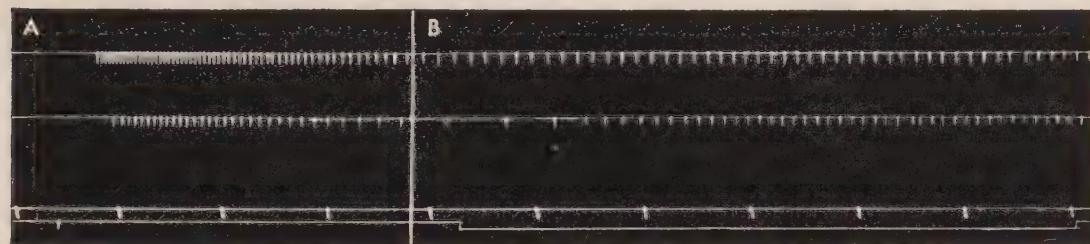


FIG. 26. As in Fig. 24, but in A, at signal, pilocarpine (0.1 mgm.) injected, and in B, 15 sec. later, during the signal, cocaine (16 mgm.) injected intravenously. (Simeone and Maes, 1939.)

*Lachrymal.* Sensitization of the lachrymal gland to pilocarpine 24 hours after removal of the superior cervical ganglion was reported by Arloing (1890) in the unanesthetized horse. On the contrary, Merz (1926) did not detect in the anesthetized rabbit a similar supersensitivity the fifth day after denervation. Arloing's results may have been due to conjunctivitis. In a single experiment on a cat Langley (1901) noted that adrenaline evoked a more abundant flow of tears on the side from which the superior cervical ganglion had been removed eight days previously, than on the normal side. The discrepancies in the testimony and its meagerness left the problem open. The task of determining sensitization of the lachrymal gland deprived of its sympathetic supply was undertaken by Maes (1938).

Cats were repeatedly tested while under temporary nembutal anesthesia. To measure the lachrymal secretion, dry strips of smooth blotting paper (1.8 cm. long, 1.2 cm. wide) were used. At the start of an experiment a strip of this paper

was placed under the upper eyelid of each eye and removed four minutes later. Any residual secretion was thus absorbed, and later tests, therefore, were made under standard conditions. Ten minutes later two new strips, which had been weighed in glass-stoppered weighing bottles, were similarly inserted under the upper eyelids and left in place for four minutes. Ten minutes later the process was repeated, and so on.

Usually two or three insertions of this character were made before the effects of drugs were tried. The drugs were injected into a femoral vein one minute before the blotting paper was set in place under the eyelids. Weighing the paper in the bottles before and after the injections measured the amount of secretion from the two glands.

Before drugs were injected the increase of weight of the inserted strips varied from 6 to 20 mgm. In all animals intravenous injection of 0.1 mgm. pilocarpine per kgm. of body weight caused a marked increase of secretion, often 3- to 6-fold the original value. Adrenaline and acetylcholine as a rule yielded a definite increase only when rather large doses were administered, i.e., 0.5 mgm. of acetylcholine per kgm., injected intravenously during a period of fifteen seconds, and 50 to 75 γ of adrenaline per kgm. of body weight, administered in the same manner.

In four animals the three drugs were tested for their effects immediately after excision of the superior cervical ganglion. The lachrymal glands responded equally on the two sides, as they had responded before the operation. The freshly denervated gland, therefore, is not sensitized to adrenaline, pilocarpine, or acetylcholine.

In a second series of animals some were deprived of the possibility of adrenaline secretion; others were left with the adrenals intact. Preliminary tests proved that in three of this series the secretion of tears evoked by the drugs was alike on the two sides; in three others the *right* gland secreted more. Absence of the adrenal medulla was without effect.

Later, under ether anesthesia, the *left* superior cervical ganglion was removed aseptically. Time was allowed for full recovery from the operation. Observations were made only if there was no evidence of conjunctivitis or inflammation of the eyelids. When the effects of the drugs were tested at various intervals, ranging from eleven days to eight weeks after removal of the superior cervical ganglion, the denervated glands invariably showed a supersensitivity to the injected drug. There was a definite response from the denervated gland to a dose which was subliminal for the normal gland, and if a dose was given which was effective on both sides the secretion from the denervated gland was always greater than that from the control. Figure 27 illustrates a

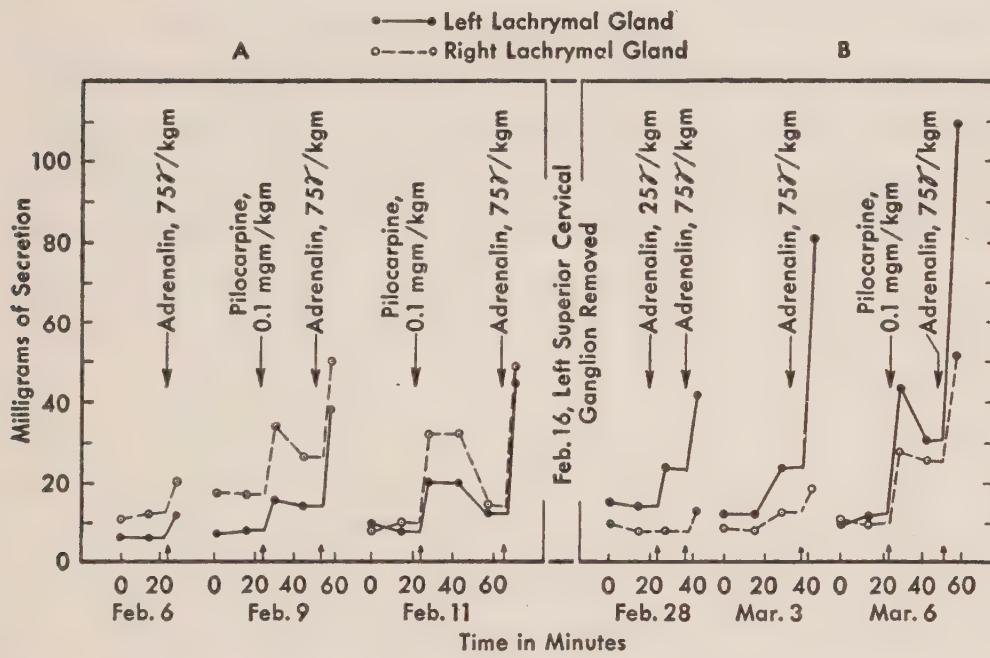


FIG. 27. Secretion of tears from the right and left lachrymal glands in response to injection of adrenaline and pilocarpine on the indicated dates. A, before, and B, after degenerative section of the ultimate sympathetic nerves. The adrenal glands were excluded from action. (Maes, 1938.)

case in which, as mentioned above, the secretion from the right gland before any operation (A) was greater than from the gland on the left side. As the graph shows, after left-sided cervical sympathectomy, the injection of adrenaline and pilocarpine reversed the relative amounts secreted by greatly increasing the flow of tears on the sensitized side.

Although the effect of acetylcholine is not illustrated in Figure 27, it likewise was more effective on the denervated side.

Again it should be noted that not only is the natural intermediary between sympathetic impulses and the effector organ, adrenaline, made more effective by denervation, but also other chemical agents, acetylcholine and pilocarpine.

*Sudorific.* In 1925, Burn reported that in the toe-pads of kittens where sympathetic fibers have been severed and degenerated, pilocarpine evokes an exaggerated secretion of sweat. It was surmised that this might not be due to a greater sensitivity of the sweat glands to the chemical agent but rather to removal of sympathetic control of blood vessels. When the external temperature was held at 37° C., however, and there was little difference in the blood flow on the two sides, sweating regularly appeared on the denervated paw earlier than on the control, after pilocarpine was administered. It seems probable, therefore, that the sweat glands can be included among those which are rendered supersensitive by being deprived of nerve impulses.

## CHAPTER IX

### THE HEART

In 1938, Sawyer, Hampel, and Ring, while testing the influence of anterior pituitary extract on the accelerator action of adrenaline on the denervated heart, incidentally reported on the results observed when adrenaline itself was administered after the denervation. In all the experiments cats were used. As a preparation for the tests the adrenal glands were rendered inactive by removal of one and denervation and destruction of the medulla of the other. The hepatic nerves, which as shown by Cannon and Uridil (1921) can cause a discharge of sympathin in an amount which accelerates the denervated heart, also were severed.

The injection of a standard dose of adrenaline ( $1 \gamma$  of a freshly prepared solution), injected into a femoral vein in a standard manner, led the investigators to the conclusion that if the heart is sensitized it becomes sensitized quite rapidly. The basis for that inference was that the control injections, made in some instances a few hours after the heart was denervated, evoked responses which, during the first half-minute after adrenaline entered the blood stream, were not strikingly different from those evoked under the same conditions ten to fourteen days later. This behavior of the heart, discordant among denervated organs, led to a further inquiry which was undertaken by Burrett (1940).

Healthy young cats were selected for the experiment. Under ether anesthesia the heart was surgically disconnected from the central nervous system. Thereafter, twenty-four hours were allowed to pass before the first tests were made. In these tests the animals were subjected to nembutal anesthesia (0.7 cc. per kgm., injected intraperitoneally), which produces a properly deep and uniform anesthetic

state for three hours or more. The heart-beat was recorded by means of a tambour placed between the board on which the cat rested and the cat's chest as the animal lay on its side. Through the air in a connecting rubber tube the pulsations of the receiving tambour affected a sensitive Marey capsule on which the recording lever rested. In the tests 1 cc. of normal salt solution containing from 0.5 to 10 γ of adrenaline was injected into a femoral vein at a constant rate during 10 seconds. The adrenaline solution was freshly prepared and its deterioration was carefully avoided. While under the anesthetic the animals were kept warm by an electric heating pad; in any experiment the rectal temperature did not vary more than 1.0° C.

Results obtained with very small doses and with large doses of adrenaline were variable and unreliable. A minimal dose of 0.05 γ was only occasionally effective; amounts greater than 2 γ occasionally evoked in the sensitized heart an arrhythmic response. The effective doses for revealing the phenomenon of sensitization were found to be in the moderate range, i.e., between 0.1 and 2 γ. The injections, after the first one was given, were repeated at seven-day intervals.

For each dose of adrenaline from 0.1 γ and including 2 γ the cardiac reactions fell into a general pattern. The percentile increase over the basal rate for a given dose rose rapidly between the first and the eighth day after the denervation. After the eighth day the percentile increase over that of the first day either increased slowly or leveled off (see Figure 28). These results and those obtained by Hampel in tests on the smooth muscle of the nictitating membrane (see Figure 8) are in close accordance.

Burrett found that not only was there a percentile increase of the heart rate over the basal during the first minute after the injection but also there was a prolongation of the increase. For example, in one animal the heart rate on the first day following the denervation increased in successive 15-second intervals after an injection of adrenaline 4, 6, 4,

and 3 beats; on the fifteenth day after the denervation the increases in the number of beats in the four successive 15-second intervals after an equal injection were 4, 13, 12, and 10 beats, the rate thus failing remarkably to decline to the basal level at the end of one minute.

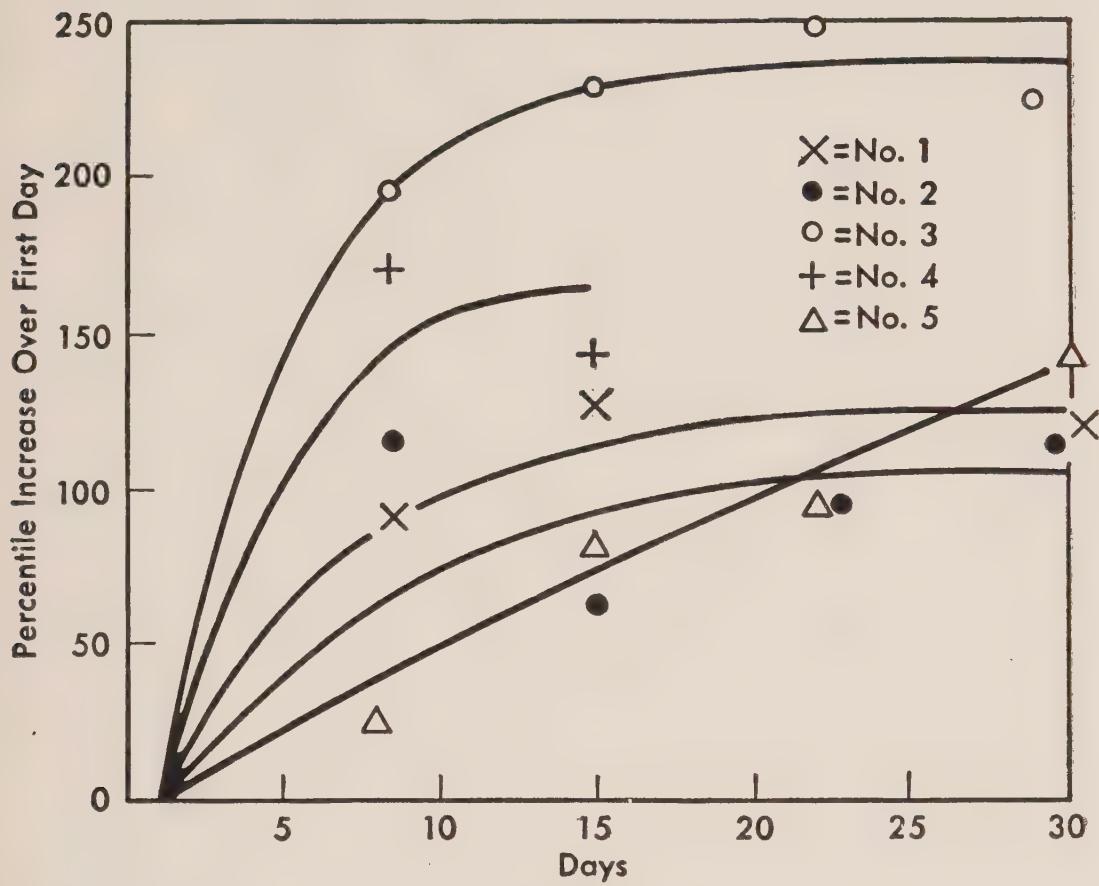


FIG. 28. Response of the denervated heart to  $0.5\gamma$  of adrenaline in five cats. The ordinates represent the percentile maximal increase of the heart rate over the increase obtained on the first day; the abscissae represent days after the denervation. (Burrett, 1940.)

The percentile increases in the heart-rate response over that on the first day varied from a minimum of 3 per cent on the twenty-ninth day, when a standard dose of  $2\gamma$  was employed, to a maximum of 390 per cent on the twenty-ninth day when the repeated dose was  $0.1\gamma$ . It was the small doses of adrenaline that gave the greatest percentile increase over the increase on the first day. This is what might be expected with augmented sensitivity as time passed. It illustrates again the importance of using sub-

maximal doses in making tests of enhanced susceptibility.

After the foregoing observations had been made by Burrett, a review of the results obtained by Sawyer, Hampel, and Ring revealed that they likewise had inadvertently recorded sensitization of the heart to adrenaline when its sympathetic supply had been severed. Though the change of heart-rate resulting from standard injections was not great during the first and the second 15-second intervals, on the twelfth day as compared with the first day after denervation, striking changes occurred in the third and fourth 15-second intervals, i.e., there were indications that the standard dose of adrenaline had a more prolonged effect in the course of time. Furthermore, in the earlier experiments attention was not paid to the percentile increase, but only to the numerical increase of the rate. Rosenblueth and Simeone showed in 1934 that the number of beats by which the heart rate changes in different experimental conditions is not an adequate quantitative criterion to judge the effects of the experimental procedure if the basal rate is variable. The appropriate measurement of the effects recorded consists in calculating the change as the per cent of the preexisting basal rate. When the results of Sawyer, Hampel, and Ring were carefully examined and the increase per minute was taken into consideration the evidence became clear that on the twelfth day the increment of the heart-rate over the basal, that resulted from the injections, was about 60 per cent greater than on the first day. This increment was not so great as that observed by Burrett except in two animals in which increases of 52 and 56 per cent on the fifteenth and the eighth days, respectively, were recorded. It is possible that anesthetization of the animals in Burrett's experiments may have made a difference.

The evidence presented above brings the heart into line with other structures which are sensitized to adrenaline when deprived of the sympathetic nerve supply. It becomes

supersensitive to adrenaline gradually, but fairly rapidly during the first week after denervation, and thereafter, as with smooth muscle, the change is slower.

The question naturally arises as to whether a supersensitive state in heart muscle could be induced by severance of the vagus nerves. The distribution of these nerves in the heart is not favorable for discriminative answer to that question. The final neurons in the vagal path, because they lie wholly within the muscle, cannot be severed independently, as is possible with the free short ciliary nerves of the iris. Furthermore, both the preganglionic and the postganglionic vagal fibers are cholinergic. If the preganglionic fibers are cut, therefore, and there is sensitization of both the cardiac muscle and the cell bodies of the ultimate parasympathetic vagal neurons, tests for supersensitivity would not reveal which of these two points is more affected, for, as already remarked and as will be shown later, depriving outlying neurons of the impulses which come to them from preganglionic fibers sensitizes these neurons to chemical agents.

Incidentally, it is of interest to note that the growth of vagal nerves into contact with cardiac muscle endows that muscle with an increased sensitiveness to acetylcholine. Armstrong (1935) found that relatively large doses of that agent (up to  $0.135 \gamma$ ), when injected in *Fundulus* embryos before the vagus nerves reached the auricle, did not inhibit the heart; after vagal innervation, however, a very small dose (down to  $0.000023 \gamma$ ) lessened the auricular contraction, and about three times that minute dose stopped both the auricle and the ventricle in diastole. Armstrong inferred from these facts that the inotropic vagomimetic action of acetylcholine on the heart is not exerted directly on cardiac muscle, but only indirectly, through the postganglionic vagal fibers. Other observers, however, have recorded direct effects of acetylcholine on the aneural heart. Thus, Plattner and Hsu (1931) and Hsu (1933) registered negative ino-

tropic and chronotropic effects upon administration of acetylcholine to chick-embryo hearts before innervation had taken place. And in 1936 Cullis and Lucas found that acetylcholine clearly slows the aneural chick heart and that this slowing is prevented by atropine and potentiated by eserine.

Armstrong's suggestion that the heart only becomes sensitive to acetylcholine after innervation has taken place is parallel to the view defended by Elliott in 1905 that smooth muscle only becomes sensitive to adrenaline after sympathetic nerves have established functional connections with it. Elliott based his inference on the following data. Langley (1901) had observed that the extent of the contraction of the blood vessels of different organs in the responses to adrenaline varied approximately with the extent of the known control by vasomotor nerves. Again, Brodie and Dixon (1904) had reported that the blood vessels of the lungs are not governed by sympathetic vasoconstrictor nerves and that they are not constricted by adrenaline. Finally, Elliott found that in two exceptional cats in which adrenaline could neither inhibit the urinary bladder nor lessen the contraction caused by stimulation of the pelvic nerves, the hypogastrics did not supply the usual fibers to the vesical plexus.

That nerve-free smooth muscle can respond to acetylcholine and adrenaline, however, has been proved conclusively. Thus, Langley showed in 1905 that the contractile elements of the amnion of the chick, which do not receive any nerves, are inhibited by adrenaline and stimulated by nicotine. In accord with these observations Baur (1928) and Ferguson (1940) confirmed the inhibitory action of adrenaline and showed that acetylcholine, like nicotine, has a stimulating action. Another non-innervated contractile tissue which has been studied is that of the vessels of the human placenta. Euler reported in 1938 that these vessels are regularly constricted by adrenaline with doses of  $2\gamma$  or more, and that the effect is sometimes potentiated by

cocaine and always abolished by ergotoxine. The effects of acetylcholine are irregular; Euler found usually no action but occasionally either a weak dilation or a pronounced constriction, sensitized by eserine and abolished by atropine.

## CHAPTER X

### STRIATED MUSCLE

In the review of the history of the Philipeaux-Vulpian phenomenon, the supersensitivity of denervated skeletal muscle was mentioned. The stimulation of normal frog muscle by acetylcholine was first described by Riesser and Neuschloss in 1921. The sensitivity of normal muscles varies considerably in different species. Thus, in the fish no responses were recorded by Rückert (1931). Frog muscles are quite responsive; Hess and Neergaard (1923) observed reactions when perfusing with dilutions of acetylcholine of 1:1,000,000 to 1:50,000,000. Avian muscle is less sensitive than that of the frog, but responses are readily recorded (Gasser and Dale, 1926). Normal mammalian striated muscle was considered for many years insensitive to acetylcholine (see Frank, Nothmann, and Hirsch-Kaufmann, 1922; Gasser and Dale, 1926; Wachholder and Ledebur, 1932). In 1930 Duke-Elder and Duke-Elder showed, however, that these muscles, like those of other species, respond to the agent. In dogs anesthetized with ether-chloralose, and also in the isolated perfused heads of dogs, all the extrinsic muscles of the eye react by brief and rapid contractions when acetylcholine is administered. Later studies (see Brown and Harvey, 1941) have shown that the eye muscles are especially sensitive, among the mammalian muscles, but it has been well established that all of these muscles respond to acetylcholine. Thus, in 1933 (a) Feldberg found that intra-carotid injections cause irregular contractions of the tongue muscles; and Simonart and Simonart (1935) recorded responses of the cat's gastrocnemii when acetylcholine (1 mgm.) was injected intravenously.

That denervation renders striated muscles more sensitive to the stimulating effects of nicotine had been shown by Heidenhain in 1883 in mammals and by Langley in 1905 in fowls. In 1922 and 1923 Frank, Nothmann, and Hirsch-Kaufmann demonstrated a similar sensitization to acetylcholine in mammalian striated muscles. The early reports seemed to establish an inverse correlation between the normal responsiveness and the supersensitivity to nicotine and acetylcholine developed after denervation. Thus, amphibian muscle, normally quite sensitive to these agents, was stated not to become importantly sensitized by denervation (Langley, 1908; Simonson, 1923; Rehsteiner, 1927). Fowl muscle, moderately sensitive to those substances, becomes moderately supersensitive (Langley, 1905; Gasser and Dale, 1926). Finally mammalian muscle, relatively insensitive in the normal conditions, becomes highly sensitized after degeneration of its motor nerves.

Recent studies disagree with the earlier observations, and are in contradiction among themselves. Thus, Brown (1937 b) reported that frog muscle is sensitized to acetylcholine by denervation, and evaluated the increased sensitivity as approximately 10 times the normal. A similar approximately 10-fold degree of supersensitivity of fowl's muscle was found by Brown and Harvey (1938). They contrast these relatively slight reactions with the approximately 1,000-fold increase which follows denervation in the mammal, according to Brown, Dale, and Feldberg (1936). In 1943, however, Kuffler observed that from three to eleven weeks after denervation of the sartorius of the Australian frog the sensitivity of the muscle to acetylcholine, nicotine, and caffeine is augmented to from 1,000 to 100,000 times the normal. These contradictory reports emphasize the difficulty of measuring accurately the changes of sensitivity. Obviously, the observations on the frog have been made with different experimental conditions. Since positive results outweigh negative findings, it may be concluded, nevertheless, that all striated muscles become supersensitive to

acetylcholine, as well as to other chemical agents, when deprived of their nerve supply.

An important observation was reported by Eccles in 1941 (*a*). As shown by Katz and Kuffler (1941) the majority of the muscle fibers in the frog sartorius receive two or more motor fibers, i.e., there are several endplates in each fiber. Section of one of the branches of the motor nerve of the muscle leads to "partial denervation" of the muscle fibers. Unlike the cells of the superior cervical ganglion, which become supersensitive upon partial denervation (see p. 142), the partially denervated muscle fibers revealed little or no increase in their sensitivity to acetylcholine, either at the region of the degenerated motor nerve endings or elsewhere along the muscle.

In his interesting discussion of the contractures of skeletal muscle, Gasser (1930) suggested that the responses of these contractile elements may be divided into two classes: the *contractions*, responses where the mechanical effects are attended by the appearance in the electrograms of muscular spike potentials, and the *contractures*, responses not attended by the development of spike potentials. The importance of this distinction lies in the recognition that agents which stimulate striated muscles may act in some cases by setting up propagated impulses, which become apparent as spike potentials, while in other cases they may act directly on the contractile system of the muscle fibers, without any intermediate muscle impulses. Although Langley had shown in 1907 and later again in 1909 that nicotine sets up two types of responses in frog muscle, rapid "fibrillary twitchings" and slow "tonic contractions," and had shown that the two modes of response are affected differently by different experimental procedures, and although the action of nicotine on voluntary muscle resembles closely the action of acetylcholine, for many years it was thought that acetylcholine can elicit only contractures in this type of muscle. The basis for that belief was the testimony of several observers that the electrograms did not exhibit any spike

potentials. Riesser and Steinhausen (1922) reported that the responses of frog muscle to acetylcholine were attended by smooth deflections of the galvanometer string. Schäffer and Licht (1926 *a* and *b*) found similar smooth excursions in denervated mammalian and in perfused frog muscle.

In 1935 Simonart and Simonart showed that acetylcholine can evoke not only slow but also brief responses in normal mammalian muscles. That the brief effects are contractions was proved by Brown in 1937 (*a*); the mechanical reactions are attended by the development of typical spike potentials. Again in 1937 (*b*) Brown showed that the responses of denervated mammalian muscles to acetylcholine consist of both contractions and contractures. Brown's results were confirmed by Rosenblueth and Luco (1937). After the motor nerve of a voluntary muscle has undergone degenerative section, the muscle fibers, for a reason still unknown (see p. 161), manifest persistent "fibrillary contractions"—minute individual shortenings wholly lacking in coördination. These fibrillary contractions are attended by the appearance in the electrical records of irregular random spikes. As illustrated in Figure 29, simultaneous mechanical and electrical records exhibit in the responses to acetylcholine a quick development of tension, attended by an outburst of spike potentials, followed by a more protracted mechanical effect during which even the spontaneous electrical activity associated with fibrillation is diminished or absent—i.e., they exhibit a contraction followed by a contracture. When successively increasing doses of acetylcholine are administered, it is found that the threshold for the contraction responses is lower than that for the contracture (Brown 1937 *b*; Rosenblueth and Luco, 1937).

As in the case of smooth muscle, the supersensitivity of denervated striated muscle develops gradually. Frank, Nothmann, and Hirsch-Kaufmann (1922) noted that an enhanced excitability of cat and dog tongue-muscles could not be detected until 4 days after section of the hypoglossal nerve. The sensitivity to acetylcholine then increased, reaching a

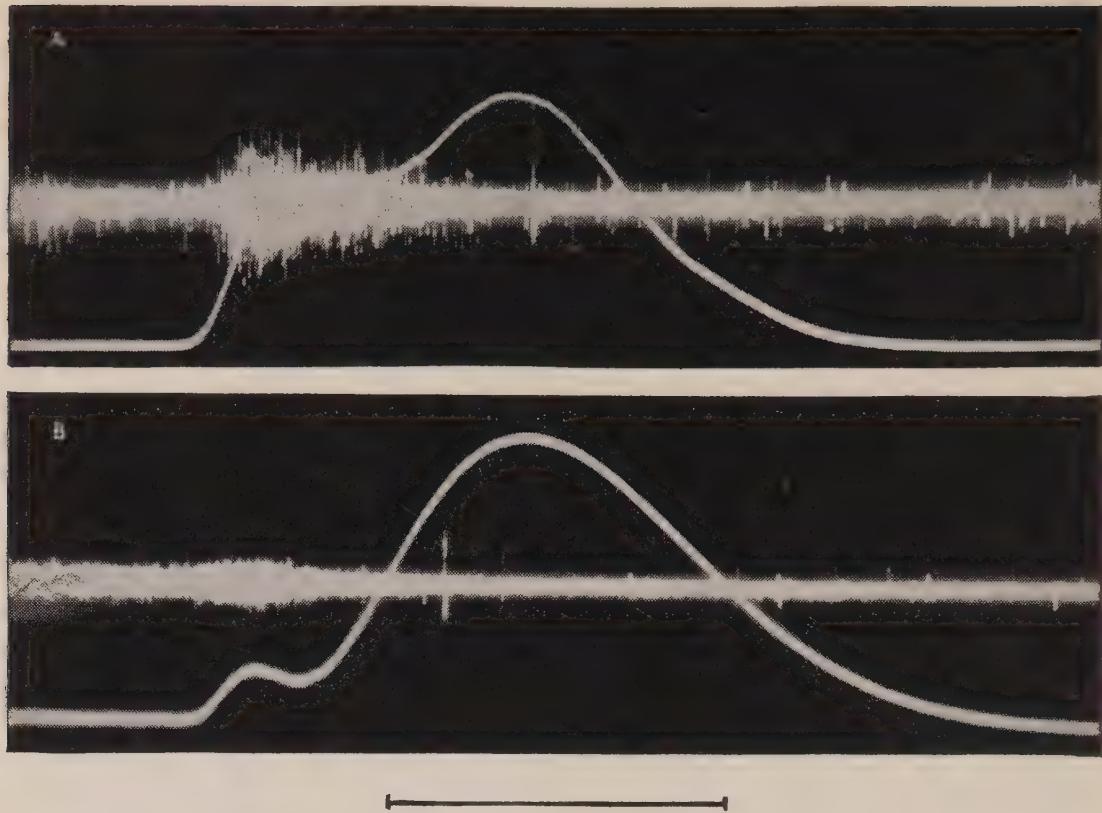


FIG. 29. Responses of a denervated muscle to acetylcholine. Cat under dial; atropine, 1 mgm. per kgm. Right sciatic nerve cut 16 days before. Records from the right Achilles-tendon muscles. Upper tracing: electrogram recorded diphasically by needles inserted into the muscles; capacity-coupled amplifier leading to a cathode-ray oscillograph. Lower tracing: isometric mechanogram. Time calibration: 2 sec.

A. Response to 0.6 mgm. acetylcholine injected intravenously. High amplification of the electrogram to show the spike potentials which attend the initial contraction response.

B. After 0.05 mgm. prostigmine. Response to 0.05 mgm. acetylcholine. Less amplification than in A in both the electric and the mechanical records. Note the relative silence of the electrogram, which attends the delayed contracture response.

maximum on about the 6th day; this maximum was thereafter stable for approximately 2 months—the time through which observations were made. In the rat, Knowlton and Hines (1937) observed that the sensitization to acetylcholine and the “contracture” appeared between the 2nd and 3rd days after a motor nerve was cut and reached a peak after 7 days; this peak was retained for about 3 weeks and then the sensitivity declined.

Brown (1937 b) found that in a cat examined on the 4th day after nerve section, when the threshold of the contrac-

tion response to acetylcholine was not less than half the normal value, the contracture response was only just visible; 6 days after the denervation the supersensitivity and the contracture appeared to be fully developed. In Rosenblueth and Luco's (1937) study, if the observations were made 5 or 6 days after the degenerative section, even large doses of acetylcholine (up to several mgm.) elicited mainly or exclusively contractions. Later (8 to 12 days after the cut) the contractures became very apparent with adequate doses, but their magnitude was approximately equal to that of the contractions—the ratio of the contraction amplitude to that of the contracture was from 0.5 to 4. When the muscles had been denervated for 15 to 31 days only small contractions were obtained, as a rule, while with large doses of acetylcholine quite large contractures developed. It appears, therefore, that the sensitivity of the contracture response to acetylcholine develops more slowly after denervation than that of the contraction response and that the increase of the contractures takes place simultaneously with a relative curtailment of the contractions.

That responses of denervated skeletal muscle can occur in the intact animal, as a result of normal activities, was shown by Bender (1938 *a*). He cut in monkeys the facial and oculomotor nerves on one side and allowed time (from 6 days to 3 weeks) for the severed nerves to degenerate. Mechanical activity of the paralytic muscles occurred whenever the monkey was angered or frightened. The effect appeared 2 to 3 seconds after the incident which caused the emotion, was of steady intensity for 20 seconds and disappeared slowly within the following 15 seconds. The responses were like slow contractures; they were persistent and never so strong as in the contralateral normal muscles. A typical observation is illustrated in Figure 30.

The contractures when the facial nerve was cut were manifested by a pulling of the nostril to the side of the paralysis, by increased wrinkling about the corner of the mouth and a curling of the lip, by almost complete closure

of the palpebral fissure (the sphincter effect), and by a downward squinting of the eyebrow. In several animals the upper lip on the paralytic side was actively raised, so that for 8 to 10 seconds the teeth and gums were exposed.



FIG. 30. Ptosis of the left eyelid resulting from severance of the oculomotor nerve. The four photographs, taken several seconds apart, show various stages in the lifting of the dropped eyelid, effected by intramuscular injection of eserine-acetylcholine. The same degree of opening was induced by frightening the animal. (Bender, 1938a.)

Effects in other muscles supplied by the degenerated facial nerve could not be observed with certainty. In places, however, the slow contracture in the muscles could be recognized by an increase in wrinkling of the overlying skin. The reaction could be obtained as long as the muscles remained denervated.

When the cervical sympathetic trunk was stimulated no changes in the denervated facial muscles were apparent. Section of the cervical sympathetic or extirpation of the superior cervical ganglion on the side of the denervation did not prevent the occurrence of the emotional reaction.

If eserine (0.05 mgm., or less, per kgm. of body weight) was injected intramuscularly the contractures of the denervated muscles induced by fright were potentiated. This effect became evident from 10 to 20 minutes after the injection. Since eserine protects acetylcholine from destruction by cholinesterase, its effect suggested that the emotional contracture might be induced by acetylcholine. Experiment confirmed this suggestion. An intramuscular injection of that agent (0.8 mgm. per kgm. of body weight), given 15 minutes after an injection of eserine, within 40 seconds

caused the denervated muscles to undergo a slow shortening which lasted from 1 to 30 minutes. The usual duration was between 5 and 10 minutes. The parallelism between the contractures obtained by frightening the monkeys and those produced by injection of acetylcholine led to the inference that the response due to fright resulted from acetylcholine, or a substance like acetylcholine, being secreted in the body during emotional disturbance.

Experiments to determine the site of formation of the acetylcholine-like substance and the manner in which it reaches the denervated muscles were undertaken by Bender and Kennard (1938). It might diffuse to the denervated structures from local tissues (e.g., from vascular muscle, as in the Philipeaux-Vulpian phenomenon), or it might be delivered from more distant sources by the blood stream. When combinations of nerves from the third to the twelfth cranial were severed and at the same time the entire ipsilateral cervical sympathetic chain was extirpated, the fright reaction was neither abolished nor diminished. This result excluded the possibility that the reaction during the emotional disturbance was due to local discharge of acetylcholine. There remained, consequently, only the blood stream as the means of bringing, from elsewhere, the acetylcholine or the acetylcholine-like substance which affected the sensitized muscles. Since acetylcholine is formed throughout the body in a display of strong emotion, or when there is a sudden muscular effort, quite possibly it diffuses from the sympathetic ganglia or the neuromuscular junctions where it serves as a chemical mediator, into the blood stream and is borne in a concentration sufficient to cause the denervated facial muscles to undergo contracture. Also, as shown by Bender (1938 *b*), animals differ in their responses to emotional stress, cats manifesting predominantly adrenergic involvement, monkeys cholinergic. Furthermore, they may differ in the degree of sensitization induced by nerve cutting. Certainly the facial muscles of the monkey are rendered extraordinarily supersensitive by denervation;

Bender and Kennard calculated that they are a better indicator for acetylcholine than leech muscle, one of the most sensitive indicators known.

In the foregoing survey of experiments on monkeys emphasis has been laid on the responses which result from severance of the facial nerve. When the oculomotor nerve is severed ptosis of the upper eyelid occurs. Excitement then causes elevation of the lid, an effect which can be duplicated by injecting acetylcholine. In an interesting case reported to one of us (W. B. Cannon) by Dr. Frank B. Walsh of the Wilmer Institute in Baltimore, there was a condition not unlike that produced experimentally in the monkey. The patient had suffered a complete paralysis of the oculomotor nerve on the left side. There was ptosis of the upper lid which, as the patient noted, was occasionally elevated momentarily and sometimes as long as a minute. He reported that washing his face in cold water or becoming angry or excited caused a lifting of the lid. An injection of acetylcholine was tried but only a small amount was used and it did not have any effect. It seems probable, nevertheless, that here was an example in man of supersensitivity of skeletal muscle that was induced by denervation.

Like other structures sensitized by denervation, skeletal muscle becomes more delicately responsive not only to its natural stimulus, acetylcholine, but also to other chemical agents. As shown by Frank, Nothmann, and Guttmann (1923), skeletal muscle lacking its normal nerve supply becomes supersensitive to potassium, and, as reported by Dale and Gasser (1926), it also becomes highly sensitive to a group of substances related to nicotine. Furthermore, Euler and Gaddum (1931) observed that if facial muscles of the dog are denervated by section of the seventh cranial nerve a slow contracture of the muscles of the upper lip occurs after an intra-arterial injection of 2 γ of adrenaline. If the adrenaline is given after stimulation of the cervical sympathetic trunk the effect is augmented. In accord with this phenomenon, is the effect reported by Bülbüring and

Burn (1936). They found that injection of 50 γ of adrenaline, after a previous dose of ergotoxine (to prevent vasoconstriction), induced contracture of the dog's gastrocnemius muscle sensitized by severance of the sciatic nerve. The suggestion arises as to whether the "fright reaction" reported by Bender might not have been the consequence of adrenaline discharged from the adrenal glands during excitement (see Cannon and de la Paz, 1911). That leaves unexplained, however, the potentiation of the "reaction" by eserine; and furthermore, Bender found that injections of adrenaline in doses ranging from 0.025 to 2.0 mgm. at times actually diminished the response due to fright or to injection of acetylcholine. The persistent action of acetylcholine in the blood of the monkey remains, therefore, a curious phenomenon.

The description has dealt so far with the results of severance of the motor nerves, i.e., of the ultimate neuron which activates striated muscles. As in the case of smooth muscle (see p. 24), however, section of penultimate neurons to the voluntary tissue leads to the development of supersensitivity to acetylcholine. In experiments to be described in detail later (see Chapter XII) Cannon and Haimovici (1939) semisected the spinal cord of cats between the twelfth and thirteenth thoracic vertebrae. This section led on one side of the cord to the degeneration of the descending nervous tracts—i.e., it led to a partial denervation of the spinal motoneurons ipsilateral to the cut and hence to the partial "decentralization" of the skeletal muscles of the corresponding hind limb. Records were taken from the two quadriceps. As shown in Figure 31, when both femoral nerves were cut and acetylcholine was injected into the aorta the response of the muscle ipsilateral to the semi-section was found markedly greater than that of its control partner.

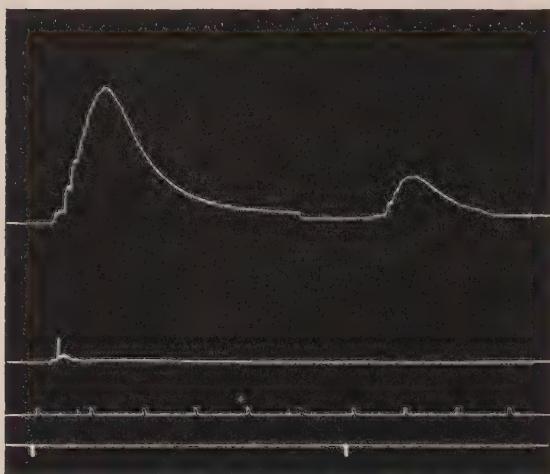
The observations of Cannon and Haimovici were confirmed by Rosenblueth, Lissák, and Lanari (1939), who ascertained, in addition, by means of electrical records, that the increased responses of the partially "decentralized" muscles

were due to the development of large contractures—the contraction responses are not significantly modified by the operative procedure.

Solandt and Magladery (1942) have also confirmed in albino rats that section of the cord at the sixth thoracic segment leads to a sensitization of the muscles of the hind limbs to the action of acetylcholine. This supersensitivity is not as marked as that obtained by section of the motor nerves.

FIG. 31. Sensitization of striated muscle to acetylcholine, caused by partial denervation of spinal neurons. Cat with brain pithed. Upper record, from right, and lower record from left quadriceps. Right semisection of the spinal cord made several days previously. Both femoral nerves cut acutely. At signals acetylcholine (0.2 and 0.1 mgm.) injected into the aorta. Time signal: 5-sec. intervals. (Cannon and Haimovici, 1939.)

The phenomenon of sensitization of skeletal muscle to acetylcholine by severance of some fibers which innervate the ultimate motor neurons appears to be analogous to the sensitization of the nictitating membrane to adrenaline by section of the preganglionic sympathetic fibers, and also to the sensitization of the submaxillary gland to pilocarpine and to adrenaline by cutting the preganglionic parasympathetic fibers in the chorda tympani nerve; in all three conditions damage to penultimate neurons results in a heightened responsiveness to chemical agents of the organs still connected with the ultimate neurons. There is some indication that this final peripheral effect may be produced at least one step further back in the neuronal series; Ascroft (1937) discovered that when the spinal cord is semisectioned at the seventh thoracic vertebra—thereby interrupting antepenultimate or perhaps more anterior neurons—the blood vessels of the foot on the cut side become especially responsive to injections of adrenaline.



## CHAPTER XI

### SYMPATHETIC GANGLIA

Long ago Elliott (1907) called attention to the resemblance between the phenomena at the junction between motor nerves and skeletal muscles and at the junction between preganglionic fibers and nerve cells in sympathetic ganglia. In both places nicotine in small doses is stimulatory and in large doses is paralytic in action, and in both places curare can block the passage of nerve impulses. When chemical mediation of nerve impulses was demonstrated another resemblance was disclosed, for acetylcholine is the intermediary at the two junctions.

As the elements of skeletal muscle are innervated by motor nerve fibers the cell bodies in sympathetic ganglia may be regarded as innervated by preganglionic fibers. Since severance of motor nerves sensitizes skeletal muscle, the question arose as to whether severance of preganglionic trunks would render ganglion cells likewise supersensitive to chemical agents. An answer to this question was sought by Cannon and Rosenblueth (1936).

The nictitating membrane of the cat was employed as an indicator. The preganglionic fibers in the cervical sympathetic supply were aseptically severed on the right side and at least a week was allowed for degeneration. At the time of the experimental tests, dial (0.8 cc. per kgm., given intraperitoneally) was used as an anesthetic. The preganglionic fibers on the left side were cut freshly, and both nictitating membranes were arranged for recording. Two methods of testing supersensitivity of the ganglion to acetylcholine were employed; it was injected into the blood stream or it was applied locally. When potassium chloride was used for stimulation, local application alone was the method.

In preparation for injecting acetylcholine the adrenal glands were first removed in order to avoid possible contractile effect on the nictitating membrane from adrenaline secreted, because acetylcholine stimulates them. Atropine (1 mgm. per kgm.) was administered intravenously to lessen the direct effects of acetylcholine on the membranes and on the blood pressure; the drug has no significant influence on the ganglion cells. A small dose of curare was used to stop the eye movements caused by acetylcholine and also to obviate muscular quivering from injected prostigmine (used instead of eserine to protect acetylcholine from destruction); curare lessens somewhat the sensitiveness of the ganglion, but not to a great extent if the dose is small. Finally prostigmine was injected.

In the local application of acetylcholine and potassium chloride no preparatory drugs were injected. Small pledgets of cotton wet with a solution of one or the other of these agents were applied directly to the superior cervical ganglion laid bare *in situ*.

In Figure 32 are presented the tracings written by the nictitating membranes recording simultaneously. The first parts (A and B) of the figure confirm the observation mentioned (see p. 24), that severance of preganglionic fibers sensitizes the nictitating membrane to adrenaline; in response to 5 γ the chronically decentralized membrane (upper record) contracts much more than the control (lower record), freshly decentralized. The second part of Figure 32A presents two responses to acetylcholine, 0.3 and 0.5 mgm., respectively. After each injection the chronically decentralized membrane contracted sharply; this quick contraction was followed by a slower contraction ending in gradual relaxation. The freshly denervated membrane showed no real contraction; the slight change was possibly due to a movement of the eyeball.

Figure 32B shows the responses which occurred when the same procedures were repeated after both cervical ganglia had been removed. Adrenaline had the effect which it had

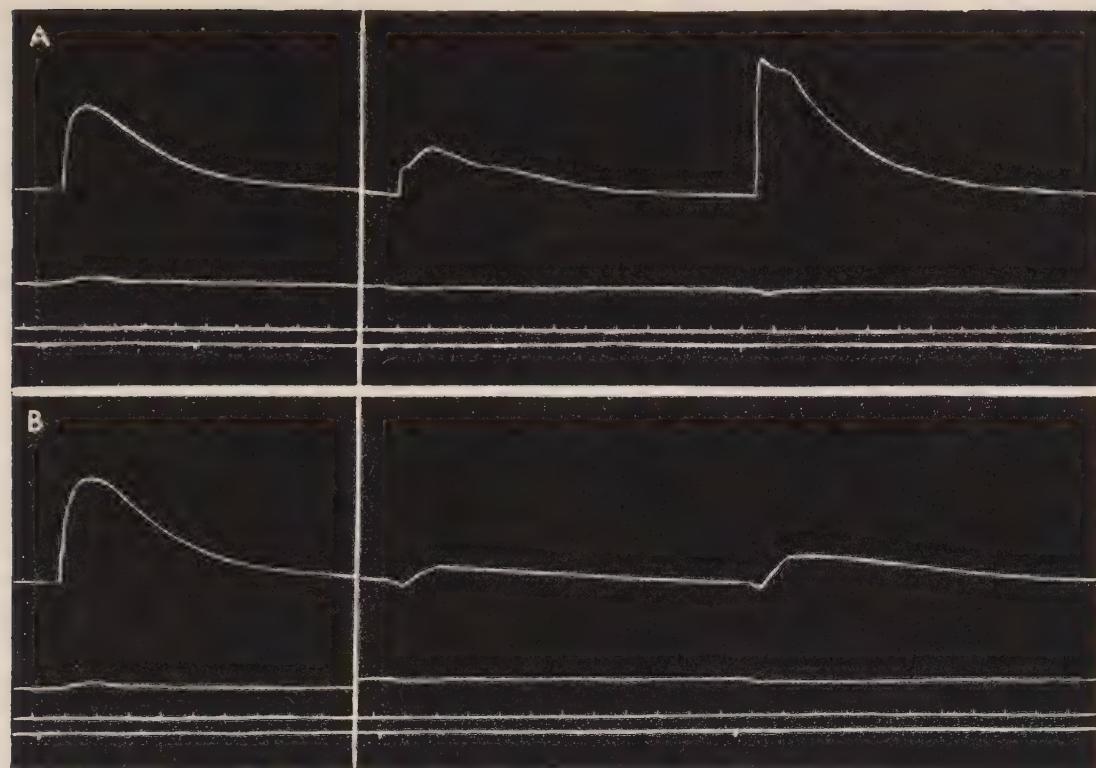


FIG. 32. Sensitization of the denervated superior cervical ganglion. Cat under dial; adrenals ligated; atropine, curare, and prostigmine. Contractions of the nictitating membranes. Right membrane (upper record) connected with ganglion denervated 9 days; left membrane (lower record) freshly decentralized. Effects of injecting intravenously first adrenaline ( $5\gamma$ ) and then acetylcholine (0.3 and 0.5 mgm.). A, before, and B, after removal of both superior cervical ganglia. Time signal: 30-sec. intervals. (Cannon and Rosenblueth, 1936.)

had previously, as was to be expected, because of its direct action on smooth muscle. Acetylcholine, however, no longer evoked the quick response on the chronically decentralized side and the degree of the response was much less as compared with that when the ganglia were present. From these and other similar observations the inference was drawn that the quick and high contraction of the membrane supplied by the denervated ganglion cells was due to a discharge from these cells. Since it did not occur on the side where the cells were freshly denervated, it was inferred that the chronically denervated cells of the ganglion had become supersensitive to acetylcholine. Rosenblueth (1932 *a*) had shown that acetylcholine can by itself stimulate the nictitating membrane. The small contractions recorded in

Figure 32B demonstrate that its smooth muscle becomes sensitized to acetylcholine by decentralization.

The defects in this evidence are two: first, the possibility of a differential effect of the preparatory drugs on the two sides; and second, the possibility of stimulating the sensitized membrane as well as the sensitized ganglion cells by the injected acetylcholine. In order to avoid these possible errors the simpler method was used—the application of cotton wet with acetylcholine (1 per cent) directly to the exposed ganglia. After a brief latent period the nictitating membrane connected with the chronically denervated ganglion underwent a sharp and prolonged contraction (see Figure 33A), while that connected with the freshly dener-

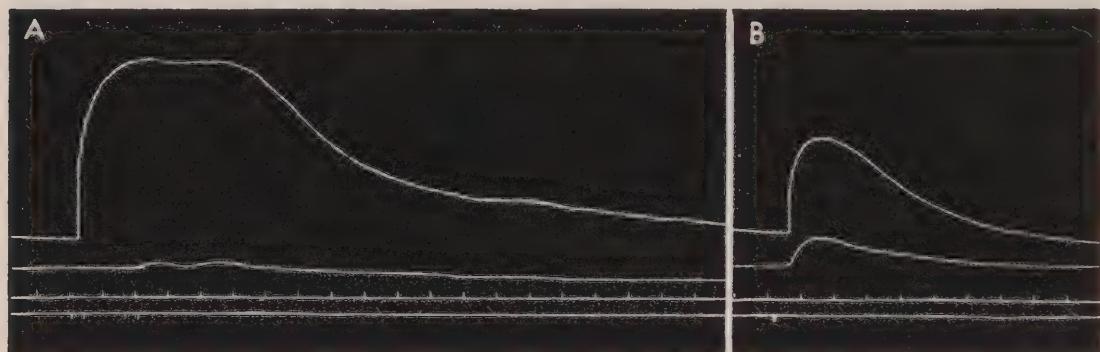


FIG. 33. Contractions of the nictitating membrane of a cat under dial. Right membrane (upper record) connected with superior cervical ganglion denervated 15 days; left membrane (lower record) freshly decentralized. A, cotton pledges wet with acetylcholine (1 per cent) applied to both superior cervical ganglia; first pair of signal marks, pledges on; second pair, off. B, adrenaline ( $5\gamma$ ). Time signal: 30-sec. intervals. (Cannon and Rosenblueth, 1936.)

vated ganglion did not respond. It should be emphasized that as soon as the contraction was well started the pledges were removed and the ganglia were promptly washed with normal salt solution. Then the test could be repeated. That the unresponsive membrane was capable of contracting was proved by injecting adrenaline and showing that it responded well (Figure 33B), even though the corresponding contraction on the sensitized side was less than that induced by the pledge on the ganglion. When both ganglia were removed and the acetylcholine was applied on pledges

placed in their former positions there was no effect. The striking response on the chronically denervated side was not therefore due to transport of acetylcholine from the region of the ganglion but resulted from direct stimulation of the ganglion cells which had been sensitized by degeneration of the fibers formerly innervating them.

As previously noted, Dale and Gasser (1926) demonstrated that denervated skeletal muscle is sensitized not only to acetylcholine but also to potassium ions. When pledges of cotton wet with potassium chloride (5 per cent) were applied to the superior cervical ganglia, the chronically denervated cells were strongly stimulated, as revealed by a large contraction of the nictitating membrane, while the freshly denervated cells were not affected.

The observations of Cannon and Rosenblueth were confirmed by Chauchard (1938) in a study of the chronaxie of the postganglionic fibers disconnected from the central nervous system. After the preganglionic nerve had completely degenerated, the postganglionic fibers were found to have a greatly augmented chronaxie. When a weak solution of nicotine (0.06 per cent), which normally does not modify the excitability of those fibers, was applied to the denervated ganglion, their chronaxie could be doubled or tripled. And when acetylcholine was used, there was again evidence of supersensitivity. Application of solutions which normally had little influence induced a marked diminution of the chronaxie in the denervated neurons. This phenomenon of sensitization, which was observable forty-eight hours after the preganglionic trunk was cut, was increased still further on the third day.

The evidence that degeneration of preganglionic fibers renders the cells of the superior cervical ganglion supersensitive to acetylcholine was doubted by Brücke (1938). From his experiments he inferred that the only effect of the denervation is to lower the limit of concentration at which acetylcholine ceases to have an excitatory action on the ganglion cells and becomes instead a depressant agent. The

results of Brücke's experiments differed from the results reported by Cannon and Rosenblueth for a number of reasons. First, in repeating our experiments of locally applying the drug to the ganglia he changed the conditions; he injected atropine (which has a depressant action on the response of the nictitating membrane), whereas we avoided use of any drug except the anesthetic. Also, in repeating our experiments he did not duplicate our conditions; he omitted the use of curare, an omission which significantly modified the results. Furthermore, he gave mainly excessive doses of acetylcholine—2 to 5 mgm. as contrasted with less than 1 mgm. used by us. (It seems necessary again to emphasize the point that differences of sensitivity cannot be determined by tests which cannot be discriminative, but have maximal effects on both the more and the less sensitive structures). Finally, Brücke failed to publish the time during which pledges wet with acetylcholine were applied to the ganglia; if that time is long the effects are like those which result from large doses; discrimination is absent because the two sides, the more and the less excitable, may both be stimulated to maximal action.

However well justified the criticism of Brücke's experiments, the difference of results called for use of another method which would be decisive. The essential need was a separation of the direct action of acetylcholine on the nictitating membrane and the indirect action by way of the ganglion. A method assuring this required condition and the results obtained by use of it were reported by Rosenblueth and Cannon in 1939.

After degenerative section of the cervical sympathetic trunk of one side, the animals (cats) were prepared for the test in the following manner. The still intact cervical trunk was cut, the adrenal glands were excluded from action, the eyeballs were removed (in order to avoid possible complication from eye movements), the *external carotid* arteries were tied immediately below the lingual arteries, and the common carotids were carefully cleaned of surrounding

tissues low in the neck. Each carotid was enclosed in a loose loop of thread, so that it could be gently lifted to receive an injection through a fine needle pressed obliquely through the arterial wall. These arrangements had two definite advantages. First, they provided that the drug would have an immediate and direct action on the ganglion, but a delayed action on the nictitating membrane because, with external carotids tied, the blood stream must take a long and devious course to reach it. The effects at the two points, therefore, would be well separated in time. Second, because of direct action on the ganglion, small injections of acetylcholine (5 to 10  $\gamma$ ) would be effective—amounts so small that, although stimulatory for the ganglion, they would be without influence on the membrane, especially when its responsiveness was depressed by atropine; thus again, the effects on the two structures would be distinct.

Adequate doses of acetylcholine injected into the common carotid artery resulted in contraction of the nictitating membrane in two stages—an initial sharp contraction having a latency of about 1 second and a slower further contraction with a latency varying from 9 to 20 seconds. Similar injections after removal of the superior cervical ganglion resulted in complete disappearance of the initial sharp contraction without any modification of the delayed component. These results are illustrated in Figure 34. Section A of the figure shows the action of adrenaline (10  $\gamma$ ), injected *intravenously*; the predominant effect is on the chronically decentralized nictitating membrane (lower record). In section B an injection of acetylcholine (5  $\gamma$ ) into the *right* common carotid artery (the *normal* side) resulted only in delayed (10 seconds, timed by stop watch) contractions of both membranes; the drug reached the membranes through the devious course, and there was no effect on the freshly denervated ganglion. In section C the same dose, injected into the *left* common carotid (the side where the ganglion was *chronically denervated*), caused a prompt response (in 1 second) of the left ganglion; the less

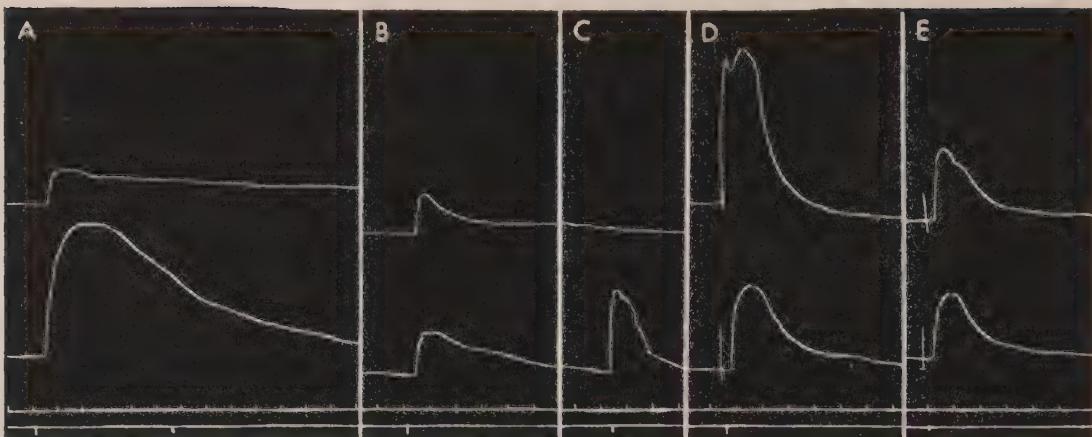


FIG. 34. Prompt and delayed contractions of the nictitating membrane resulting from injections of acetylcholine into the carotid artery. Cat under dial. Left membrane (lower record) connected with superior cervical ganglion denervated twelve days; right membrane (upper record) freshly decentralized. Time signal: 30-sec. intervals.

- A. Adrenaline ( $10\gamma$ ) injected intravenously.
- B. Acetylcholine ( $5\gamma$ ) injected into right carotid (normal side).
- C. The same dose of acetylcholine as in B, but injected on the left (denervated) side.
- D. As in B, but  $40\gamma$  acetylcholine.
- E. As in D but after removal of the two superior cervical ganglia. (Rosenblueth and Cannon, 1939.)

sensitive right membrane did not contract. In section D is shown the immediate response (delay of 1 second) of the normal, right ganglion when a much larger dose of acetylcholine ( $40\gamma$ ) was injected into the right carotid artery; the left membrane contracted only after a delay (10 seconds), coincident with an increase of contraction on the right side. The simultaneous ordinates indicate the time relations of the two effects. In section E, finally, is shown the disappearance of the prompt component when the same large dose of acetylcholine as in D was repeated after removal of the two cervical ganglia.

When atropine (1 mgm. per kgm.) was injected it reduced to a slight degree the magnitude of the sharp initial component but did not delay it, while it abolished the later effect on the membrane. These results are illustrated in Figure 35. Section A shows the prompt contraction (delay, 1 second) of the nictitating membrane (lower record) connected with the left superior cervical ganglion (denervated

14 days previously), when acetylcholine ( $5\gamma$ ) was injected into the left carotid artery before atropine was administered. A similar injection into the right carotid did not produce any response. Between sections A and B atropine (1 mgm. per kgm.) was administered. In section B (first mark) an injection of acetylcholine ( $10\gamma$ ) into the right carotid (i.e.,

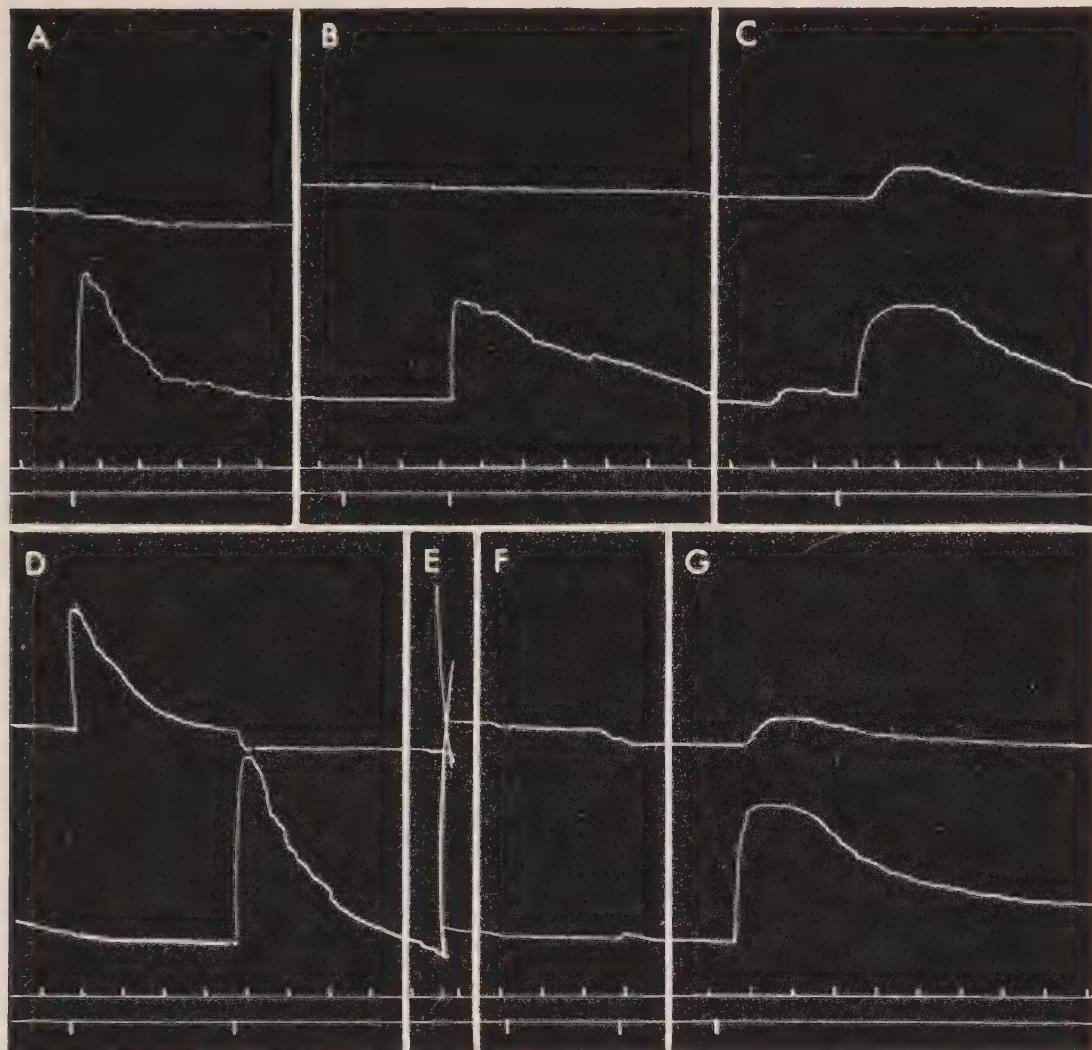


FIG. 35. Difference of sensitivity to acetylcholine between chronically and freshly denervated superior cervical sympathetic ganglia. Cat under dial. Lower record, left nictitating membrane, decentralized 14 days; upper record, right membrane, freshly decentralized. Time signal: 30-sec. intervals.

A. Acetylcholine ( $5\gamma$ ) injected into left carotid. A similar injection into the right carotid caused no response. Between A and B atropine (1 mgm. per kgm.) injected intravenously.

B. Acetylcholine ( $10\gamma$ ), first into right, then into left carotid.

C. Adrenaline ( $10\gamma$ ), intravenously.

D. Acetylcholine ( $40\gamma$ ) first into right, then into left carotid.

E. Removal of the superior cervical ganglia (with standing drum).

F. Acetylcholine as in D.

G. Adrenaline as in C. (Rosenblueth and Cannon, 1939.)

on the freshly denervated side) was without effect; the same dose introduced (second mark) into the left carotid was stimulatory to the ganglion, and the membrane contracted. Section C shows the usual effects of an intravenous injection of adrenaline (10 γ). In section D are recorded the effects of injecting a large dose of acetylcholine (40 γ) first into the freshly sectioned right side, and second into the chronically sectioned left side (compare with section B, Figure 34). Note that large doses of acetylcholine resulted in approximately equal discharges from the decentralized and the control ganglia. In section E are recorded the contractions of the membranes when their nerves were stimulated by removal of the ganglia while the recording drum was standing still. In section F the absence of contraction when acetylcholine (40 γ) was injected successively (at the marks) into the carotids, again proves the blocking effects of atropine at the membrane. Section G is a repetition of section C; the effect of injecting adrenaline (10 γ, intravenously) demonstrates that the membranes were still capable of responding.

Repeated tests on seven animals proved that the previously denervated ganglion was consistently about four times more sensitive than its freshly denervated companion, i.e., the threshold concentration of acetylcholine was only about one-fourth as high. This augmentation of sensitivity consequent on degenerative severance of the controlling nerves is not nearly so great as that seen when skeletal muscles are sensitized by denervation. The evidence clearly demonstrates, however, that the final sympathetic neurons, bereft of preganglionic fibers by section and degeneration, become sensitized to their natural stimulating agent, acetylcholine.

The observations of Cannon and Rosenblueth that cotton pledges wet with acetylcholine (1 per cent), when applied locally to the cervical ganglia, revealed a marked difference of sensitivity between the chronically denervated and the control ganglion, were not confirmed by Brücke. In the later study reported by Rosenblueth and Cannon the pre-

vious observations, however, were consistently confirmed. Whether the eyeballs were intact, emptied, or excised, the phenomenon was present. It was found, furthermore, that the period of application of the pledges was important. In the earlier observations they were removed soon after the response began, i.e., in about 45 seconds. In the later experiments a similar brief application evoked a response on the denervated side but not on the control side. The same solution applied for 90 seconds, however, resulted in a delayed response on the control side also. It is possible that inattention to the time during which acetylcholine acted on the ganglion was the source of Brücke's failure to repeat the results obtained by Cannon and Rosenblueth. The reasonableness of that suggestion cannot be assured, however, for he did not publish a time-scale for his records, nor did he tell how long he left the pledges in place.

## CHAPTER XII

### SPINAL NEURONS

The sensitizing of ganglion cells by denervation naturally suggested that the nerve cells of the brain and spinal cord might be likewise sensitized if they were "denervated," i.e., deprived wholly or in part of the nervous connections from which they routinely receive impulses. A research by Cannon and Haimovici (1939) was undertaken to learn about that possibility.

Cats were used, weighing about 3 kgm. As a region for testing this idea the lower portion of the spinal cord was selected, for various reasons. Semisection is a relatively simple operation and is well borne. Furthermore, the quadriceps, attached to the patella and supplied by readily accessible nerves, can be easily isolated and used as an indicator of nervous discharges. In addition, the direct blood supply of the muscle can be promptly and temporarily shut off while that to the cord is continued. And finally, injection into the lower thoracic aorta permits chemical agents to be delivered locally to both sides of the cord in a concentration which does not affect the rest of the nervous system.

The semisection of the cord was always made on the *right* side, most commonly between the twelfth and thirteenth thoracic vertebrae. After the spinal canal was opened a needle, pressed directly downward through the mid-line of the cord, served as a guide for the sharp blade of fine scissors which, on being closed, completed the semisection. To assure time for degeneration of the isolated portion of the severed fibers the interval between the semisection and the acute experiment was usually between five and eight days. In one case when the interval was only two days it proved to be too short.

Since the experiment involved the possibility of sensitization of the partially denervated half-cord to chemical agents, avoidance of anesthetics at the time of the acute experiment was deemed desirable for they might be more effective on the sectioned than on the normal side. Accordingly operations for the final tests were made as rapidly as possible under ether, whereupon the brain (including the medulla) was pithed through the foramen magnum and etherization was stopped. Thereafter the preparation was left for at least thirty minutes to permit artificial respiration to remove the ether vapor.

For the acute experiment a strong thread was looped around the aorta just above the iliac branches and passed through a glass tube of small diameter; the mid-abdominal section was closed with a loose continuous suture; a simultaneous pull on the loop and a push downward on the tube (which reached to the aorta), closed the direct blood flow to the leg muscles. Threads looped under the femoral nerves allowed them to be readily lifted and cut whenever that was desired. For injection into the branches of the aorta which deliver blood to the spinal cord the chest was opened on the left side between the tenth and eleventh ribs, the ribs were separated 4 or 5 cm. by means of an adjustable spreader, the left lung was pushed upward and held away from the opening by dry absorbent cotton, the diaphragm was drawn away from the aorta, and, when the vessel was cleared of its pleural covering, a thread looped around it permitted it to be lifted slightly for insertion of a fine syringe-needle. Doubtless much of the injected fluid entered the splanchnic vessels; it seemed wise, however, to allow this escape rather than to prevent it by a deeply disturbing evisceration. In order to protect the thoracic structures from cooling and drying, a pad of cotton was laid over the opening in the chest wall and removed only at times of injection.

For simplifying the muscular responses most of the leg muscles were paralyzed by severance of the sciatic nerves.

Drills were screwed into the two ends of each femur and so fixed in rigid clamps (supported on rigid uprights) that the bone was held vertical. The freed tendon of each quadriceps, attached by means of a strong string to the short end of a firmly supported lever, was stretched by a thick rubber band. The tension was approximately alike for the two muscles. The two levers had equal magnification—about 10-fold. An electric heating pad kept the preparation warm.

An important question was whether the unoperated side of the cord should be left intact or sectioned at the time of the immediate test. No influences could come from the brain, for that had been pithed. A number of reasons led to avoidance of any further operations, i.e., completing semi-section of the cord. Equivalent effects were obtained on the two sides when the initial semisection was only two days old—a delay too brief for nerve degeneration—and the left side was not cut across. Furthermore, in animals not previously operated upon, acute semisection was found to depress the irritability of the freshly separated neurons; such a depression, if produced by section of the still intact side at the time of testing, would favor the idea that the chronically sectioned side was more sensitive than the other—the very idea which was suggested by previous evidence of supersensitivity from denervation and which must not be favored. Confidence was felt, therefore, in the propriety of leaving intact the uncut side of the cord when the relative sensitiveness of the two sides was being examined.

As stimulating procedures, solutions of strychnine sulphate, acetylcholine chloride, and sodium carbonate (half-saturated) were injected into the aorta, and also asphyxia was employed.

*Effects of Strychnine.* Two types of responses were registered—single twitches and a more or less prolonged clonus. Strychnine (0.4 to 0.5 mgm.), injected into the aorta, evoked one or the other of these reactions. The contractions of

the quadriceps were more marked on the cut side. For example, after an injection of 0.4 mgm., there were in one experiment 71 twitches in fifteen minutes on that side, and none, or the slightest signs of them, on the intact side.

More striking were the clonic contractions. These sometimes appeared on the two sides; in such instances they started later on the intact side, were intermittent and were less persistent, ending in one case 47 minutes after the start while the clonus on the cut side continued for seventeen minutes longer. In another animal injection of strychnine called forth, on the cut side only, a clonus which lasted 68 minutes; the contraction rate varied from 13 per second in the early stages to 14.5 in the later. At the rate of 13.5 per second the quadriceps on the right side contracted without intermission about 55,000 times (see Figure 36) before it ceased.

*Effects of Acetylcholine.* Acetylcholine injected into the aorta might act in various ways. By direct stimulation it might cause the quadriceps to shorten and thus might start a series of clonic contractions; or just as it excites the ultimate autonomic neurons it might excite spinal neurons; or, as shown by Schweitzer and Wright (1937), it might depress them.

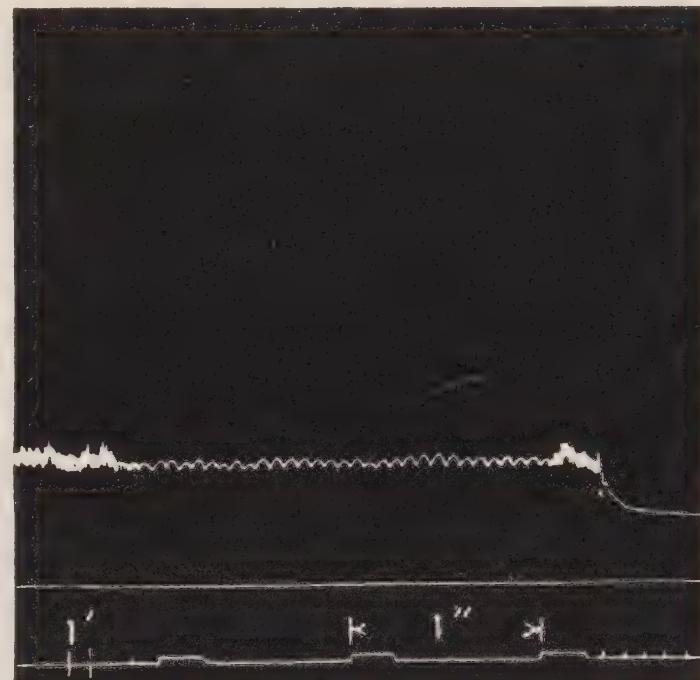


FIG. 36. Sensitization of partially denervated spinal neurons. Cat with brain pithed. Right spinal semisection performed several days before. Upper record, right quadriceps controlled by partially denervated neurons; lower record, left quadriceps controlled by intact side of the cord. Termination of a unilateral clonus lasting 68 min. after an injection of strychnine into the aorta. Time signal: 1-min. and 1-sec. intervals. (Cannon and Haimovici, 1939.)

If at the testing time the femoral nerve on the side of the spinal semisection was severed, or if both femorals were severed, an intra-aortic injection of an adequate dose of acetylcholine evoked a contracture on the right side and little or no response on the left (see p. 103). The exaggerated response of the quadriceps on the semisected side raised the question whether such a response when the femoral nerve was intact might not be due to sensitization of the muscle to acetylcholine without involvement of the nerve cells in the cord. Two types of evidence showed that acetylcholine can stimulate the spinal neurons below the semisection: first, a contraction of the right quadriceps when the direct blood flow to the legs is stopped by clamping the aorta above the iliacs; and second, a peculiar response, different from that of the muscle alone.

In Figure 37 is shown a quadriceps contraction after an injection of acetylcholine while the direct circulation was blocked by closing the aorta about fifteen seconds before.

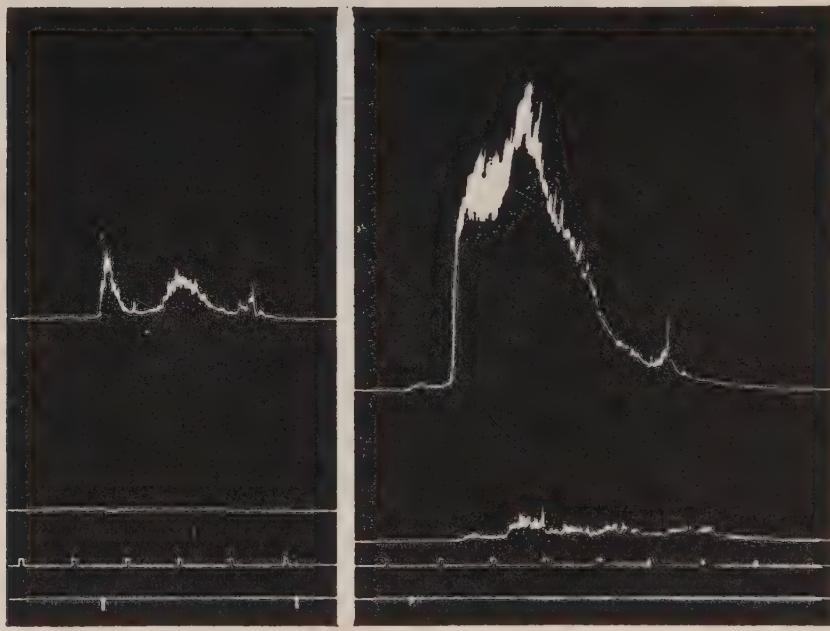


FIG. 37 (left). Preparation and records as in Fig. 36. At first lower signal, acetylcholine (0.2 mgm.) injected into the aorta clamped above the iliacs; at second signal-mark clamp removed. Time signal in this and other records through Fig. 40: 5-sec. intervals. (Cannon and Haimovici, 1939.)

FIG. 38 (right). As in Fig. 36. At lower signal, acetylcholine (0.3 mgm.) injected into aorta; nerves and blood supply intact. Strychnine (0.1 mgm.) previously injected. (Cannon and Haimovici, 1939.)

There was a sharp response on the right side (upper record) and none on the left. The secondary effect was unexplained, though the depression between the two rises might have been due to excess of the drug. That the left (intact) side was capable of responding was proved by the presence of the knee jerk. After the right femoral nerve had been cut, acetylcholine caused a slight contracture of the denervated quadriceps but had no effect on its opposite control.

The second kind of response was registered when acetylcholine was injected while the blood and nerve supplies to the quadriceps were not interrupted. It appeared to be a combination of the effects of acetylcholine on the spinal neurons and on the muscle. As shown in Figure 38, there was a quick contraction, similar to that recorded in Figure 37, followed by a persistent contracted state in which clonic contractions rose from a curve like that in Figure 31. It is possible that the clonus was started by a muscular contraction resulting from the direct effect of acetylcholine; the sharp initial rise in Figure 38, as contrasted with the slow rise in Figure 31, and also the presence of twitches on the intact side (lower record, Figure 38) indicate that the drug acts on the spinal neurons as well as on the muscle of the semisected side.

There may be some surprise that relatively large doses of acetylcholine (200 to 300  $\gamma$ ) were given in these experiments. It should be remembered, however, that no protective eserine was administered, and that doubtless most of the acetylcholine injected did not enter the small vessels leading to the spinal cord but escaped into the general circulation by way of the splanchnic arteries.

*Effects of Sodium Carbonate.* Strong solutions of sodium salts are known to have a convulsant effect by action on the central nervous system. An injection of a small amount (0.1 to 0.2 cc.) of a half-saturated solution of sodium carbonate into the thoracic aorta causes a discharge of nerve impulses.

Figure 39 displays the result of 0.1 cc. injected while the

aorta above the iliacs was closed, to prevent any blood flow to the leg muscles. The marked muscular response on the right side (upper record) shows that the stimulant action was especially pronounced on neurons partially denervated.

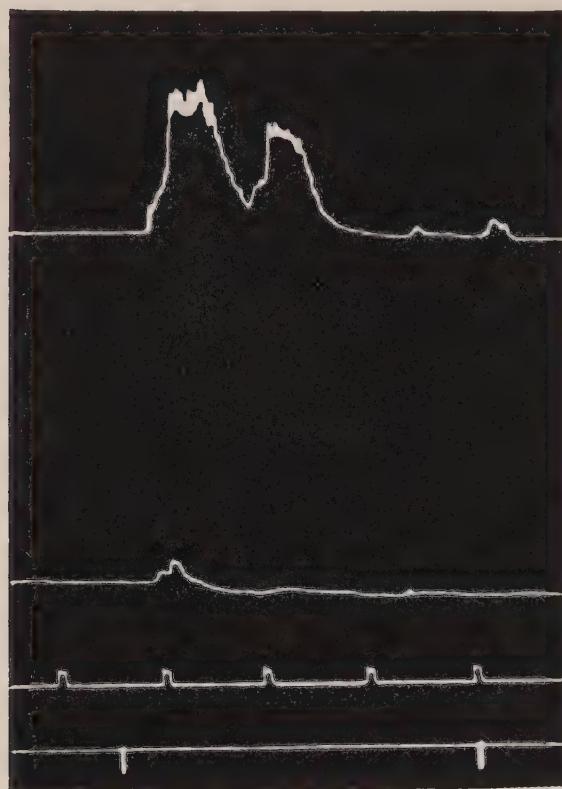


FIG. 39. As in Fig. 37. At first signal-mark, sodium carbonate (0.1 cc., half-saturated solution) injected; at second signal, clamp removed from aorta. (Cannon and Haimovici, 1939.)

cells. It is so little disturbing that it can be repeated a number of times without much change in results. In the experiments of Cannon and Haimovici asphyxia was easily induced by merely stopping artificial respiration. The effects were various. In some animals and in some tests the discharge was solely on the cut side of the cord. In others it was on both sides, but then the discharge came earlier on the cut side, was more pronounced, and also more lasting than on the intact side.

Figure 40A presents an example of pure unilateral effect. The quadriceps innervated by the partially isolated neurons was brought abruptly into contraction when the air was shut

*Effects of Asphyxia.* That asphyxia can cause increased excitability of spinal neurons was shown by Russell (1893). In experiments on dogs and rabbits he found that in the early stages of asphyxia there was an exaggeration of the knee jerks until a single tap on the patellar tendon evoked a clonic response. This exaggeration occurred quite as well when asphyxia was induced in animals whose spinal cords had been divided above the lumbar enlargement.

Undoubtedly partial asphyxiation is the simplest way of exciting spinal nerve

off for about 30 seconds, whereas the muscle controlled by the neurons on the intact side was not affected. This reaction was twice repeated within eight minutes, once with the aorta clamped. That the uncut side of the cord was fully capable of responding was proved by repeated tests of the knee jerk.

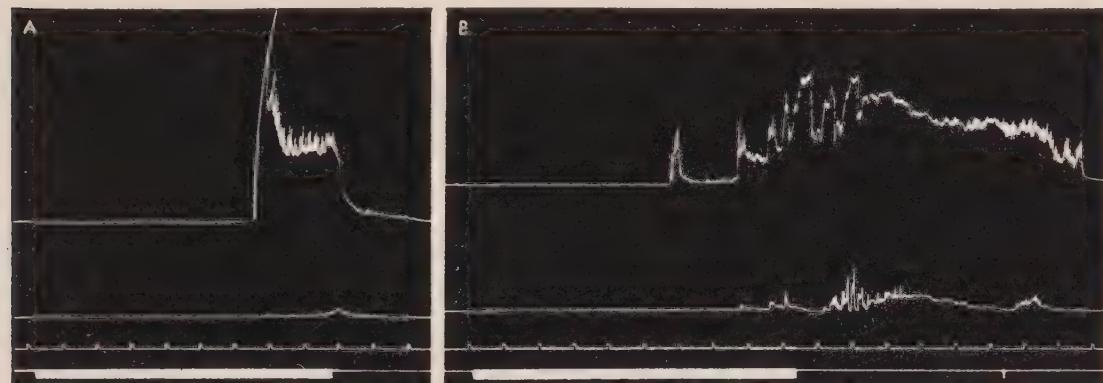


FIG. 40. As in Fig. 36. A, effect of asphyxia induced during period marked by the broad line. B, another type of response to the same conditions, but with the aorta clamped above the iliacs; at second signal-mark, clamp removed. (Cannon and Haimovici, 1939.)

Figure 40B shows the other type of response. In this case the lower aorta was closed without causing contraction of the asphyxiated muscles; the excitatory effects which were recorded were due solely to neuronal responses. Obviously the asphyxial state has a much more prominent influence on the semisected than on the intact side of the cord.

From the foregoing results the inference may be drawn that as denervation of the nerve cells of the superior cervical ganglion (see p. 114) sensitizes them to the action of chemical substances, so partial denervation of spinal nerve cells sensitizes them to chemical agencies which are stimulatory. Thus neurons in the central nervous system may be added to neurons of the autonomic system, and also to glands and to smooth and skeletal muscle, and to the heart, as obeying a general law that degeneration of the nerves which normally deliver impulses to a structure is attended by a lowered threshold to submaximal chemical stimuli applied to that structure.

## CHAPTER XIII

### COÖRDINATING CENTERS

The evidence that partial denervation of spinal neurons renders them more susceptible to stimulation by chemical agents raised the question as to whether disconnecting other parts of the central nervous system from a superior control would have a similar effect. With this possibility in mind Stavraky (1943) removed various portions of the brain in aseptic operations, waited for complete recovery of the animals, and then tested the effects of injecting acetylcholine.

In ten cats he performed cerebral operations ranging from extirpation of one frontal lobe to a complete supratentorial decerebration. In four animals the frontal lobe was excised on one side with inclusion of the motor cortex; the resection line passed through the neighborhood of the sulcus ectosylvius anterior. In all animals the removals extended to the base of the brain, well in front of the optic chiasma. In two cats complete semidecerebrations were performed. The incision then was made in the sagittal plane through the corpus callosum and the diencephalic structures about two to three millimeters off the midline, so as not to damage the optic chiasma, the stalk of the hypophysis cerebri and the hypothalamic nuclei of the opposite side.

Acetylcholine bromide dissolved in about 0.5 cc. of distilled water was injected into the femoral vein or into the great saphenous. The injections were carried out without anesthesia or any previous preparation except shaving the inner surface of the thigh. The animals manifested no discomfort, and though the injections were repeated at first once a week (later at longer intervals), there were no ill effects and several of the animals lived for more than a year.

The quantities injected were not related to the weight of the animal since no close relation to weight was found.

As controls, acetylcholine was injected into full-grown normal cats in doses between 0.1 and 0.2 mgm. and in larger doses of 0.2 to 0.3 mgm. The smaller doses had little influence on the normal animals—there was a brief phase of unsteadiness or slight twisting of the extremities; the cat then lay down and lowered its head. Momentary slight dilation of the pupils occurred in the initial stage of the response and some salivation was observed. The larger injections caused slow, spastic contractions of the extremities of the athetoid type. In sensitive animals these movements might end in a short period of rigidity. Again there was a moderate initial dilation of the pupils preceding the muscular contractions.

*Effects of Acetylcholine after Removal of One Frontal Lobe.* In the early stages of recovery from the operation (from ten days to two weeks) the most prominent features of the reaction of the animals to acetylcholine were a markedly greater initial dilation of the contralateral pupil, slight lachrymation on that side, and an increased tendency to convulsions. Gradually a completely asymmetrical response to the injections of acetylcholine developed on the two sides of the body. This development reached a maximum in five or six weeks after the operation and persisted thereafter.

Small doses of acetylcholine (0.05 to 0.1 mgm.), which before the operation were practically ineffective, now produced, after a short period of latency, a forward thrust of the contralateral front paw and a turning of the head toward the side of the operation. A transient dilation of the pupils preceded these changes and was more pronounced on the contralateral side (see Figure 41B).

Injections of 0.1 to 0.2 mgm. of acetylcholine caused a greater dilation of the contralateral pupil and a contraction of the muscles of the whole side of the body opposite to the removal. The head was drawn down and the face turned away by a powerful contraction of the sternocleidomastoid

muscle; the front paw was raised over the head and often the claws, which were protruded, were pressed into the skin behind the ear. The back paw was drawn forward into a semi-flexed position and the body of the animal was curved with the concavity on the side opposite to the side of the operation (see Figure 41C). Often there was some erection

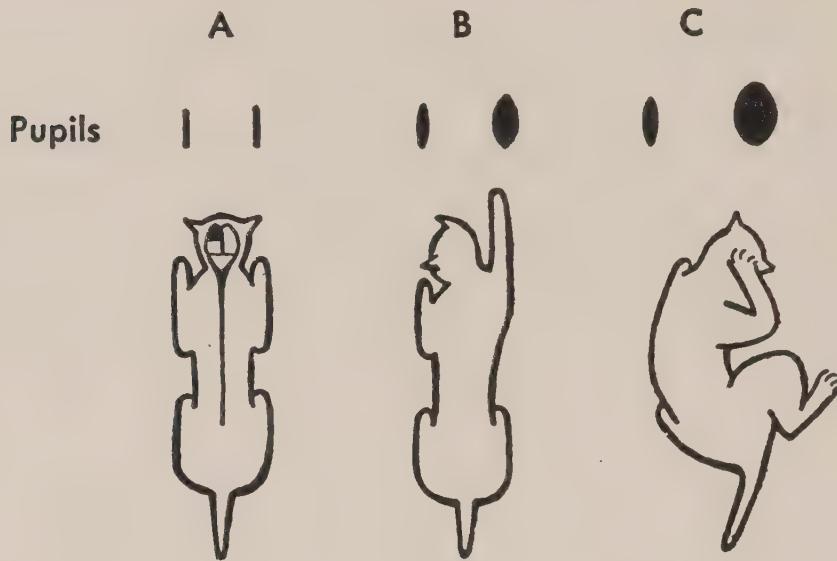


FIG. 41. Outlines of a cat from which left frontal lobe, including the motor cortex, was removed. The diagrams show the movements and postures resulting from injections of subconvulsive doses of acetylcholine. A, pupils and posture before injection. B, pupils and posture after a small dose of acetylcholine (0.05 to 0.1 mgm.). C, pupils and posture after a larger dose (0.1 to 0.2 mgm.). (Stavraky, 1943.)

of the hair over the spastically contracted extremities. The tonic contraction lasted four or five minutes and toward the end a coarse tremor occasionally appeared in the rigid limb. Though the effect gradually wore off, turns of the head toward the side of the operation and continued greater dilation of the contralateral pupil might persist for several hours.

When still larger doses of acetylcholine were administered (0.2 to 0.3 mgm.) the effects appeared on both sides. It is noteworthy, however, that when the animal came out of the rigid state the ipsilateral limbs quickly resumed their normal posture and their natural tone, while the muscles contralateral to the partial decerebration manifested a

characteristic contraction which produced the body curvature shown in Figure 41C.

Incidentally it may be noted that when Stavraky removed both frontal lobes he found that acetylcholine (0.2 to 0.3 mgm.) produced an exaggeration and prolongation of the tonic muscular contraction and a great accentuation of all the features of sympathetic discharge. There was a brisk dilation of both pupils; the back was arched, the head retracted, the four extremities rigidly extended, the tail dorsiflexed, the pupils remaining in continued dilation, and the hair standing erect all over the body.

*Effects of Acetylcholine after Semidecerebration.* Semidecerebrated animals proved to be very sensitive to acetylcholine. A small dose (0.1 mgm.), practically ineffective before the operation, caused a response such as is seen in normal animals only when a much larger dose is given. All four limbs were rigidly extended, with claws protruding, and with pupils widely dilated. The initial pupillary dilation was greater on the side opposite the decerebration. Toward the end of the convulsive seizure a slow tonic contraction of the muscles developed in the extremities on the contralateral side and was accompanied by slight rhythmic movements; the front paw was raised and was gently worked up and down; the hind limb was held rigid in a semi-flexed position until it began a movement suggesting an incomplete scratch reflex.

In the foregoing observations there are a number of points to be noted especially. First, after an operation on the brain, the full discriminative effect of injections of acetylcholine appears, not at the end of five or six days, as was found by Cannon and Haimovici in semisection of the spinal cord, but only after a delay of five or six weeks. The reason for this long period before maximal sensitization developed is not explained. It is noteworthy, also, that with discriminative small doses of acetylcholine muscular contractions of a coördinated character are limited to the side of the body opposite to the removal of the frontal lobe—

there are no contractions on the ipsilateral side. Another point to be emphasized is the evidence of sensitization of neurons which engage in discharge of sympathetic impulses. Especially is this noteworthy when both frontal lobes have been removed. Whereas injections of 0.2 to 0.3 mgm. evoke in normal animals only a slight initial dilation of the pupils as a manifestation of sympathetic activity, the same doses after frontal lobectomy cause maximal and persistent pupillary dilation and general erection of the hairs.

A consideration which must be kept in mind when there is sensitization of superior neurons by denervating them is that subordinate neurons may likewise be sensitized, though to a minor degree. A stimulatory chemical agent, therefore, may be especially effective on the immediately denervated nerve cells and may also have a stimulatory action at a number of points in the subordinate series of nerve cells.

*Effects of Adrenaline after Removal of One Frontal Lobe and after Semidecerebration.* Recently Stavraky (1946) has reported that injections of adrenaline into animals previously semidecerebrated or unilaterally frontal-lobectomized lead to asymmetrical responses similar to those elicited by acetylcholine. Doses of adrenaline of 0.3 mgm. per kgm. or less, administered intravenously to the unanesthetized animals, caused a predominant dilation of the pupil and some extensor rigidity in the extremity contralateral to the operation. When injected with or before acetylcholine, moderate doses of adrenaline counteracted the initial generalized convulsions and accentuated the secondary motor manifestations evoked by acetylcholine. In large doses adrenaline counteracted both these effects of acetylcholine.

*Effects of Mecholyl in Human Subjects with Brain Lesions.* In 1944 Fisher and Stavraky reported observations on male patients with lesions of the rostral portions of the cerebral hemispheres or with signs of involvement of upper motor neurons, when these patients were injected with acetyl-beta-methylcholine chloride (mecholyl). The various lesions were: two brain tumors, one frontal lobectomy for a tumor,

seven head injuries with damage to the frontal lobe extending in some cases to the motor cortex, one case of unilateral lesion of the pyramidal system after cerebrospinal meningitis, and one case of spinal cord injury. The tested subjects were males of excellent physique who were associated with the armed forces of Canada. The mecholyl (8 to 10 mgm.) was dissolved in 0.3 cc. of distilled water and injected into the deltoid muscle; the doses were repeated in each subject on at least two or three occasions, always with similar results.

In control tests on normal males it was found that the sensitivity of individuals varied considerably. As a rule, within twenty or thirty seconds after an injection the subject experienced a sensation of heat in the face; this was followed by a flushing of the face and neck which rapidly spread to the chest and back and then to the extremities. As it spread toward the lower part of the body, it decreased in intensity. The flush reached its maximum about 2 minutes after the injection and was accompanied by wide-spread sweating and often by secretion from the nose, salivation, and lachrymation. Later in the response shivering usually occurred and occasionally a slight increase of tendon jerks. In two very sensitive subjects the first signs of flushing were accompanied by a slight transient dilation of the pupils. Most of the features of the reaction disappeared within five or ten minutes after the injection.

In eleven patients with lesions of the frontal lobe, with or without involvement of the motor cortex, the injection of mecholyl produced an asymmetrical response. In nine of them the flush spread more rapidly on the side of the lesion and stopped at the wrist or in the hand, the hand or the fingers then becoming very pale. In the lower extremity the flush extended to the ankle or to the dorsum of the foot. Contralaterally the spreading of the flush was delayed and it stopped high in the limbs. Frequently the arms beyond the elbow and the entire leg became pale; this pallor developed more slowly than the flush on the ipsilateral side. It became pronounced two minutes after the injection and

reached its acme about a minute and a half later, when it gradually replaced the initial redness. In three patients the pallor spread over almost the whole side of the body opposite to the lesion; on that side only the face and the chest above the nipple line still retained the flushed state. Within four minutes after the injection the contralateral blanching was often followed by a delayed flush which occurred after the general redness had largely subsided (see Figure 42).

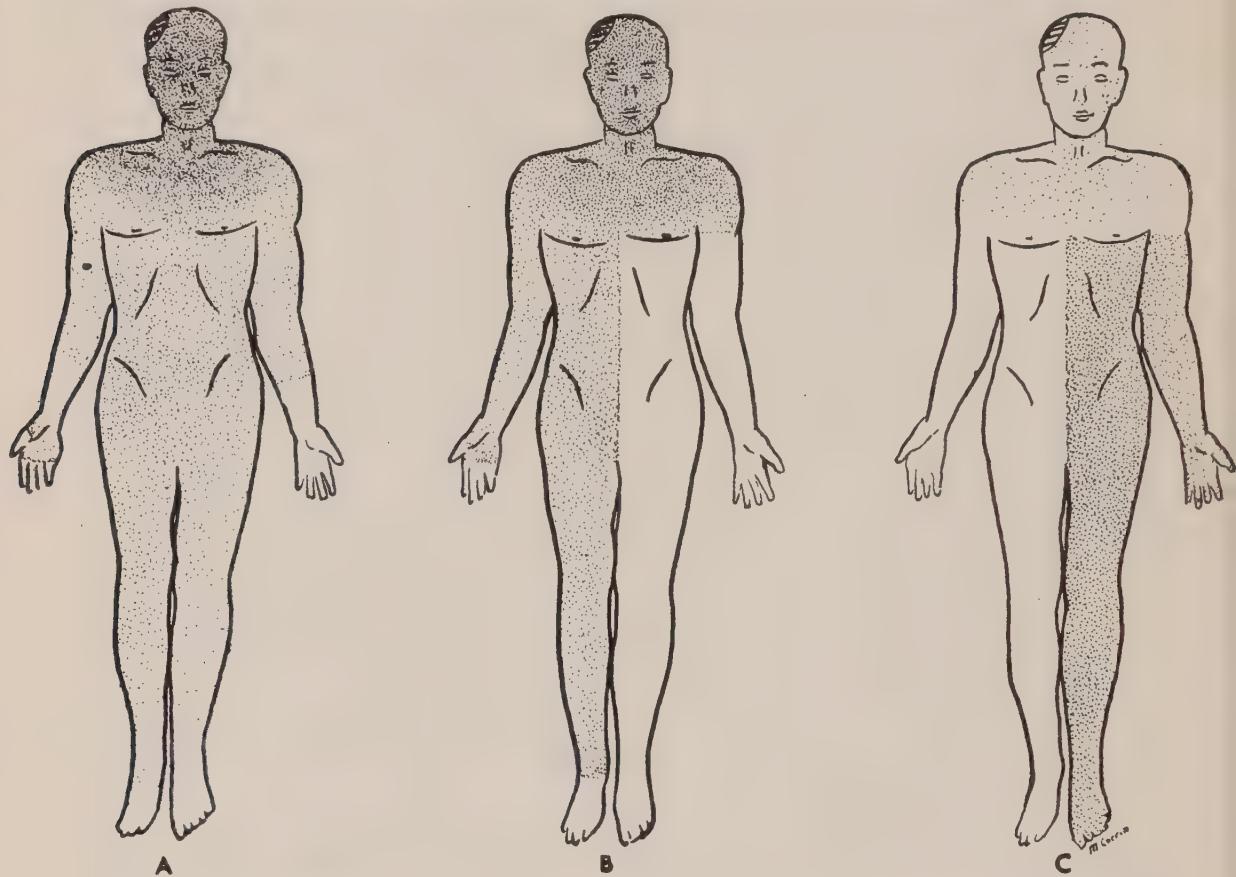


FIG. 42. Distribution of a flush on intramuscular injection of mecholyl (8 mgm.) in a patient with a left-sided hemiplegia due to destruction of the motor area of the right cerebral hemisphere (for description of the case see text). A, distribution of the flush 1 to 2 min. after the injection. B, distribution of the flush 3.5 min. after the injection; the pallor of the left side of the body is to be contrasted with the flush on the right side. C, disappearance of the initial flush about 4 min. after the injection, and appearance of brief secondary flush over the previously pale regions on the left side. (Fisher and Stavraky, 1944.)

Supersensitivity of the nervous control of skeletal muscles on the side opposite the cerebral lesion was seen in these human cases as in Stavraky's experimental animals. When the lesions extended to the motor or premotor cortex,

mecholyl evoked, on the side of the body opposite to the cortical damage, tremors, slight muscular movements, an increase in spasticity, pronounced accentuation of reflexes, and clonus of the wrist, ankle, and patella. These phenomena appeared in the later stages of the reaction.

The evidences of sympathetic sensitization in the human cases likewise were analogous to those of the experimental animals. During the reaction to mecholyl the contralateral extremities were markedly colder than the ipsilateral—a natural consequence of the vasoconstriction. Also sweating was less pronounced on the side of the body opposite the lesion than on the control side. In seven patients a transient dilation was noted in the contralateral pupil; in four both pupils dilated slightly but the reaction was more marked on the contralateral side.

An illustrative case was that of a soldier who on April 1, 1942, received a gunshot wound on the right side of the head. He suffered a complete left hemiplegia, involving the lower face, the arm, and the leg. On November 5, 1943, an intramuscular injection of 8 mgm. of mecholyl was followed in 20 to 25 seconds by a sensation of heat on the right side of the face and by a slight but definite dilation of the left pupil. Immediately thereafter a flush appeared on the face, chest, and extremities and soon spread to the hand and the foot on the right side. On the left side it extended to a region halfway between the wrist and the elbow and to about five inches above the ankle; the left hand and the left foot became very pale and a sharp line of demarcation developed between the pallor and the flush. The pallor gradually ascended; three and a half minutes after the injection it had risen to seven inches above the elbow on the left arm and involved the whole left leg and left lower portion of the body (see Figure 42). The pale parts remained cold and dry while the flushed portions were warm and covered with beads of sweat. In half a minute the initial redness largely subsided as the pale parts suddenly reddened. The delayed flush on the paralyzed side lasted less than one minute. At this time

the spasticity of the paralyzed limb was much greater than before the injection and there were tremors and slight movements in the left arm. On another occasion, when 16 mgm. of mecholyl were injected, the tremors were much more pronounced and were accompanied by athetoid-like movements in the paralyzed limbs and by a slight lifting of the stiffened semi-flexed arm.

Normally mecholyl has a predominant parasympathomimetic action, i.e., its effects are largely on peripheral effectors. It appears from the observations above recorded that damage to the motor and the premotor cortex, as well as to the cortical representation of the sympathetic system, results in a sensitization of the next subordinate links in the chains of descending nervous connections. Consequently the sensitized neurons are stimulated by mecholyl to a degree which results in an overwhelming of the usual peripheral effects by the discharge of nerve impulses. Thus the phenomena observed in the human cases could be accounted for. The initial flush would result from the vasodilation due to the peripheral effect of the mecholyl. Thereupon vasoconstriction overwhelms the dilator effect because mecholyl stimulates the sensitized sympathetic center—contralateral to the lesion because of the crossed control. Finally, when the dominant vasoconstriction, imposed from the center, subsides, there is a secondary flush in the regions which have been pallid—an effect possibly due to a persistent peripheral influence of mecholyl causing relaxation of the vessels.

The movements which occur in the paralyzed limb are explicable, as in the experiments of Stavraky, in terms of sensitization of coördinating motor centers. Both the experiments on the lower animals and the tests on human subjects can be adequately explained on the assumption that removal of superior neurons, ordinarily governing centers which are located in basal ganglia or are more peripherally situated in the central nervous system, sensitizes these centers to stimulatory chemical agents.

## PART III. SUPERSENSITIVITY TO NERVE IMPULSES

### CHAPTER XIV

#### SMOOTH MUSCLE. SUBMAXILLARY GLAND. ADRENAL MEDULLA

*Smooth Muscle.* In Chapter III it was shown that decentralization of the pupil by degenerative section of the preganglionic fibers in the cervical sympathetic renders the nictitating membrane and iris retractor more sensitive than normally to the stimulating action of injected or secreted adrenaline. The degeneration of the preganglionic axons and terminals does not cause a corresponding degeneration of the ganglionic neurons and postganglionic axons—i.e., these elements persist indefinitely, conducting impulses when appropriately stimulated. It has been proved in relatively recent years that most postganglionic fibers exert their control of the effectors to which they distribute by the liberation from their nerve endings of acetylcholine or adrenaline or an adrenaline-like substance (see for references Cannon and Rosenblueth, 1937). It is to be expected, consequently, that the smooth muscle of the nictitating membrane and iris, as well as that in other regions of the organism, should become more sensitive than normally to the effects of postganglionic nerve impulses after degeneration of the preganglionic fibers. This expectation is substantiated by the data.

Already in 1905 Elliott reported an experiment on a cat in which the cervical preganglionic fibers had degenerated on one side as a consequence of the removal of the stellate ganglion. Fifteen days after this operation, under ether anesthesia, some of the sympathetic nerves were stimulated in the orbit. Since only a fraction of the nerve supply to the iris was stimulated on each side, local pupillo-dilation resulted. The response on the chronically decentralized side

showed a more persistent contraction, outlasting the period of stimulation for two minutes or more, than that of the control side, which subsided within fifteen seconds after the cessation of the stimuli.

In the experiment cited, Elliott used repetitive stimulation with tetanic frequency, from an induction coil. The responses were thus maximal, and no difference in the amplitude of the effects on both sides was seen. Although the experiment has not been performed, to our knowledge, it is probable that if single shocks were applied in similar circumstances the amplitude of the response would be importantly greater on the decentralized than on the control iris.

In 1936 Rosenblueth and Cannon studied the responses of the chronically decentralized nictitating membrane of the cat when the postganglionic fibers of the superior cervical ganglion were stimulated in the neck. In the experiment illustrated in Figure 43 the left cervical sympathetic had been severed aseptically nine days previously. Electrodes were placed on the postganglionic filaments on both sides, after crushing centrally, toward the ganglia.

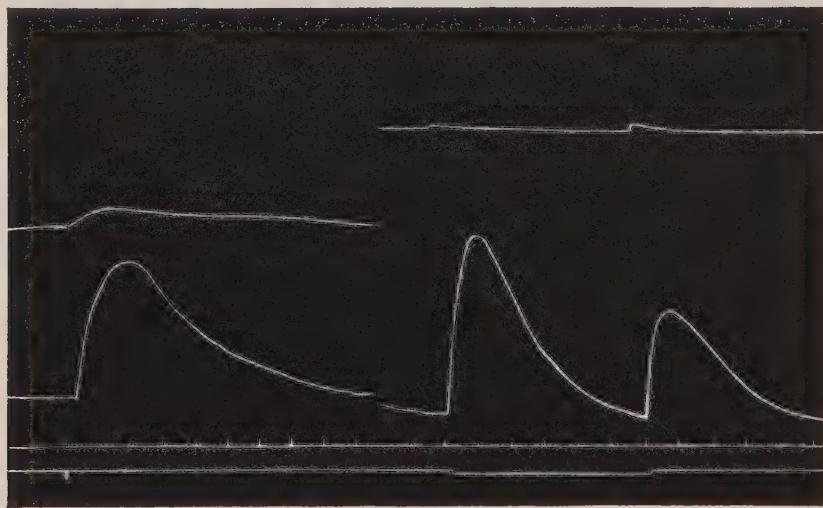


FIG. 43. Sensitization of the decentralized nictitating membrane to the action of sympathetic nerve impulses. Cat under dial. Right cervical sympathetic cut nine days before; left sympathetic cut freshly. Lower tracing, right decentralized nictitating membrane; upper tracing, left control membrane. Responses first to adrenaline ( $2\gamma$ , intravenously), and then to a make and a break induction shock applied to the postganglionic nerves on both sides. Time signal: 30-sec. intervals. (Rosenblueth and Cannon, 1936.)

The records show the contractions of the two nictitating membranes in responses, first to an intravenous injection of adrenaline (2 γ) and then to a make and later a break induction shock applied to both postganglionic nerves simultaneously. Not only are the responses of the left, decentralized membrane (lower tracing) more prolonged than those of the control muscle (upper tracing), but they are also much more ample. It is noteworthy that the difference of responses on the two sides in Figure 43 is approximately the same when adrenaline and when postganglionic nerve impulses were delivered to the muscles.

Although the retractor of the iris and that of the nictitating membrane are the only two smooth muscles which have been studied from the present standpoint, and although these two muscles receive an adrenergic nerve supply—i.e., although no systems with a cholinergic nerve distribution have been tested—the logic of the argument presented on p. 133 justifies the general conclusion that decentralized smooth muscle becomes supersensitive to the excitatory or inhibitory action of the nerve impulses delivered to it by its postganglionic nerves.

*Submaxillary Gland.* In 1923 Maeovsky showed that several days after section of the chorda tympani the parasympathetically decentralized submaxillary gland becomes supersensitive to the secretory effects of sympathetic nerve impulses. When he compared the two glands, one chronically and the other acutely disconnected from the central nervous system, he found that weaker electrical stimuli applied to the cervical sympathetic on the previously operated side elicited an outflow of saliva equal to that obtained by stronger stimuli on the control side. He also found that the responses were much longer lasting on the chronically than on the acutely operated gland. Maeovsky's results were confirmed by Fleming and MacIntosh in 1935. This supersensitivity to the action of sympathetic nerve impulses is in accord with the increased sensitivity of the submaxillary to the action of adrenaline after section of the chorda tym-

pani, mentioned on p. 80, and with the knowledge that the postganglionic sympathetic fibers involved are adrenergic—i.e., that they act by liberating an adrenaline-like mediator.

*Adrenal Medulla.* As will be shown later, partial denervation of the superior cervical ganglion renders it supersensitive to nerve impulses which come to it through remaining preganglionic fibers. The chemical mediator between preganglionic axons and the cell bodies of the outlying sympathetic neurons is acetylcholine. There is a similar situation in the adrenal medulla. Embryologically, the cells of this gland are derived from the same type of neuroblasts that elsewhere develop into the ultimate sympathetic neurons. Furthermore, the medullary cells are innervated by the equivalent of preganglionic fibers and the chemical mediator is acetylcholine. The question arose as to whether partial denervation of an adrenal gland, like partial denervation of a superior cervical ganglion, would have a similar sensitizing effect. Experiments to answer that question were performed by Simeone (1938 a).

In a number of cats under ether anesthesia the right or left major splanchnic nerve was severed aseptically in the chest. In others the minor splanchnic nerve and the upper abdominal sympathetic chain were excised on one side or the other. A week later the right or left superior cervical ganglion was removed in order to sensitize the nictitating membrane, the response of which to adrenal secretion is a reliable indication of the concentration of adrenaline in the circulating blood (Rosenblueth, 1932 b).

The test experiments were performed between three and six weeks after the partial denervation. Under dial anesthesia the nerve fibers on the still normal side were severed acutely in a manner corresponding to the previous section of the opposite side. Thereafter the adrenal gland that had been in part chronically denervated was stimulated electrically through the still intact nerve, which was crushed centrally in order to avoid any possible reflex secretion. Maximal stimuli were always used; they were either rapidly repeated

shocks from an induction coil, for varying durations, or groups of single maximal condenser discharges at the rate of 4 per second. The resultant contraction of the sensitized nictitating membrane was recorded on a kymograph. By injecting adrenaline and duplicating the responses of the membrane to adrenal secretion it was possible to quantify the output from the adrenal gland on either side, one with fresh and the other with chronic partial denervation.

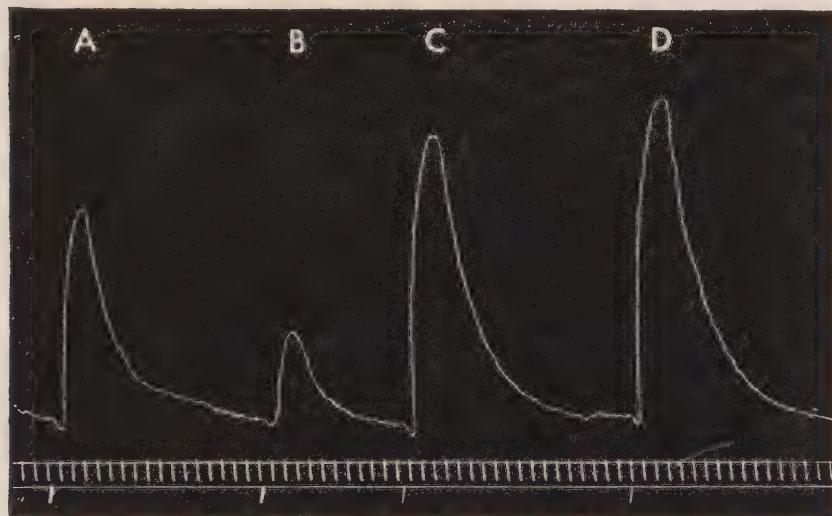


FIG. 44. Sensitization of the partially denervated adrenal medulla to the influence of impulses over the remaining nerves. Cat under dial. Left abdominal sympathetic chain and minor splanchnic resected 4 weeks previously; same resection on right, acutely. Responses of a nictitating membrane, sensitized by the extirpation 3 weeks before of the corresponding superior cervical ganglion, to adrenaline secreted by the adrenal medullae. Time signal: 30-sec. intervals.

A. Response to stimulation of the left major splanchnic (20 maximal condenser discharges in 5 sec.)

B. Response to the similar stimulation of the right major splanchnic.

C and D. Faradization of first the right and then the left major splanchnics for 1 sec.

Weight of right adrenal gland: 0.21 gm.; weight of left gland: 0.19 gm. (Simeone, 1938a.)

Adrenaline was not secreted in detectable amounts when fewer than 10 maximal condenser discharges in 4 seconds were applied to the nerves. With 20 to 40 stimuli the previously denervated gland liberated as a rule more adrenaline than did the control (see Figures 44 and 45). Using that degree of stimulation, Simeone found that in seven out of nine cats equivalent stimuli on the two sides liberated from

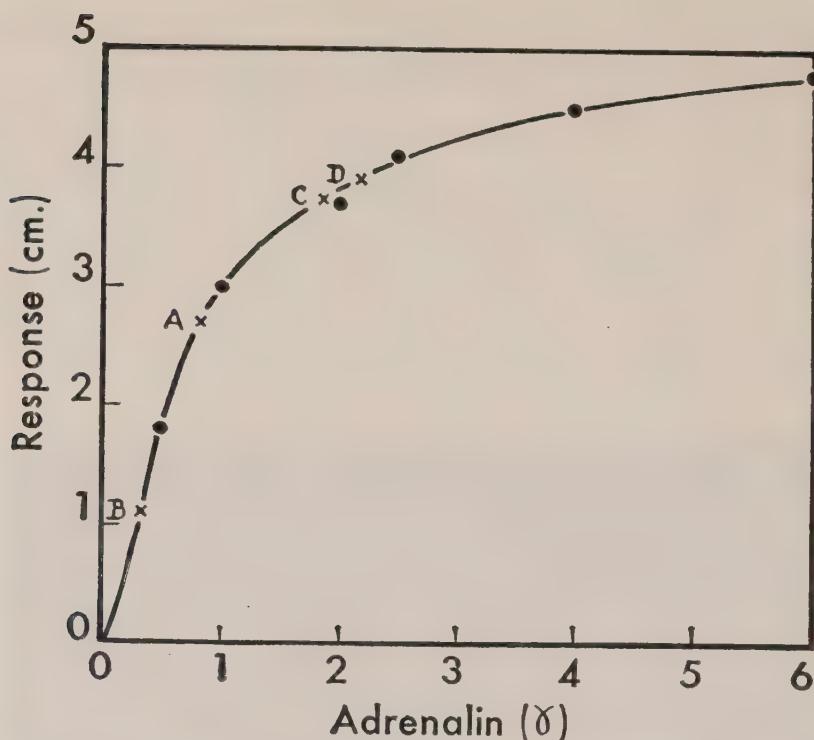


FIG. 45. Dots: responses to graded doses of adrenaline of the nictitating membrane used in Fig. 44. The crosses, A, B, C, and D, represent the points on the curve at which the corresponding responses in Fig. 44 fall. (Simeone, 1938a.)

2 to 8 times (average 3.7 times) more adrenaline from the chronically partially denervated adrenal than from the control gland. In one of the two cases in which the results were not consistent, the adrenal on the chronically denervated side, instead of being approximately the same weight as that of its fellow (which is the normal relation), weighed only two-thirds as much. In the other case there was a possibility that nerves had regenerated, for the test was made about a month and a half after the operation.

As shown in Figures 44 and 45, when the stimuli were rapidly repeated for a relatively long period of time (tetanizing frequency for 1 second) the difference between the two glands was much less striking than when less stimuli were delivered to the nerves.

The results obtained by Simeone cannot be attributed to anatomical differences between the right and left adrenals. Either side was used as a control in the different experiments and, except in the two cases cited, the gland chronically

denervated consistently gave greater responses than the normal. In an extreme instance the partially denervated gland secreted 8 times the amount secreted by the control when the two were subjected to similar stimuli. On the assumption that the secreting cells of the medulla act like the outlying neurons of the sympathetic system, the larger response of the partially denervated gland can be explained either by activation by comparable stimuli of a larger number of cells on the denervated than on the normal side or else by a secretion from the same number of cells on the two sides but with longer or more abundant activity from the gland chronically deprived of a fraction of its nerve supply. These possibilities will be discussed further in relation to the increased sensitivity of the partially denervated ganglion when it is subjected to nerve impulses.

## CHAPTER XV

### SYMPATHETIC GANGLIA. STRIATED MUSCLE

*Sympathetic Ganglia.* Experiments performed by Simeone, Cannon, and Rosenblueth (1938) have a significant bearing on the results above described and also on evidence of sensitization of neurons in the spinal cord, to be reported in the next chapter. They examined the effect of partial denervation of the superior cervical ganglion. Adult cats were used. The right or left superior cervical ganglion was partially deprived of its nerve supply by severing within the chest the *rami communicantes* which connect the first and second thoracic nerves with the stellate ganglion. It was expected that this procedure would lead to degeneration of 50 per cent or more of the preganglionic fibers which cause contraction of the nictitating membrane through action on the ganglion cells. Three to six weeks later, under dial anesthesia, the *rami* from the first and second thoracic nerves were cut on the control side, in correspondence to the earlier operation; the thoracic sympathetic chains were then crushed on each side below the connection with the fourth thoracic nerve, and shielded electrodes were placed in corresponding positions on the chains so as to include and stimulate the fibers from the third and fourth thoracic nerves. To avoid spread of current, dry cotton was packed around the electrodes and if necessary, because of excess moisture, it was changed during the experiment. The effects of stimulation were registered mechanically as contractions of the nictitating membranes, equally magnified, and also electrically by recording the action potentials of the post-ganglionic fibers.

As shown in Figure 46A, the response of the nictitating membrane on the side partially denervated three weeks

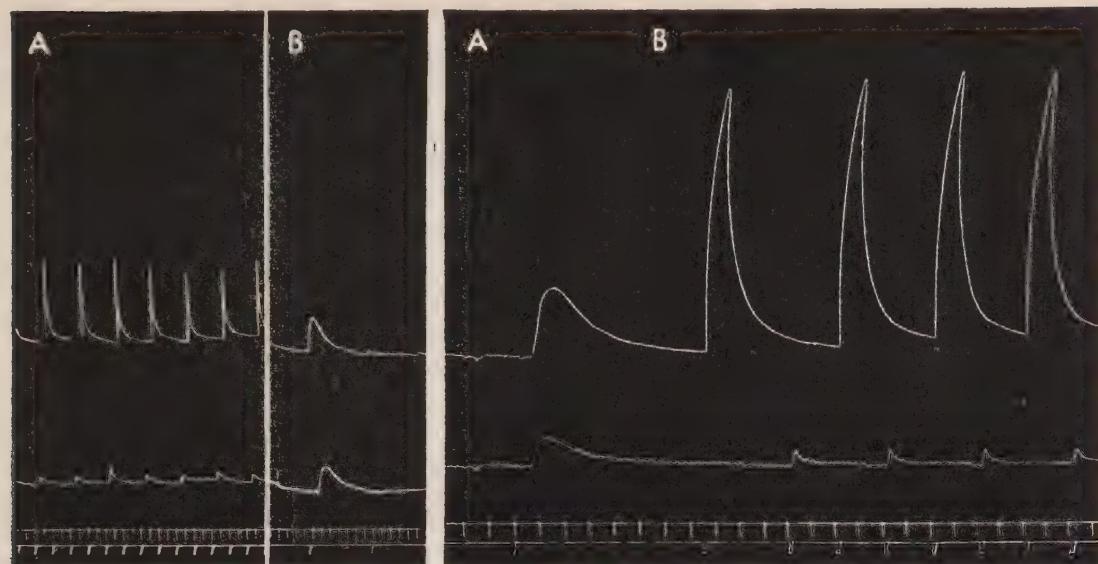


FIG. 46 (left). Sensitization of the partially denervated superior cervical ganglion to the action of impulses over the remaining preganglionic fibers. Cat under dial. Right superior cervical ganglion partially denervated 3 weeks previously by section of the rami  $T_1$  and  $T_2$ . Fibers from  $T_1$  and  $T_2$  cut acutely on left. Both thoracic chains crushed below  $T_4$ . Stimulating electrodes on both sides so as to include fibers from  $T_3$  and  $T_4$ . Upper tracing, right, and lower tracing, left nictitating membrane. Time signal: 30-sec. intervals.

A. Responses to groups of 4 maximal condenser discharges applied on both sides at a rate of 1 per sec.

B. Responses to adrenaline (10 $\gamma$ , intravenously). (Simeone, Cannon, and Rosenblueth, 1938.)

FIG. 47 (right). Preparation and records as in Fig. 46, but in another animal.

A. Responses to adrenaline (2 $\gamma$ ).

B. Extreme difference in the responses to groups with constant rate and number of maximal condenser discharges applied alternately to the two sides at the signals. (Simeone, Cannon, and Rosenblueth, 1938.)

previously is much greater than on the control side. The difference can in part be attributed to the slight degree of increased sensitivity of the membrane to liberated adrenaline. This difference, however, could only be minimal, as demonstrated by the comparative responses to injected adrenaline (10  $\gamma$ , Figure 46B). Furthermore, stimulation of the postganglionic fibers after the ganglia were crushed yielded practically equal responses on the two sides. It may be concluded, therefore, that the greater response on the side where the ganglion had been previously partially denervated was due to consequent sensitization of the ganglion cells.

Similar results were obtained in seven animals. Usually

the response was at least double that on the normal side (Figure 46A). The most extreme instance is illustrated in Figure 47. It should be emphasized that since maximal shocks were used in all experiments, any spread of the electrical stimuli would favor the normal side because some of the freshly cut fibers from the first and second thoracic nerves might thereby be excited. The maximal responses of the two sides when tetanizing currents were applied were usually equal, and sometimes that on the non-sensitized side was actually greater. This effect excluded the possibility that the larger contraction from single shocks on the sensitized side might be due to differences in the recording apparatus or to a greater reactivity of one membrane as compared with the other.

The greater contraction on the sensitized side might be explained as due to repetitive discharge from cells in the isolated ganglion because the cholinesterase, known to disappear from the fully denervated ganglion, becomes insufficient for rapid destruction of acetylcholine which is liberated at the nerve terminals. Or the greater contraction might be explained as a consequence of stimulation of a larger number of slightly innervated ganglion cells—cells ordinarily in the “subliminal fringe”—because they have become sensitized. Whatever the explanation may be, the important fact emerges that partial denervation of the outlying neurons renders them more highly responsive. This is an effect which, from what we know of total denervation, can reasonably be attributed to degeneration of the neurons which normally deliver to them activating impulses.

*Striated Muscle.* The sensitization of denervated striated muscles to the influence of nerve impulses is revealed by the Philipeaux-Vulpian and the allied phenomena, described in the historical introduction in Chapter I. These phenomena may not be analogized strictly with the cases of supersensitivity to nerve impulses reported in the preceding chapter and earlier in this one. In those cases the nerve impulses whose effects were increased after partial denerva-

vation or after decentralization were impulses delivered directly to the effectors. The corresponding observations in skeletal muscle would require the demonstration of an enhanced action of the normal motor nerve impulses through some unsevered motor axons after degenerative section of other motor fibers or else they would require the comparison of effects of stimulating the motor nerve to a "partially decentralized muscle" (e.g., after semisection of the cord; see p. 104), with those elicited by stimulation of the opposite non-decentralized control. Hines, Wehrmacher, and Thomson (1945) have reported that 9 to 15 days after unilateral section of some ventral roots the partially denervated gastrocnemius yields considerably greater mechanical responses upon stimulation of the remaining roots than those obtained when the stimuli are applied only 3 days after the sections. They observed these effects in both rat and cat muscles. Although Hines and her collaborators consider the possibility of a supersensitization, and dismiss it as unlikely, their data do not disprove that hypothesis. Only further study will decide the issue.

Partial denervation of striated muscles is possible only in muscle fibers with multiple innervation, i.e., in fibers receiving each more than one motor nerve ending. The existence of such muscle fibers has been much debated (see Fulton, 1926; Hinsey, 1934). In 1941 Katz and Kuffler reported convincing evidence that the frog sartorius has abundant multiple innervation, i.e., that most of the fibers of the muscle receive 2 or 3 motor nerve terminals. This muscle is consequently suitable for the performance of the observations mentioned. Since the sensitized responses to acetylcholine of "partially decentralized" muscles are mainly contractures (see p. 104), it is difficult to surmise whether the contractions in response to motor nerve stimulation would be increased in these muscles.

In the Philipeaux-Vulpian and allied phenomena the nerve impulses whose effects on the muscle are sensitized by motor denervation are not delivered directly to the

muscle fibers. Although claims to the contrary have been made, the histological evidence is quite conclusive in showing that the only axon terminals which make connections with the muscle fibers are those from the ventral root motor axons (see Hinsey's excellent review of the subject, 1934). Notwithstanding this significant difference between the two sets of phenomena it is legitimate to include the "pseudomotor" effects here, for they reveal a supersensitivity of denervated structures to the action of nerve impulses, even though this action be relatively indirect. After what was stated in Chapter I, only some additional data and comments need be presented.

In 1918 Van Rijnberk reported that the Sherrington phenomenon could not be obtained unless the dorsal roots were cut, centrally to the spinal ganglia, as well as the ventral roots. This claim was proved erroneous by Hinsey and Gasser (1928); in this, as in the other related phenomena, the fibers whose degeneration is important for the development of the supersensitivity are the ventral root motor fibers exclusively.

Sherrington (1894) observed that stimulation of the sympathetic was not necessary for the production of the pseudomotor responses and inferred that the important nerve fibers are the dorsal-root vasodilators. Van Rijnberk (1918) and Hinsey and Gasser (1930) came to the same conclusion. In 1933, however, Hinsey and Cutting reported that stimulation of *postganglionic* sympathetic fibers, not of somatic afferent fibers, is the necessary condition for the appearance of the Sherrington phenomenon; stimulation of preganglionic sympathetic nerves they found ineffective. Their observations agree with the inference that acetylcholine is the chemical mediator involved in the phenomenon, since, as already mentioned, there exist cholinergic sympathetic vasodilator nerves, while the nature of the transmission of the antidromic dilator impulses is still controversial (see for references Rosenblueth, 1949).

The responses in the Philipeaux-Vulpian and allied phenomena were assumed for many years to be contractures.

Indeed, the term "pseudomotor contractures" was often employed to designate them collectively. In 1937 Rosénblueth and Luco showed, however, that these responses may consist exclusively of contractions or else may include contractions followed by contractures. In Figure 48 are pre-

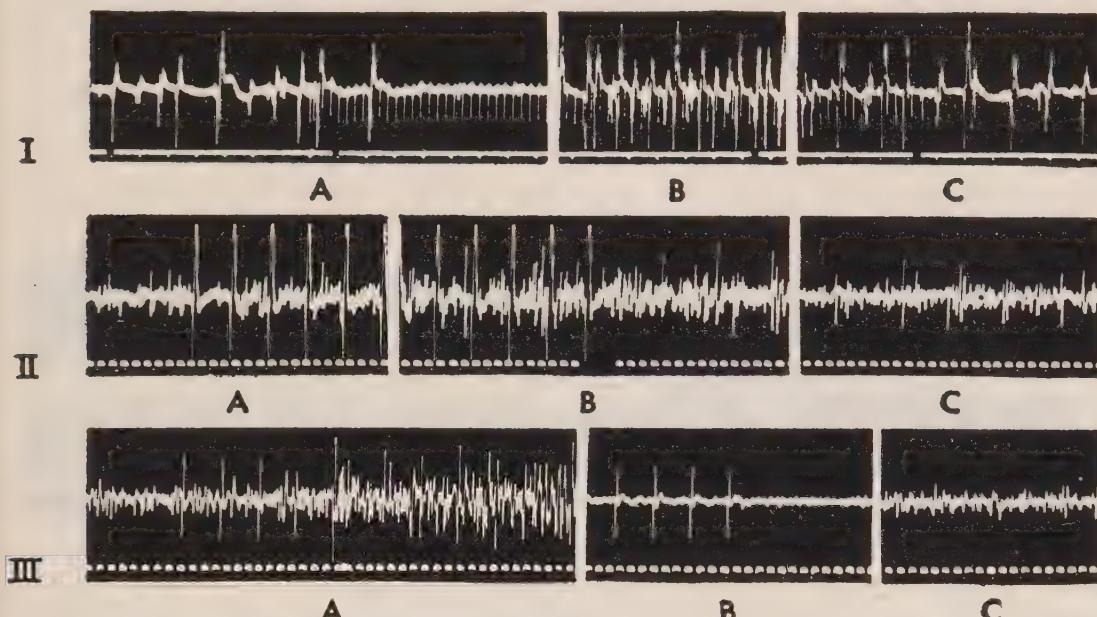


FIG. 48. Contraction and contracture responses in the Philipeaux-Vulpian and allied phenomena. Cats under dial. Electrical records from denervated striated muscles. Cathode-ray oscillograph; capacity-coupled amplifier.

I. The Rogowicz phenomenon. Posterior auricular muscle denervated by section of the facial nerve 13 days previously. Stimulation of the cervical sympathetic at the rate of 30 per sec. for 10 sec. Record with concentric electrodes. Time signal: 1-sec. intervals. The fine descending lines are the stimulus artifacts. A, beginning of stimulation; B, 3 sec. later; C, end of stimulation. The spontaneous activity of the muscle was first abolished by the stimuli. The numerous spikes in B indicate the development of a contraction response.

II. The Philipeaux-Vulpian phenomenon (without eserine). Right hypoglossal cut 6 days previously. Atropine (1 mgm. per kgm.); left hypoglossal cut acutely. Concentric electrodes in right side of tongue. Right lingual nerve stimulated 30 sec. at the rate of 30 per sec. as shown by the large stimulus artifacts in A and B. Time signal: 10-msec. intervals. A, beginning of stimulation; B, end of stimulation; C, 10 sec. later. The abundant spikes in B prove the development of a contraction response.

III. The Philipeaux-Vulpian phenomenon (after eserine). Right hypoglossal cut 7 days previously. Atropine (1 mgm. per kgm.); adrenals ligated; left hypoglossal and both facial and lingual nerves cut; eserine (3 mgm. per kgm.). Stimulation of the right lingual nerve for 10 sec. at the rate of 30 per sec., as shown by the diphasic stimulus artifacts (see B). Time signal: 10-msec. intervals. A, beginning of stimulation; B, end of stimulation; C, 1 min. later. The increase of spike-potential activity at the end of A demonstrates contraction; the silence in the electrogram in B shows that this contraction was followed by a contracture. This last response did not occur in II without eserine. (Rosénblueth and Luco, 1937.)

sented characteristic electrograms from a facial muscle (I) and from the tongue muscles of the cat (II and III), previously denervated, in typical responses to stimulation of vasodilator nerves. The marked increase of electrical activity in the records IB, IIB, and IIIA testify to the development of contractions; the silence of the electrogram in record IIIB indicates the appearance of a contracture of the denervated tongue muscles when the lingual nerve was stimulated tetanically after eserine (3 mgm. per kgm.) had been administered to the cat. As shown in Figure 49, even

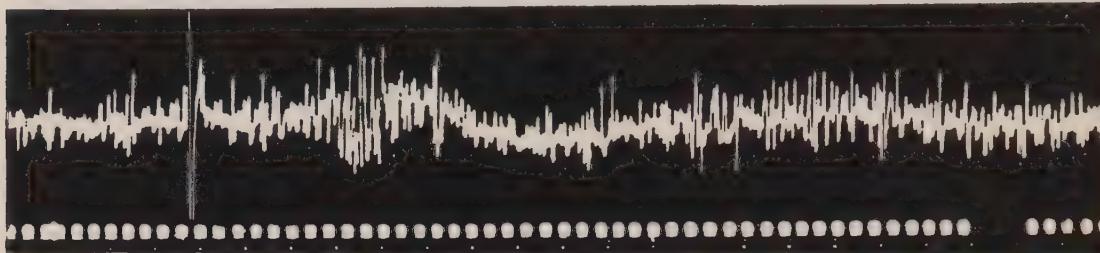


FIG. 49. The Philipeaux-Vulpian phenomenon in response to a single nerve volley (after eserine). Cat under dial; right hypoglossal cut 7 days previously; atropine (1 mgm. per kgm.); eserine (1 mgm. per kgm.); left hypoglossal cut acutely. Single maximal shocks applied to the right lingual nerve as shown by the large first diphasic excursion in the record. Time signal: 10-msec. intervals. Concentric electrodes in the right side of the tongue. After a latency of about 60 msec. a burst of spike potentials is succeeded by a period of decreased activity, showing fewer waves than the original spontaneous background; this quiet period is followed shortly by renewed enhanced deflections which subside slowly (5 to 15 sec.). (Rosenblueth and Luco, 1937.)

single-shock stimulation of the vasodilator nerve fibers in the lingual nerve may elicit a clear response from the denervated and eserinized tongue.

The anatomical, pharmacological, and physiological evidence available clearly indicates that the Philipeaux-Vulpian and allied phenomena are usually contraction and sometimes contracture responses of striated muscles rendered supersensitive by denervation to the stimulating action of acetylcholine liberated at some distance from them by cholinergic nerve fibers distributed to the blood vessels within the muscle. These responses differ from those of normal muscles to motor nerve impulses only in that the

activating acetylcholine has to diffuse to the endplates from a relatively distant region in the first instance, while it is delivered directly at the endplates by the motor nerves in the second. The term "pseudomotor contractures" is thus seen to be a misnomer—the responses are motor and are in general not contractures.

## CHAPTER XVI

### SPINAL NEURONS

The evidence, presented in Chapter XII, that partial denervation of spinal neurons by semisection of the cord renders them supersensitive to the stimulating influence of several chemical agents, together with the evidence that partial denervation of sympathetic ganglia causes an increased sensitivity of the ganglion cells to the action of preganglionic nerve impulses, detailed in the preceding chapter, naturally raised the question whether the partially denervated spinal neurons become supersensitive also to the influence of impinging nerve impulses. Bremer (1928; 1932) had shown that the partial denervation of spinal elements consequent to the intradural section of dorsal roots renders these elements more responsive than normally to the excitatory action of nerve impulses delivered by higher centers. Thus, he found that a deafferented hind limb of a cat exhibits a surprising hyperreactivity when vestibular discharges are initiated by appropriate movement of the animal with respect to its surroundings. Only relatively incidental observations were available, however, on the responses of partially denervated spinal neurons to afferent nerve impulses (see Mott, 1892; Moldaver, 1935). A study of this problem was undertaken by Cannon, Rosenblueth, and García Ramos in 1945.

In adult cats a spinal semisection at a variable level (from  $C_2$  to  $T_8$ ) was made with aseptic precautions on either side. A careful exploration of the placing and hopping reactions, of the walking ability of the animals, of the hind limb reflexes, and of the presence or absence of a respiratory hemiplegia in the cats in which the semisection had been made above  $C_4$ , revealed whether the semisection was com-

plete. These tests were supplemented by a gross anatomical examination of the operated region of the cord, made in the course or at the end of the acute experiments. The only results considered significant were those obtained from cats in which those tests indicated that the semisection had been complete and that the cut had not extended much beyond the midline. For the acute experiments, under ether anesthesia, the carotid arteries were ligated, the cranium was amply trephined, and the animals were decerebrated. The anesthesia was then discontinued and a complete transection of the cord was made, above or below the previous semi-section, or by completing the early cut so as to include the other half of the cord. Another neurological exploration of the animals was then carried out. In a second group of normal cats, used as controls, the preparation was similar to that employed for the previously semisected animals.

The tendons of the quadriceps and semitendinosus muscles were freed on both sides and were later tied by means of thick relatively inextensible threads to recording myographs. The muscles pulled against heavy rubber bands; the graphs were thus mainly tension records, upward excursions denoting contraction. Shielded stimulating silver-wire electrodes were applied to both peroneo-popliteal nerves, after peripheral crushing.

The following spinal reflexes were studied: extensor and flexor phasic myotatic reflexes, elicited by dropping a constant weight from a constant height on the recording levers; ipsilateral and crossed, flexor and extensor reflexes; inhibition of the knee-jerks by single ipsilateral afferent volleys.

The comparison of the spinal reflexes on both sides of cats with a previous spinal semisection revealed significant differences. As shown in Figure 50 the knee-jerks were more ample on the operated than on the opposite side. This difference was regularly seen also in the preliminary neurological exploration of the animals, after complete transection of the cord; the jerks on the previously semisected side were ampler and showed a greater tendency to

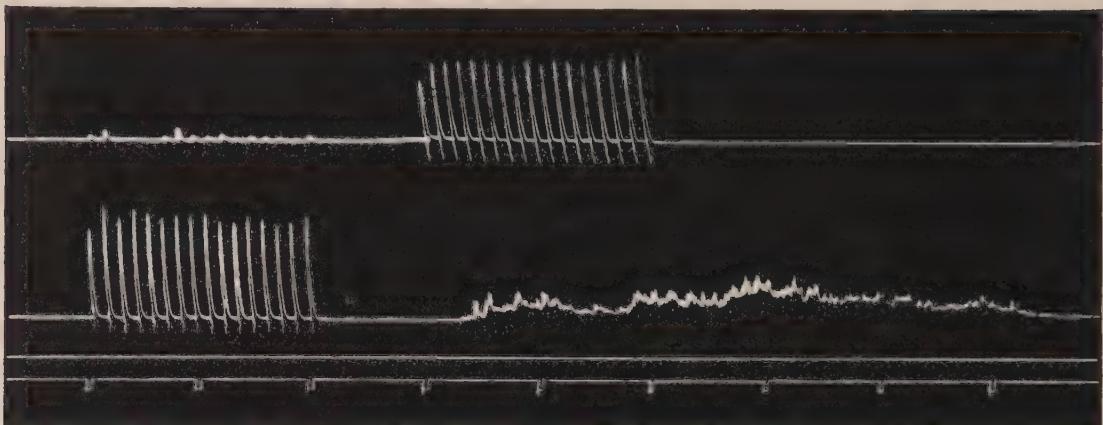


FIG. 50. Sensitization of partially denervated spinal neurons to the excitatory action of afferent nerve impulses. Decerebrate cat. Right spinal semisection at  $T_8$  made 25 days previously. Complete spinal transection at  $T_9$ . Records of the left (upper tracing) and right (lower tracing) quadriceps; same magnification on both sides. Myotatic reflexes evoked by dropping equal weights from equal constant heights on the recording levers (see the artifacts indicated by the downward excursions), first on the right and later on the left side. Time signal: 6-sec. intervals. (Cannon, Rosenblueth, and García Ramos, 1945.)

develop into clonus than in the other leg. Furthermore, percussion of the patellar tendon of the non-semisected side caused contractions of the crossed quadriceps, while the symmetrical observations resulted in minimal or absent crossed contractions (see Figure 50). Like the knee-jerks, the myotatic flexor reflexes were more striking on the previously semisected than on the opposite side (see Figure 51).

The ipsilateral flexor reflex was more ample and revealed a more prolonged after-discharge on the chronically semisected than on the uncut side (see Figure 52). A crossed flexor reflex was seen in about one-half of the semitendinosus muscles studied; it occurred more commonly on the semisected than on the opposite side (see Figure 52B).

The ipsilateral extensor reflex was regularly observed on both sides of the operated animals, not only in the responses to repetitive afferent stimulation but also in those elicited by single shocks. As illustrated in Figure 53, the responses to single shocks were as a rule more ample on the operated than on the previously uncut side. The crossed extensor reflex was also observed regularly on both sides

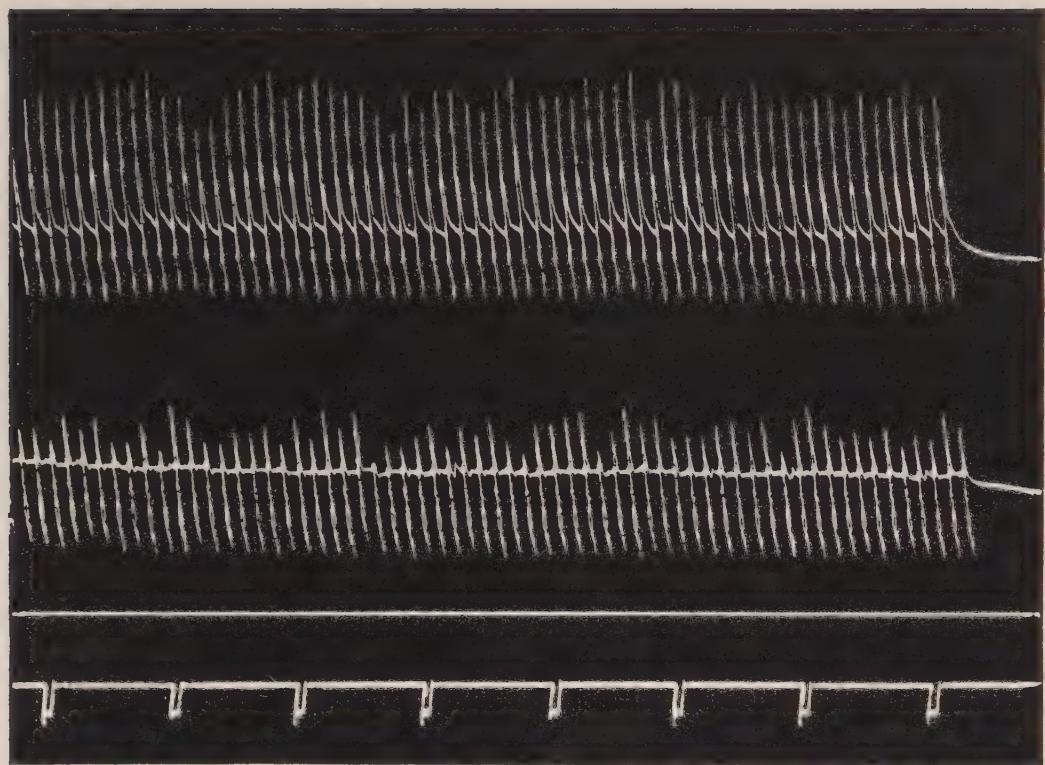


FIG. 51. Preparation as in Fig. 50, but left spinal semisection 11 days previously. Records of the left (upper tracing) and right (lower tracing) semitendinosus. Myotatic reflexes evoked as in Fig. 50. (Cannon, Rosenblueth, and García Ramos, 1945.)

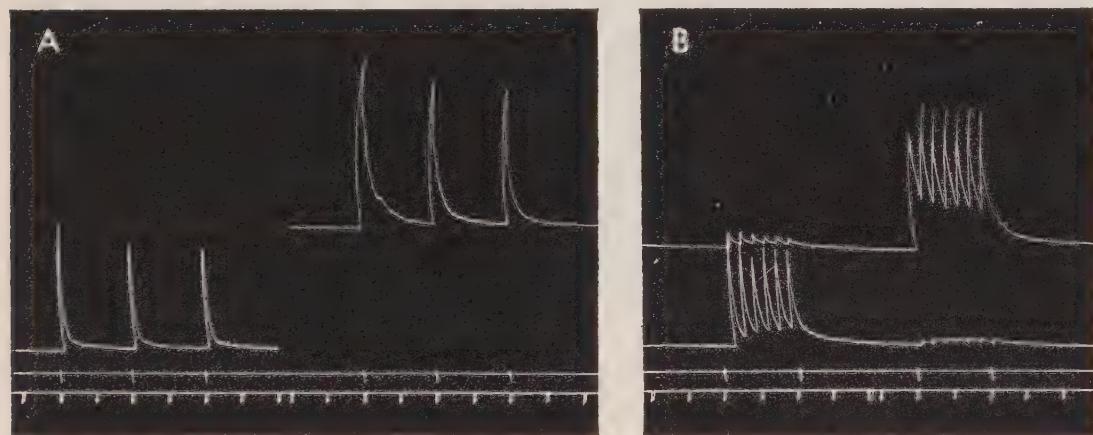


FIG. 52. Preparation and records as in Fig. 51.

A. Afferent stimulation of the peroneo-popliteal nerve by means of single shocks at the rate of 1 per 12 sec., first on the right, then on the left. The recording of the contralateral muscle was omitted.

B. Afferent stimulation for 12 sec. between each pair of signals, first of the right and then of the left peroneo-popliteal nerve, at the rate indicated by the responses. (Cannon, Rosenblueth, and García Ramos, 1945.)

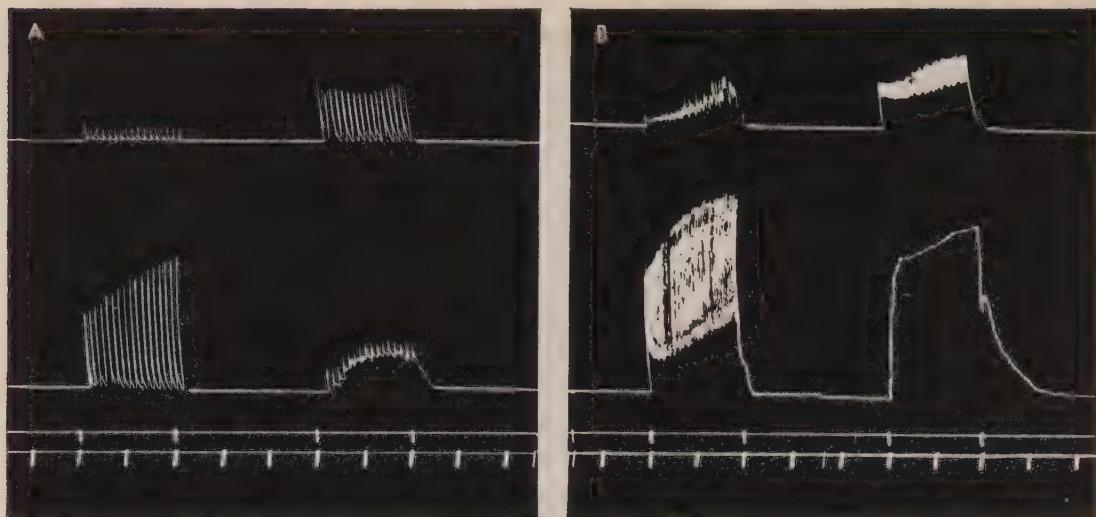


FIG. 53. Preparation and records as in Fig. 50, but right spinal semisection 10 days previously. Afferent stimulation for 12 sec. first of the right, then of the left peroneo-popliteal nerve. A, frequency 2 per sec.; B, frequency 6 per sec. (Cannon, Rosenblueth, and García Ramos, 1945.)

in response to single-shock stimulation; no consistent differences were found between the chronically and the acutely semisected sides.

As shown in Figure 54, the extensor responses to repetitive

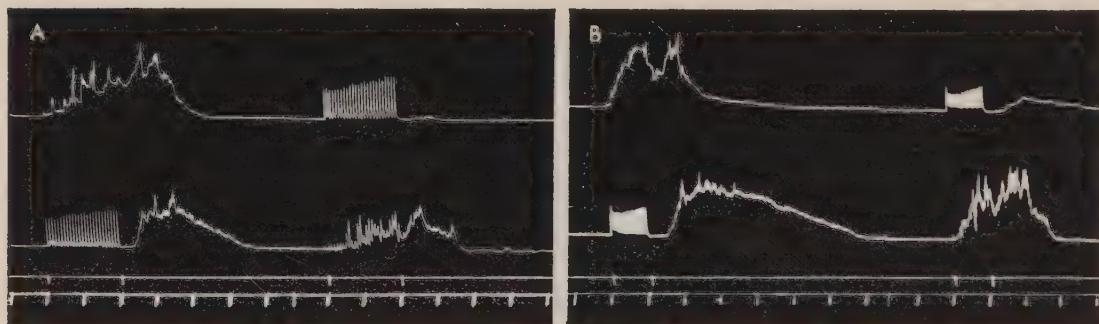


FIG. 54. As in Fig. 53, but right spinal semisection 8 days before. Afferent stimulation first of the right, then of the left peroneo-popliteal nerve. A, frequency 1.5 per sec.; B, frequency 10 per sec. (Cannon, Rosenblueth, and García Ramos, 1945.)

stimulation of the sciatic often exhibit two parts, one which develops during the period of application of the stimuli and the other which appears only later, some time after the stimuli have ceased (see Rosenblueth, García Ramos, and Cannon, 1945). The rate of stimulation necessary for summation of the first part of the reflexes was lower for both

the crossed and the ipsilateral responses of the muscle on the previously semisected side than for the symmetrical muscle. Again the minimal rate of stimulation which elicited the second part of the ipsilateral extensor reflex was lower on the operated than on the other side (see Figure 54).

In order to evaluate these data it is important to compare the reflexes which involve the muscles of the operated animals on the side not previously semisected with those which may be elicited from decerebrate cats made spinal acutely, i.e., with the normal controls mentioned earlier. Cannon, Rosenblueth, and García Ramos found that all the reflexes they studied could be elicited more readily on either side of the previously semisected animals than in the controls. Thus, the knee-jerks of the operated animals became readily clonic in character if the stimuli were repeated; as is well known, clonic knee-jerks are not common in freshly spinal cats. Moreover, the crossed extensor responses to stretch of the patellar tendons illustrated in Figure 50 were not seen in the control animals.

Again, the ipsilateral flexor reflex exhibited more prolonged after-discharge in both sides of the operated cats (see Figure 52) than in the acute spinal preparations. The crossed flexor reflex, often seen in the operated animals, is exceptional in the spinal cat. Similarly, both the ipsilateral and the crossed extensor responses to single afferent volleys are but rarely seen in the acute spinal animal, while they were regularly obtained in the semisected cats (Figures 53 and 54). The late component of these reflexes (see above) could be elicited with lower frequencies in the operated animals, on both sides, than in the controls.

In confirmation of earlier reports on the inhibitory effects of single ipsilateral afferent nerve volleys (see for references Creed *et al.*, 1938), this inhibition was found to last regularly about 1.2 seconds—i.e., the knee-jerks, at first totally inhibited by the afferent volley, recovered to about 90 per cent of their original amplitude in 1.2 seconds, on the average; the extreme variations in the control series were 0.7 and 2

seconds. In the previously semisected cats there was no consistent difference between the inhibitory effects on the two sides, nor was the average duration of the inhibition different in these from the average of the control animals. Unlike the results obtained in the latter, however, many instances of quite brief (0.3 seconds, and less) or quite long (3, 4, and even 7 seconds) duration of the inhibitory effects were often encountered in the operated animals. Figure 55 illustrates an instance of prolonged inhibition.

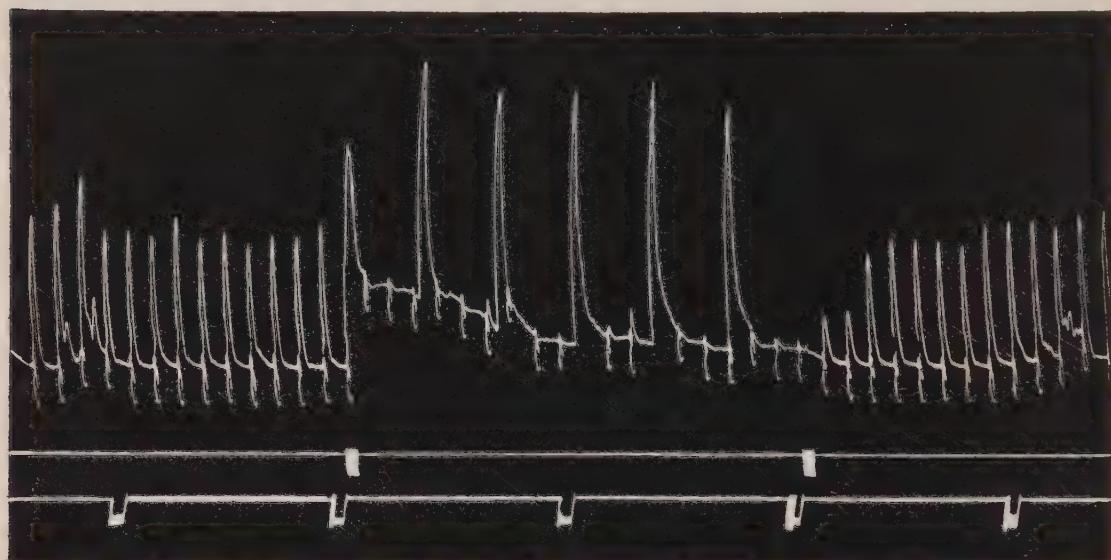


FIG. 55. Inhibition of the knee-jerk by ipsilateral single afferent volleys. Right spinal semisection 11 days before. Record from the right quadriceps. Knee-jerks evoked regularly throughout the graph as in Fig. 50. Between the two upper signal-marks 6 maximal single shocks were applied centrally to the right peroneo-popliteal, as indicated by the large ipsilateral extensor reflexes evoked by the corresponding nerve volleys. (Cannon, Rosenblueth, and García Ramos, 1945.)

An appraisal of these results requires some preliminary considerations. The semisections performed caused a relative immobilization of the muscles of the hind limbs, especially of those on the side homonymous to the cut. For several days or weeks those muscles were used less than normally. A disuse atrophy may thus have developed, which tended to decrease the responses of the muscles on the operated side. This probable source of asymmetry in the results registered may have been at least partially balanced by the probable supersensitivity of the muscles on the cut side.

to the influence of the efferent motor nerve impulses (see p. 143). In any case, the influence of disuse atrophy on the observations would oppose the inference of sensitization on the cut side.

Afferent nerve impulses may exert not only an excitatory but also an inhibitory action on spinal neurons. If partial denervation should cause a supersensitivity to both influences, then the increased sensitivity to excitation might be masked in some of the responses by the also augmented inhibitory effects. This possibly confusing factor is again opposed to the inference of the existence of supersensitivity to excitatory impulses.

Crossed reflexes involve the activation of neurons in both sides of the cord. Augmented contralateral responses might therefore be due to a supersensitivity of elements on either side. This consideration suggests that the observations on ipsilateral reflexes are more significant for the solution of the problem on hand than the observations on crossed reflexes.

Although the majority of the descending spinal tracts have an ipsilateral distribution in the cord of the cat, some fibers, e.g., those in the direct pyramidal tract, cross below the semisected level and distribute to neurons on the opposite side in lower segments. A spinal semisection causes therefore the partial denervation of nerve cells on both sides of the cord although the degree of denervation is probably greater on the side homonymous to the section.

From these considerations it is clear that negative results do not allow any conclusions to be drawn on the changes of sensitivity of the nervous elements in the spinal cord, and also that the important observations are those made on ipsilateral reflexes. As positive results with non-crossed responses in the operated animals may be cited the greater amplitude and duration, on the previously semisected side, of the myotatic reflexes (Figures 50 and 51), the ipsilateral flexor reflex (Figure 52), and the ipsilateral extensor reflex (Figures 53 and 54). These results justify the inference that

the spinal neurons on the semisected side become supersensitive to the influence of excitatory afferent nerve impulses as a consequence of partial denervation. This inference is in accord with data reported by McCouch, Hughes, and Stewart in 1943. In a study of 14 monkeys with initial semisection and subsequent transection of the spinal cord, they made the following observations. Reflex recovery after the transection was always more rapid in the previously paretic lower extremity. Three animals developed crossed reflexes on the chronic side in response to stimulation of the acute side. Crossed flexion of digits was recorded a few hours after the transections; crossed extension of the leg only two or more days later. This greater reactivity of the chronic as opposed to the acute side suggests that in the monkey, as in the cat, spinal semisection supersensitizes the partially denervated spinal neurons ipsilateral to the cut.

The differences found by Cannon, Rosenblueth, and García Ramos between the muscles on the non-semisected side of the operated animals and those of the normal controls justify the further inference that, in the cat, spinal semi-section leads to the development of supersensitivity to excitatory afferent nerve impulses not only of the spinal neurons on the side homonymous to the section, but also of neurons on the opposite side. Indeed, it is apparent that had not the latter reflexes been also more readily evoked than normally, the differences between the two sides of the operated animals would have been far more striking than they were in fact. The supersensitivity in question may be attributed in part to the degeneration of fibers which cross at the level of the segments to which they distribute, and in part also to a possible "penultimate" partial denervation of elements on the uncut side (cf. p. 104).

The much prolonged period of inhibition of the knee-jerks by ipsilateral afferent volleys seen in some animals (p. 154) and illustrated in Figure 55 indicates that the semisections sensitized not only the excitatory but also the inhibitory effects of afferent nerve impulses. That this pro-

longation of inhibition was not seen in all instances may be attributed to the concomitant supersensitivity to excitation. But the observations of marked inhibition, like that of Figure 55, are especially significant, precisely because the knee-jerks were more ample than normally.



## CHAPTER XVII

## THE SPONTANEOUS ACTIVITY OF DENERVATED STRUCTURES

The observations to be reported in this and the following chapter reveal phenomena which may bear some relationship to the sensitization of structures after denervation. In all cases, the effects are seen after severance of centrifugal nerves; those effects are distant; they are enduring; they are not directly due to the abolition or initiation of nerve impulses by the cut. For these reasons a consideration of these phenomena is included here. This inclusion, however, does not imply that a strict similarity of mechanism is assumed. The phenomenological analogy may be merely gross and misleading. Only future studies will decide whether all the diverse phenomena presented in this monograph have a common basis or whether some are fundamentally different from others.

*The Fibrillary Activity of Denervated Striated Muscle.* The random, persistent activity of denervated fibers of voluntary muscle has been already mentioned in Chapter X. First observed by Schiff in 1851, it has been repeatedly studied since as one of the most outstanding features elicited by motor denervation. According to Langley and Kato (1915) it begins in the cat on the fifth day after section of the nerve; this time for the onset has been confirmed by Denny-Brown and Pennybacker (1938). In the denervated rat's gastrocnemius, fibrillation starts sooner—on the third day after the cut (Hines and Knowlton, 1933). While a few scattered fibers are at first active, within a few days all the elements join in the fibrillary display. The activity then persists as long as there are viable muscle fibers to

contract. Thus, Langley and Kato observed fibrillation up to 71 days from the time of the degenerative cut; and Tower (1939) reports observing random muscular activity one year or more after denervation.

Fibrillation may not only be seen as contraction of some elements, it can also be recorded electrically (see Figures 29, 48, and 49). This electrical activity has been studied by Schäffer and Licht (1926), Brown (1937 *b*), Rosenblueth and Luco (1937), and Denny-Brown and Pennybacker (1938). There is now unanimous agreement that it denotes the random development of spike potentials, i.e., the fibrillary effects are contractions; there is no evidence that there also occur spontaneous contractures of denervated muscles.

The rate of fibrillation per fiber has been fixed as 2 to 7 per second by Brown. He assumed that the unit of action is the entire muscle fiber. Langley and Kato, on the other hand, observed that the rate and magnitude of the activity differed in different muscles, and in different regions of a given muscle, but that the rhythm was regular in any one region. They described the contractions, observed visually, as localized, extending only 0.5 to 1 mm., as if occurring in limited regions of the fibers, and not spreading throughout the elements. Tower states that her experience is in accord with this opinion, as opposed to that of Brown and Denny-Brown and Pennybacker, who believe that the activity propagates through each entire muscle fiber. Recently Eccles (1941 *a*) has reported that the rhythm of discharge in cat muscles is about 10 per second. He states also that electrical recording at different points along the length of small bundles of fibers shows that this rhythmic discharge is initiated at foci often widely separated from the endplate regions, and from these foci the impulses are propagated normally along the whole length of the fiber.

It is interesting to note that the fibrillation of denervated muscles results in the development of little or no tension. The "atonic" flabby condition of the affected muscles in

patients with lesions of a motor nerve is well known. Experimentally, a cessation of the fibrillary electric discharges is readily obtained with large doses of acetylcholine (Brown, 1937 *b*; Rosenblueth and Luco, 1937). This silence of the electrogram can long outlast the contraction and contracture responses elicited by the agent. It is possible thus to observe a denervated muscle at rest. The comparison of its tension with that which it exerts when fibrillation is later renewed confirms that the fibrillary discharges do not develop any significant degree of tension. This observation is opposed to the view that the impulses spread throughout each active muscle fiber; for if that were the case, a measurable tension would develop in the experimental circumstances cited. As pointed out by Tower (1939) in her comprehensive review of the topic, partial contractions, with elongation of the inactive segments of the fibers, account better for the minor degree of tension than unitary fiber activity.

The source, i.e., the stimulus, of the fibrillary activity of denervated striated muscle is still obscure. Rosenblueth and Luco considered in 1937 the possibility that acetylcholine might be the cause of the fibrillary twitches, but they dismissed this hypothesis as improbable. In 1938 Denny-Brown and Pennybacker adopted this interpretation. They adduced Bender's observations on the release of acetylcholine during emotional excitement (see p. 100) as an argument in support of it. Bender's study was made on monkeys, however, and this species is probably exceptional in its sensitivity to acetylcholine. In other animals the attempts to demonstrate a release of acetylcholine into the blood have failed when made without the use of protective eserine.

Magladery and Solandt (1942) have recently again defended the view that acetylcholine is the stimulus for fibrillation, and have also considered that potassium ions play a part in its production. Their argument is the following: denervated skeletal muscle is supersensitive to acetylcholine and, less markedly, to potassium chloride; small concentrations of these agents produce action potentials com-

parable to and superimposed on those of fibrillation; these excitatory effects are abolished by quinidine in doses which arrest fibrillation; acetylcholine, potassium, or both, may be therefore the causative agent or agents of fibrillation. This argument is fallacious. Nicotine also stimulates denervated muscles, and since quinidine blocks the excitatory action of acetylcholine it probably also prevents stimulation by nicotine; yet nicotine is not the source of fibrillation, for it does not occur normally in the mammalian organism. The considerations presented by Magladery and Solandt are necessary but not sufficient conditions for the inference under discussion.

As shown by Rosenblueth and Luco, prostigmine and eserine increase transiently the fibrillary activity of denervated muscles (see Figure 56). Indeed, this increase leads

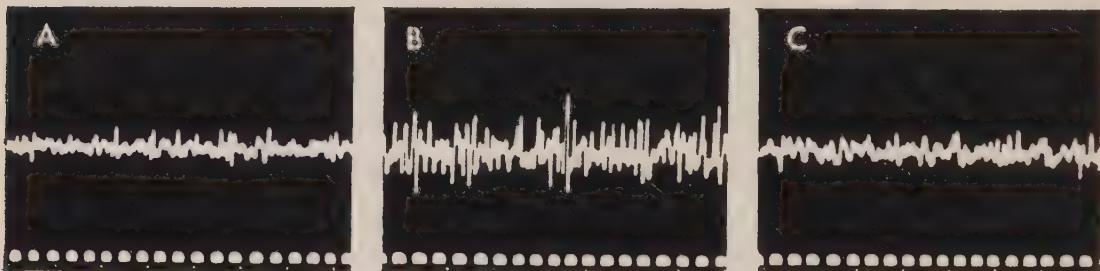


FIG. 56. Action of eserine on the spontaneous electrical activity of a denervated tongue. Cat under dial. Right hypoglossal nerve cut 9 days previously. Atropine (1 mgm. per kgm.); adrenals ligated; left hypoglossal cut acutely. Electrodes in the right side of the tongue. Capacity-coupled amplifier and cathode-ray oscillograph. Time signal: 10-msec. intervals.

A, before eserine; B, 30 sec. after injecting eserine (0.5 mgm. per kgm.); C, 1 min. later. (Rosenblueth and Luco, 1937.)

to a development of tension that may be readily recorded with a myograph. This action might be interpreted as favorable to the view that acetylcholine is the stimulus of fibrillation. Several arguments can be opposed to that view, however, as follows. Curare depresses considerably the sensitivity of striated muscle to acetylcholine (see for references Rosenblueth, 1949). More specifically, curare raises considerably the threshold of both the contraction and the contracture responses of denervated muscles to acetylcholine

(Brown, 1937 *b*; Rosenblueth and Luco, 1937). Yet curare does not modify significantly the fibrillary activity of denervated muscle (Rosenblueth and Luco, 1937; Eccles, 1941 *a*). Also, as pointed out by Eccles, fibrillation is due to impulses arising anywhere along the muscle fibers, not only at the endplate zone, the region of the fibers most sensitive to acetylcholine. The conclusion emerges that although denervated striated muscle is supersensitive to many chemical agents the existence of a specific substance responsible for the appearance and persistence of fibrillation has not been demonstrated.

The changes of electrical excitability of denervated muscles have been the object of several studies. The evidence on the point, however, is still incomplete (see Adrian, 1916, 1917; Watts 1924; Pollock, Golseth and Arieff, 1944). The bulk of this evidence indicates that the electrical excitability of the muscles is either unchanged or only slightly decreased after denervation. It is of interest to contrast this slight change with the marked supersensitivity to chemical agents. Another interesting contrast is that between this decreased electrical excitability and the prolonged repetitive discharges elicited by single-shock direct stimulation of the denervated muscles. It has long been known that such stimuli evoke relatively long-lasting mechanical effects. The phenomenon was designated as a contracture response of the muscles. Rosenblueth and Luco showed in 1937 that these responses are not contractures, but contractions, since they are attended throughout their development by a burst of spike potentials. Eccles (1941 *a*) confirmed this observation and reported that the contraction is due to repetitive discharges of impulses which resemble fibrillation in that their frequency is about 10 per second and in that they begin anywhere along the muscle fiber.

The relations between the fibrillation and the concomitant atrophy of denervated muscles have been variously interpreted. Langley (1916) suggested that the constant activity of the fibers is the cause of the atrophy, i.e., that

the muscle wastes because of overactivity. This view has been accepted in recent years by Tower. The observations of Solandt and Magladery (1940) do not support the hypothesis, however, for they found that quinidine may abolish the fibrillation in rats, while the atrophy is still marked. In the atrophy caused by the disuse of muscles (see p. 189), Tower (1937 b) found no concomitant fibrillation; her report has been confirmed by Tower, Howe, and Bodian (1941) and by Eccles (1941 a).

*The Spontaneous Discharges of Denervated Neurons.* In the stellate ganglion Govaerts (1935, 1936) has reported the appearance of persistent spontaneous discharges 3 to 7 days after severance of the preganglionic nerves. When both ganglia were isolated from the central nervous system in a cat and the heart rate was recorded some days later, after decerebration, it was found relatively high, e.g., 194 per minute. Removal of the ganglia then led to a slowing of the heart, e.g., to 164 per minute. This slowing was interpreted as indicative of a continuous accelerator discharge from the ganglia, not due to reflex activity, for the outgoing nerve impulses persisted, and could be recorded electrically, if the postganglionic fibers were cut at the time of the acute observation. Such electrical records usually showed persistent random series of impulses.

The spontaneous random continuous activity of denervated sympathetic ganglia is clearly analogous to the fibrillary activity of denervated striated muscles. In contrast with the behavior of these two denervated structures is that of partially denervated spinal neurons, as observed by Tower in 1937 (a). With aseptic conditions, under ether anesthesia, the lumbar and sacral dorsal roots were cut intradurally on both sides and the cord was transected above and below the deafferented segments, in four puppies. The animals survived from two to six months thereafter. As revealed by post-mortem anatomical examination, and also by the motor responses elicited upon mechanical stimulation of the

isolated segments of the cord, the majority of the anterior-horn motor cells and their efferent axons were in excellent functional condition. Yet the paralysis of the attached muscles indicated that there were no persistent random discharges of the motoneurons. Tower inferred that autochthonous activity is not a property of the mammalian spinal cord. Her observations have been confirmed by Tower, Bodian, and Howe (1941) in monkeys, and by Eccles (1941 *a*) in cats.

Although section of the descending tracts and of the dorsal roots should cause the denervation of many elements in the spinal cord, this denervation is only partial, as opposed to the total denervation of sympathetic ganglion cells when all the preganglionic fibers are severed, and the equally total denervation of striated muscles when their motor nerves are cut. The difference in the two groups of observations might therefore be quantitative, not qualitative. Recently Keller (1946) has reported observations pertinent to this problem. In cats he made two transections of the brain stem, one above and the other below the oculomotor nuclei and nerves, thus deafferenting completely those nuclei. The pupils remained markedly constricted as long as 11 weeks after the operation, unless the sympathetic nerve supply to the iris became active. Local application of an atropine solution to one eye resulted in a unilateral mydriasis. Although Keller did not test the effect of a later section of the IIIrd nerve, his conclusion appears justified that the deafferented, and thus denervated, oculomotor nuclei are capable of spontaneous persistent discharges.

*The "Paralytic" Secretion of Saliva.* Claude Bernard, in 1864, was the first observer to report that two to three days after complete denervation of the submaxillary gland of the dog—i.e., after section of the chorda tympani and the cervical sympathetic—a continuous secretion of saliva was initiated. This secretion lasted several weeks (five or six) and declined only when the parasympathetic fibers of the

chorda had regenerated. Bernard's explanation of this puzzling effect was that the nerves exert normally an arresting (inhibitory) action on the gland.

The development of this "paralytic" secretion was confirmed by Heidenhain in 1868. He showed that total denervation of the gland is not necessary, but that it is sufficient to cut the chorda tympani for the phenomenon to appear. He found furthermore not only that severance of the chorda on one side led to abundant paralytic secretion of the corresponding gland, but that a clear, though less abundant, flow of saliva occurred from the opposite, normally innervated gland.

Heidenhain's observations were successfully repeated by Langley in 1885. Since the nondecentralized gland also exhibits a paralytic secretion Langley inferred that section of the chorda leads to an increased irritability of the salivary centers and hence to a tonic outflow of secretory impulses. He did not suggest a possible mechanism for this central sensitization.

Acute severance or resection of the sympathetic supply was followed in his observations by a decrease of the paralytic secretion. This resection, however, did not totally abolish the secretion of the parasympathetically decentralized gland. Langley suggested that the activity of the gland now entirely disconnected from the central nervous system was due to a persistent discharge of impulses by the intact post-ganglionic parasympathetic neurons. He assumed that the decentralization, i.e., the denervation, of those nervous elements rendered them supersensitive to the  $\text{CO}_2$  in the blood, and that the persistent discharges were due to the continuous stimulation of these supersensitive elements.

Whether or not  $\text{CO}_2$  be an appropriate stimulus for denervated neurons, Langley's suggestion is in harmony with the observations of Govaerts, cited above. The evidence mentioned, however, does not exclude the possibility that the decentralized secretory cells are capable of spontaneous activity in the absence of impinging nerve impulses.

On the basis mainly of histological evidence, Babkin (1944) infers that in the submaxillary glands the serous, demilune cells are innervated by the sympathetic and the mucous cells by the parasympathetic nerve supply. He further concludes that the paralytic secretion after severance of the chorda is due exclusively to the activity of the demilune cells, sensitized to the action of sympathetic nerve impulses by the continuous discharge of parasympathetic impulses from the isolated postganglionic neurons. That parasympathetic discharges enhance the action of sympathetic impulses was shown by Langley in 1889 and has been confirmed by Goldenberg (1924) and by McIntosh and Rawlinson (1935). In support of the concept that the paralytic secretion is originated in the demilune cells, Babkin mentions the observation made by Bradford in 1888 that section of the parasympathetic secretory nerve of the parotid gland does not lead to the development of a paralytic secretion. As is well known, the parotid contains only one type of secretory cells, but these cells are of the serous (demilune) variety. Bradford did not see any secretory activity in the parotid when he stimulated the sympathetic after destruction of the tympanic plexus. These observations, therefore, do not bolster Babkin's inferences. The inferences fail to explain why the parasympathetic discharges do not cause secretion of the mucous cells, instead of merely sensitizing the action of the sympathetic on the demilune elements. They fail also to explain the persistence of paralytic secretion when not only the chorda but also the sympathetic is severed. The mechanism of paralytic secretion remains thus obscure.

## CHAPTER XVIII

### THE CROSSED PHRENIC PHENOMENON. THE DEFICIENCY OF THE DISCHARGES OF THE CUT PHRENIC

*The Crossed Phrenic Phenomenon.* Like the paradoxical pupillary dilation and the Philipeaux-Vulpian response, the crossed phrenic phenomenon constitutes a mysterious problem handed on from the nineteenth century physiologists to those of the present century. Unlike the other two puzzles, which have been largely cleared up, this riddle remains unsolved.

In all the mammals studied so far, semisection of the spinal cord above  $C_3$  causes the appearance of a respiratory hemiplegia which affects the ipsilateral hemidiaphragm and other respiratory muscles, including the intercostals. In 1887 Langendorff, and shortly thereafter Girard, in 1890, observed contractions of the hemidiaphragm homonymous to a high cord semisection if the phrenic nerve on the opposite side was also cut. Schiff (1894) showed that the crossing of the respiratory impulses to the phrenic motoneurons did not take place until the phrenic nerve on the opposite side was cut; to account for the crossing he suggested that the section of the phrenic acts as a "specific enhancing agent" of the activity of the symmetrical nerve. Schiff also laid stress on the rôle of asphyxia as a factor for the crossing. In his careful study of the phenomenon in the rabbit, Porter, in 1895 confirmed Schiff's observation that the crossed discharges do not occur until the opposite phrenic is cut. He showed furthermore that the crossing of the impulses is effected in the cord at the level of the phrenic nuclei, not above or below. Porter suggested that some dendrites of the phrenic motoneurons cross the midline, that the impulses they

carry are not normally sufficient to cause a contraction of the opposite side of the diaphragm, but that after section of the phrenic nerve a greater portion, perhaps the whole of the "descending impulse" of that side, passes through the crossed dendrites into the phrenic cells of the opposite side. This explanation, reminiscent of Descartes's (1677) theory of the drainage of nerve impulses, is not too different from that proposed by Barcroft in 1934. Barcroft speaks of the respiratory impulse coming down the side where the phrenic is cut as being "thwarted" along its usual path, and therefore "pushing" across to the opposite phrenic along which it discharges.

No further work was carried out on this problem after Porter's study in 1895 until Rosenblueth and Ortiz renewed the enquiry in 1936. Their study was undertaken with the purpose of obtaining an explanation of the crossed phrenic phenomenon in terms of the known properties of nerve fibers and synapses. That purpose was not achieved. The phenomenon apparently reveals new, previously unsuspected properties of nervous elements.

Rosenblueth and Ortiz recorded the movements of both halves of the diaphragm as follows. Under dial anesthesia a midline abdominal incision was made from the umbilicus to the base of the xiphoid cartilage. Two long clamps were placed across the abdominal walls, immediately below the ribs. Sometimes a third clamp was fixed to the xiphoid cartilage. The clamps were supported vertically, so that the diaphragmatic region of the animals was slightly raised from the animal board. This procedure was found not to interfere with the respiration. The abdominal viscera were pressed caudad with cotton, thus exposing the ventral half of the diaphragm. Serrefines were then placed symmetrically toward the center of each diaphragmatic dome and connected, via pulleys, to the recording levers. Downward excursions in the record denoted inspiration.

Comparative observations on different mammals showed that the appearance of crossed diaphragmatic contractions

is not a general phenomenon. Thus, as shown in Table I, while these contractions appear in dogs, cats, rabbits, and woodchucks, they do not occur in monkeys (spider and macaque) and guinea-pigs. Figure 57 illustrates the absence

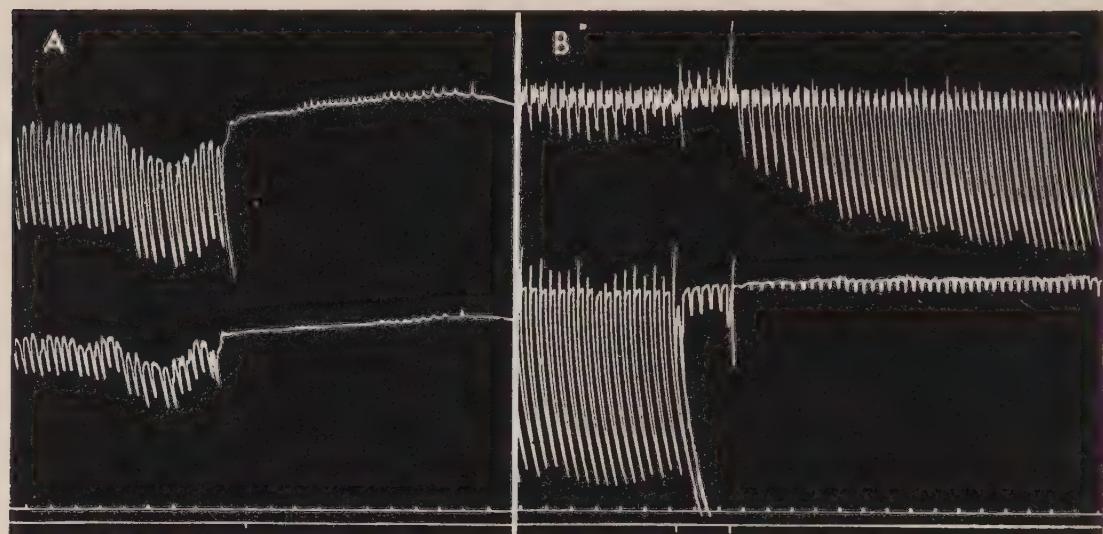


FIG. 57. Absence of the crossed phrenic phenomenon in the monkey, and presence in the dog. Animals under dial. Left spinal semisection at  $C_2$  that caused a left respiratory hemiplegia, and complete transection at  $C_7$ . Records from both hemidiaphragms; downward excursions denote contraction. Time signal: 5-sec. intervals.

A. Macaque monkey. At the start of the record the movements of the lower recording lever were transmitted from the right, active hemidiaphragm (upper record). At signal right phrenic cut low in the neck—a total paralysis of the diaphragm ensued. The slight movements of the recording levers were transmitted from the neck and head muscles. The animal died of asphyxia.

B. Dog. Upper record, left, and lower record, right half of the diaphragm. At the two signal-marks, first a part and then the remainder of the right phrenic nerve was cut. (Rosenblueth and Ortiz, 1936.)

of crossed contractions in a macaque (A) and their development in a dog upon severance of the initially active phrenic (B). Again, as indicated in Table I, crossed intercostal contractions do not develop in any of the species mentioned, whether the active phrenic alone be severed or whether the section be extended also to other respiratory nerves on the direct side, including the intercostal nerves.

The appearance of the crossed diaphragmatic contractions requires the section of the majority or all of the fibers in the phrenic on the direct side. Schiff had reported that the

TABLE I

SPECIES DIFFERENCES IN THE CROSSED PHRENIC PHENOMENON  
(Rosenblueth and Ortiz, 1936)

	MON-KEYS	Dogs	CATS	GUINEA PIGS	RAB-BITS	WOOD-CHUCKS
Respiratory hemiplegia on spinal semisection .....	Yes	Yes	Yes	Yes	Yes	Yes
Crossed diaphragmatic contractions on cutting the vagi, cervical sympathetics and depressors .....	No	Yes	No	No	No	No
Crossed diaphragmatic contractions with asphyxia (see text for exceptions) .....	No	No	No	No	No	?
Crossed diaphragmatic contractions on cutting the active phrenic .....	No	Yes	Yes	No	Yes	Yes
Crossed costal movements .....	No	No	No	No	No	No
Unilateral costal respiration adequate .....	Yes	Yes	Yes	No	No	?
Unilateral diaphragmatic respiration adequate .....	Yes	No	Yes	Yes	Yes	Yes

important component of the nerve was that contributed by the sixth cervical root. Rosenblueth and Ortiz found, however, that the crossing only took place upon section of the last root of the nerve, regardless of the order in which these roots were cut. Thus, if the cervical nerve  $C_6$  was first cut, then  $C_5$  and  $C_4$ , and finally  $C_3$ , the crossed contractions only appeared upon section of this last nerve.

As illustrated in Figure 58 reversible blocks of the active phrenic, produced by a local application of ether to the nerve low in the neck, lead to the transient appearance of crossed contractions. As can be seen in the record, the crossed discharges do not occur until all the motor fibers in the active nerve have been blocked. During the gradual return of conduction in the blocked fibers, on the other hand, both hemidiaphragms may contract simultaneously. Block of the active phrenic by applications of direct or alternating currents also leads to the appearance of crossed contractions. In

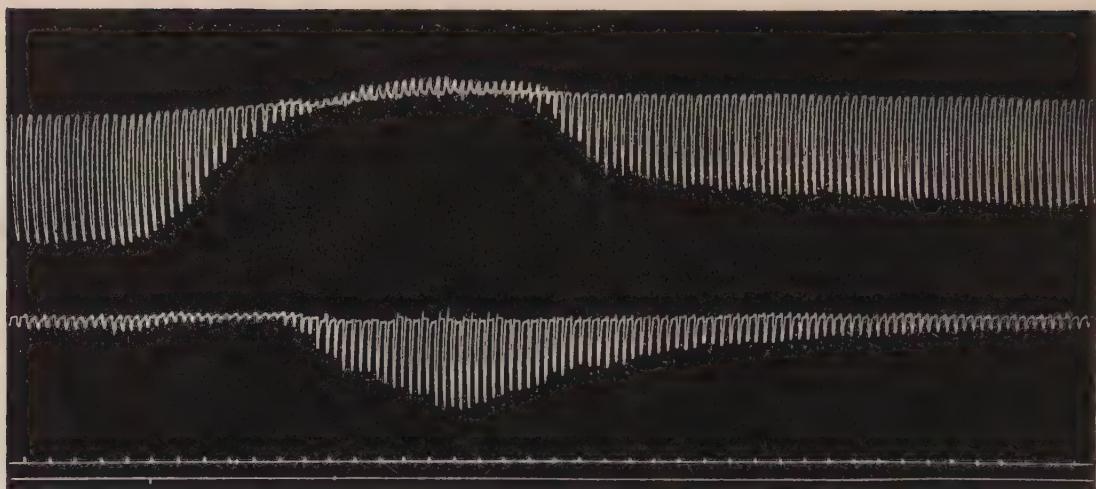


FIG. 58. The crossed phrenic phenomenon elicited reversibly by a transient ether block of the phrenic. Rabbit under dial. Left spinal semisection at C<sub>2</sub>. Vagi cut. Upper record, right, and lower record, left half of the diaphragm (inspiration downward). Between signals, application of a pledget of cotton soaked with ether to the right phrenic, low in the neck. (Rosenblueth and Ortiz, 1936.)

Figure 59 are illustrated the contractions of the two diaphragmatic slips of Head in a rabbit; the slips were attached directly to the recording levers, so that upward excursions

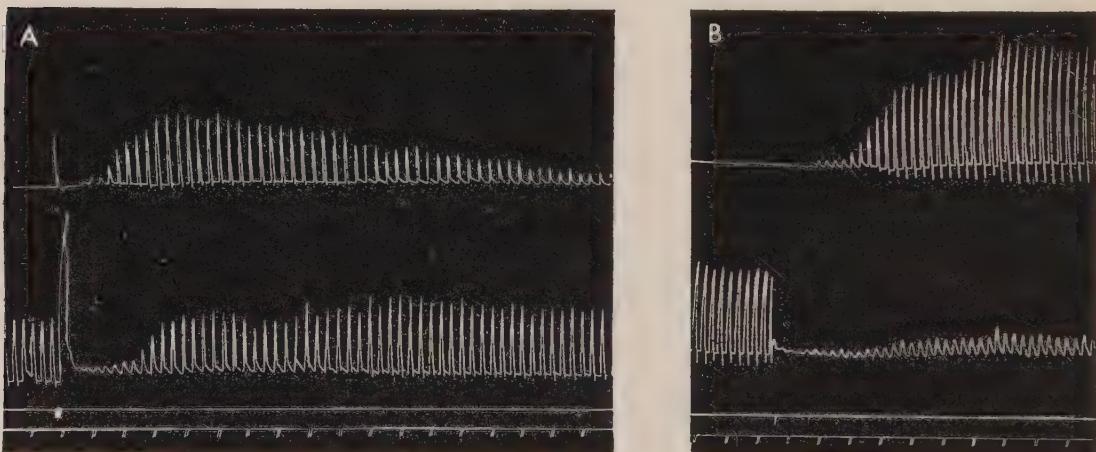


FIG. 59 The crossed phrenic phenomenon elicited reversibly by a transient alternating-current block of the phrenic. Rabbit under dial. Left spinal semi-section at C<sub>2</sub>. Electrodes on intact right phrenic below C<sub>6</sub>; C<sub>7</sub> cut centrally to the phrenic. Records from Head's diaphragmatic slips, left above and right below; upward excursions denote contraction. Time signal: 5-sec. intervals.

A. Strong faradic shocks (alternating current) applied to the right phrenic for 1 sec., at signal. After the stimulation of the nerve a block ensued, as shown by the transient lack of contractions of the right hemidiaphragm. This block resulted in the appearance of crossed contractions.

B. At signal, right phrenic cut below the electrodes. (Rosenblueth, Klopp, and Simeone, 1938.)

denote contraction. As a consequence of a left spinal semisection at  $C_2$  the left slip (upper record) was paralyzed. Application of strong faradic shocks from an induction coil (Harvard inductorium, primary 5 v., coil distance 7 cm.) to the right phrenic first stimulated, but later blocked the nerve transiently. A few seconds after the block had developed the left slip began to contract. It then was active for about two minutes, although the block of the right phrenic only lasted about 15 seconds.

Rosenblueth and Ortiz tested the influence of blocks by curare. In three rabbits the two phrenic nerves were dissected low in the neck and electrodes were applied to them between  $C_5$  and  $C_6$  after a spinal semisection at  $C_2$ . The action potentials on the active side were amplified from the corresponding pair of electrodes and led to a loudspeaker and a cathode-ray oscillograph, so that they could be heard, seen, and photographed. Curare was then injected in a dose sufficient to paralyze all respiratory movements. Artificial respiration was administered, adjusting the ventilation to very slightly less than that which would cause apnea. The phrenic on the direct side was found to continue discharging nerve impulses rhythmically, as before curare was injected. The crossed phrenic remained inactive in these circumstances. Turning off the artificial respiration for 20 to 30 seconds merely intensified the discharges on the direct side, but did not produce any crossed activity. Severance of the active phrenic below the recording electrodes, under constant artificial respiration, led to bilateral simultaneous respiratory discharges, i.e., the crossing then took place and the nerve impulses on the side originally active were not apparently modified.

In 1938 Rosenblueth, Klopp, and Simeone tested the influence of the passage of time on the production of the crossed phrenic phenomenon. In 19 rabbits and 9 cats one phrenic nerve was cut below  $C_7$  and its connection with that nerve was severed, thus freeing the central end of the phrenic. This central end was then sutured either to the pe-

ripheral end of the cut ipsilateral cervical sympathetic, or, more frequently, to the sterno-mastoid muscle, denervated by section of the spinal accessory nerve. One to 25 weeks later the animals were anesthetized with dial, a spinal semisection was made at C<sub>2</sub> on the side opposite to the previously cut nerve, the abdomen was opened and the dia-phragm was either observed directly or hooked up for recording. The effects of a new section of the previously cut phrenic and of section of the vagi were studied. Regrowth of the anastomosed phrenic, when present, could be detected by activity of the iris or the sterno-mastoid, coincident with the respiration.

The purpose of these experiments was two-fold; first, to see whether crossed contractions would appear when the spinal semisection was performed several weeks after cutting the phrenic; and second, to investigate the influence which the functional connections of the cut nerve with some structure other than the diaphragm would have on the phenomenon. The results are summarized in Tables II and III. In the columns marked *Regrowth*, 0 stands for no detectable functional connections of the anastomosed phrenic; the + signs indicate slight, medium, or marked functional re-growth. In the columns marked *Results of semisection* the + signs denote the appearance of crossed diaphragmatic contractions immediately after the semisection of the cord, i.e., they indicate that the corresponding hemidiaphragm did not stop breathing. The sign 0 stands for complete paralysis of the diaphragm. The columns marked *Results of cutting phrenic, or vagi*, indicate the changes in the amplitude of the contractions of the crossed hemidiaphragm—i.e., of the side ipsilateral to the semisection of the cord, with the intact phrenic—upon section of the anastomosed phrenic or the vagi; the sign 0 stands for no detectable change and the + signs denote either the appearance of crossed contractions, if the diaphragm was previously paralyzed, or the increase of pre-existing crossed contractions.

The following results may be emphasized. a) With only

**TABLE II**  
**THE CROSSED PHRENIC PHENOMENON IN RABBITS WITH A PREVIOUSLY  
 CUT PHRENIC NERVE**  
 (Rosenblueth, Klopp, and Simeone, 1938)

ANIMAL No.	TIME AFTER OPERATION (WEEKS)	REGROWTH	RESULTS OF SEMISECTION	RESULTS OF CUTTING PHRENIC	RESULTS OF CUTTING VAGI
1	1	0	++		
2	2	0	++		
3	4	0	++		
4	4	0	++		
5	5	0	++		
6	6	0	++		
7	13	0	0	0	++
8	22	0	++		+
9	24	0	++		
10	13	+	0	0	+
11	13	+	0	0	0
12	13	+	+	+*	+
13	24	++	+		
14	13	+++	+		++
15	13	+++	0	0	0
16	20	+++	+?	Sl. +*	++
17	24	+++	++	0	++
18	24	+++	0	+**	++
19	24	+++	0	0	0

\* The section of the regrown phrenic followed section of the vagi.

\*\* The section of the phrenic was incomplete; later completion led to no further change.

**TABLE III**  
**THE CROSSED PHRENIC PHENOMENON IN CATS WITH A PREVIOUSLY  
 CUT PHRENIC NERVE**  
 (Rosenblueth, Klopp, and Simeone, 1938)

ANIMAL No.	TIME AFTER OPERATION (WEEKS)	REGROWTH	RESULTS OF SPINAL SEMISECTION	RESULTS OF CUTTING PHRENIC	RESULTS OF CUTTING VAGI
1	25	0	+	+	+
2	23	0	0	0	+
3	23	0	++		
4	16	0	+++		
5	23	+	0	0	+
6	20	+	++	+?	++
7	19	+	+++		
8	18	++	+	0	
9	16	++	0	0	+

two exceptions (rabbit 7 and cat 2), if the cut phrenic had not regrown, spinal semisection resulted in prompt crossed contractions. *b*) In 7 out of the 15 animals with regrowth, spinal semisection did not elicit crossed contractions; in 4 of these 7 animals a crossing occurred later. *c*) Up to 24 weeks after cutting the phrenic, spinal semisection may lead to immediate crossing (rabbit 9, cat 1). *d*) Immediate crossed contractions may take place upon semisection even when good functional regrowth of the cut phrenic has been established (rabbit 17, cats 7 and 8). *e*) Section of the regrown phrenic may lead to crossing (rabbit 18). *f*) Section of the vagi may cause crossing after the phrenic has been cut, even though there has been no obvious regrowth (rabbit 7, cat 2), or when such regrowth has taken place (rabbit 10, cats 5 and 9).

It is likely that the severance of the phrenic in the preliminary operation immediately opened the path for the crossed contractions, i.e., the opposite hemidiaphragm was contracting after that section in response to both direct and crossed respiratory impulses. According to this interpretation when the later semisection did not paralyze the diaphragm the reason was that the crossed path had remained open throughout the postoperative weeks or months. The data indicate, however, that when the cut phrenic has established some functional connections with another structure the crossed path closes; it may be reopened again if the regrown fibers are cut anew.

Rosenblueth and Ortiz considered the possibility that the crossed contractions upon section or block of the active phrenic, in experiments such as those illustrated in Figures 57, 58, and 59, might be due merely to an increase of the respiratory discharges consequent to relative asphyxia. This hypothesis, however, does not account for the data. First, asphyxia produced before severing the phrenic failed usually to evoke contractions of the paralyzed hemidiaphragm. As may be seen in Figure 57B the crossing elicited by cutting the active phrenic is immediate, and within about 10 seconds

the crossed contractions are ample. A rubber balloon containing expired air and attached to the tracheal cannula for 30 seconds or more led to minor or to no crossed contractions. Again, in some experiments the phrenic was cut while constant artificial respiration was applied; a crossing-over occurred in these cases although there was no asphyxia and no change of the discharges of the respiratory center. Finally, in the experiments performed under curare the output of the respiratory center was constant, yet crossing ensued upon section of the active phrenic.

When the active phrenic is cut, in addition to a certain degree of asphyxia, some nervous effects take place. Afferent impulses in the phrenic, arising at the previously active hemidiaphragm, are probably interrupted by the section. Also, since the paralysis of a hemidiaphragm entails changes in the expansion of the corresponding lung, afferent impulses arising from that lung will be interrupted. These impulses travel in the vagus and possibly the sympathetic—the nerves distributed to the lungs. In some experiments Rosenblueth and Ortiz transected the cord at  $C_6$  and the dorsal roots  $C_3$ ,  $C_4$ , and  $C_5$  on both sides, in addition to making a semisection at  $C_2$ . The observations were not modified by this procedure, i.e., no crossed contractions appeared until the active phrenic was severed. This eliminates the afferents in the phrenic as a source of the phenomenon. In accord with this conclusion are the observations that stimulation of the central stump of the cut phrenic, or direct stimulation of the paralyzed hemidiaphragm before cutting the phrenic, failed to inhibit the contractions of the opposite side.

Inhibitory afferent impulses in the vagi or sympathetic, arising at the lungs, were eliminated in experiments in which the vagi were cut in the neck and the sympathetic thoracic chains were removed. These operations, and also the denervation of the carotid sinuses, do not influence the phenomenon significantly. Some observations made by Rosenblueth, Klopp, and Simeone are apposite here. In five cats the vagi, cervical sympathetics, and depressors were

cut in the neck. The carotids were ligated and the spinal accessory nerves cut. The cervical nerves  $C_3$  to  $C_7$  were severed peripherally to the phrenics on both sides. A left semisection of the cord was performed at  $C_3$  and a complete transection immediately below  $C_7$ . The animals were then breathing only with their right hemidiaphragm. Artificial respiration was started. The cerebellum was removed. A midline longitudinal section of the medulla and cord was made from the upper part of the 4th ventricle to the level of  $C_2$ , and also two left semisections at the upper and lower limits of this midline incision, so that a segment on the left side was isolated. This segment was removed, eliminating the left half of the respiratory center. Stimulation of either the right respiratory center, by applying electrodes to the medial surface of the sectioned medulla just above the tip of the *calamus scriptorius*, or of the right respiratory tract, by means of electrodes applied to the right lateral surface of the cord at  $C_1$  or  $C_2$ , caused marked contractions of the right hemidiaphragm with no involvement of the left side. The right phrenic was then cut. The same stimuli elicited now strong contractions of the left hemidiaphragm; the right side was of course paralyzed. Completion of the semi-section of the cord at  $C_3$ , i.e., complete transection at that level, abolished the diaphragmatic responses, thus proving that the crossed left contractions were not due to spread of the stimuli to the left respiratory tract below  $C_3$ .

From these data the inference may be drawn that although asphyxia, and changes in the afferent nerve impulses which attend respiration, may modify the crossed phrenic discharges, they are not the conditions which determine the crossing. The important factor is the block or section of the majority or all of the active phrenic motor fibers. Peripheral block, e.g., by curare, does not cause the crossing; it is necessary to interrupt the motor impulses or the axons in the course of the fibers.

Rosenblueth, Klopp, and Simeone showed that once the crossed path has been functionally opened, it tends to re-

main open. This statement is based on the effects of some experimental procedures when carried out before or after the application of a reversible block to a phrenic. Figure 60

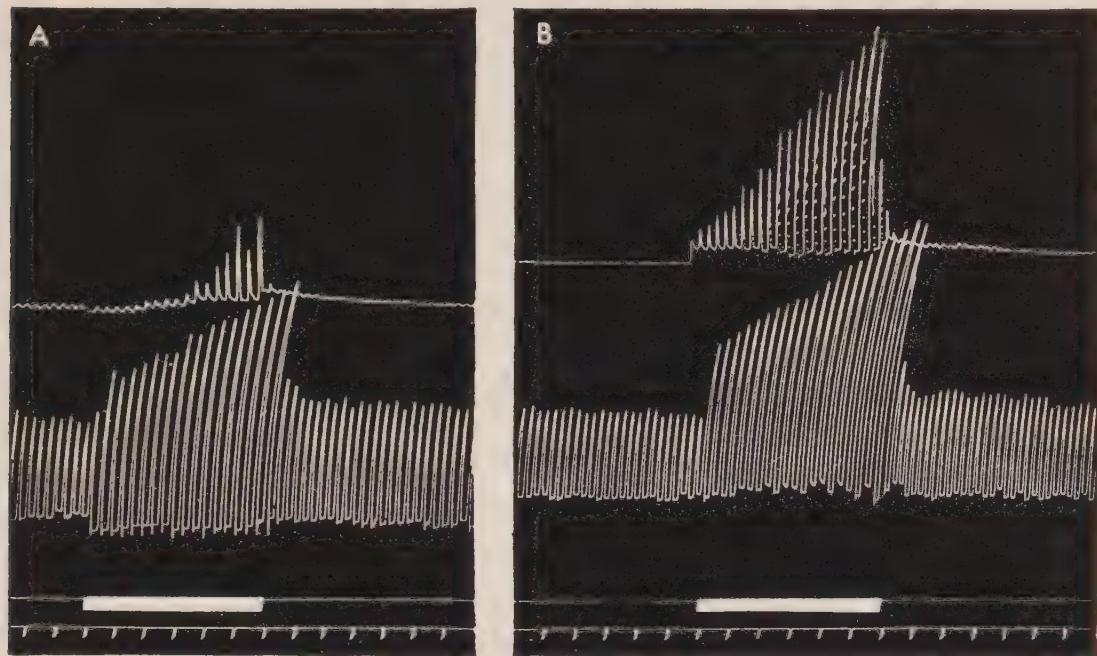


FIG. 60. Effects of asphyxia before and after reversible crossings of the respiratory impulses. Cat under dial. Left spinal semisection at  $C_2$ . Records from Head's slips, left above and right below. Time signal: 5-sec. intervals. Tracheal cannula occluded through continuous signal-mark.

A. Before any reversible crossing.

B. After 3 reversible crossings caused by the application of alternating current as in Fig. 59A. (Rosenblueth, Klopp, and Simeone, 1938.)

illustrates a typical observation. While asphyxia caused only delayed and slight contractions of the crossed diaphragm when applied, in A, before any reversible blocks had been carried out, the same degree of asphyxiation elicited, in B, prompt and marked crossed contractions after the path had been opened by blocking the active phrenic reversibly with alternating current three successive times.

The data available are not sufficient for the elaboration of a plausible theory of the crossed phrenic phenomenon. The following considerations, however, are pertinent. Severance of the phrenic motor axons has a central repercussion. This central influence is not due to a change in the ability of the severed axons to conduct impulses, nor is it due to a change in the number of impulses conducted by them, for,

as mentioned above (p. 173) and as observed also by Gasser and Newcomer (1921) and by Adrian and Bronk (1928), severance of the phrenic neither interrupts nor apparently modifies the respiratory output up to the cut region.

The central repercussion transcends the synaptic confines of the severed neurons. This is proved by the slowing of the respiratory rate commonly observed by Rosenblueth, Klopp, and Simeone after cutting the phrenic.

Crossed impulses are probably always present, at least in the species where the crossed phrenic phenomenon can be produced, but are subliminal in normal conditions, and only attain threshold after a phrenic has been cut. This assumption explains the possibility of causing slight crossed contractions by means of asphyxia even though the phrenics be intact. It is also in accord with the observations made by Seligman and Davis in 1941 on the effects of some drugs on the crossed phrenic phenomenon. They found that eserine or prostigmine, acetylcholine and strychnine can produce or promote crossed respiratory contractions. In some of their observations transient crossings were seen without section of either phrenic. There was no evidence of an increased respiratory nervous output during these crossings. They inferred, therefore, that the action of the drugs was exerted at the neuronal mechanisms of the spinal cord concerned in the crossing, and that the drugs probably enhance transmission in the synapses involved.

In broad terms, the crossed contractions indicate one or both of the following changes: (*a*) an increase (temporal or spatial) of the number of crossed respiratory impulses; (*b*) an increase of the sensitivity of the phrenic motoneurons on the crossed side, so that the normal, subliminal crossed nerve impulses become effective for activation of the sensitized neurons. A possible assumption (see Rosenblueth and Ortiz) is that the dendrites of the phrenic motoneurons cross over, as suggested by Porter, that these dendrites have normally only unidirectional conduction, as postulated by Gad in 1884, and that blocking the axon makes the dendrites

capable of cellulifugal conduction, whereupon they can activate the opposite phrenic nuclei, with which they establish synaptic connections. That dendrites may activate other neurons was first defended and later (1909) rejected by Cajal, but the hypothesis has not been disproved so far.

The possibility that the crossed diaphragmatic contractions may be due to an increased sensitivity of the phrenic motoneurons is the reason for including the crossed phrenic phenomenon in this monograph. If such sensitization takes place in fact, it differs considerably from that produced by denervation, for it develops rapidly and may disappear promptly, unlike the supersensitivity caused by anterograde nerve degeneration.

Another important aspect of the crossed diaphragmatic contractions was emphasized by Rosenblueth and Ortiz. The phenomenon reveals two properties of the nervous system: the functional opening of a potentially pre-existing but previously unused path, and the tendency for the new path to remain open once it has been used. These properties appear also in other unsolved problems of central nervous functions: the establishment of conditioned reflexes and the persistence of learned patterns of reaction.

*The Deficiency of the Respiratory Discharges of the Cut Phrenic Nerve.* This phenomenon is in some respects the counterpart of the crossed phrenic phenomenon. It was repeatedly stated above that the respiratory output in an active phrenic nerve is not modified by cutting that nerve. This is true in tests made within 7 days after the section. In 1942 Acheson, Lee, and Morison reported, however, that from the 8th to the 21st day after the cut there is a progressive decrease of the amplitude of the respiratory discharge recorded from the previously severed phrenic. As shown in Figure 61, by the end of the second week this discharge is only about a third of that from the control, acutely cut nerve. After three weeks there is a gradual recovery of discharge from the previously cut nerve, and between the 40th and the 78th day the recovery is complete,

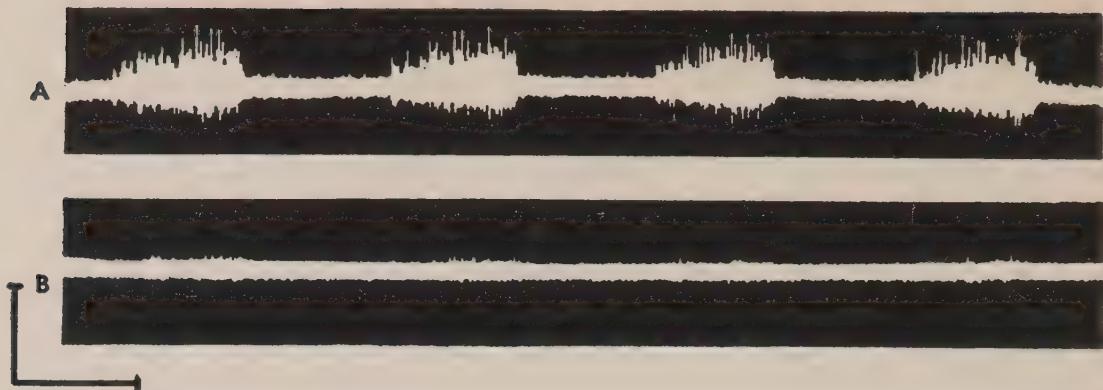


FIG. 61. Deficiency of respiratory discharge recorded from the right phrenic, which had been cut close to the diaphragm 15 days previously. Cat under dial. Capacity-coupled amplifier and cathode-ray oscillograph. Electrodes on both phrenics in the neck, cut peripherally. Calibrations: 50  $\mu$ v and 1 sec. A, left control, and B right test phrenic. (Acheson, Lee, and Morison, 1942.)

i.e., the discharge is alike on the two sides. Figure 62 illustrates the time course of the phenomenon.

As shown in Figure 62 the results are the same whether the phrenics be cut low, near the diaphragm, or high, at the

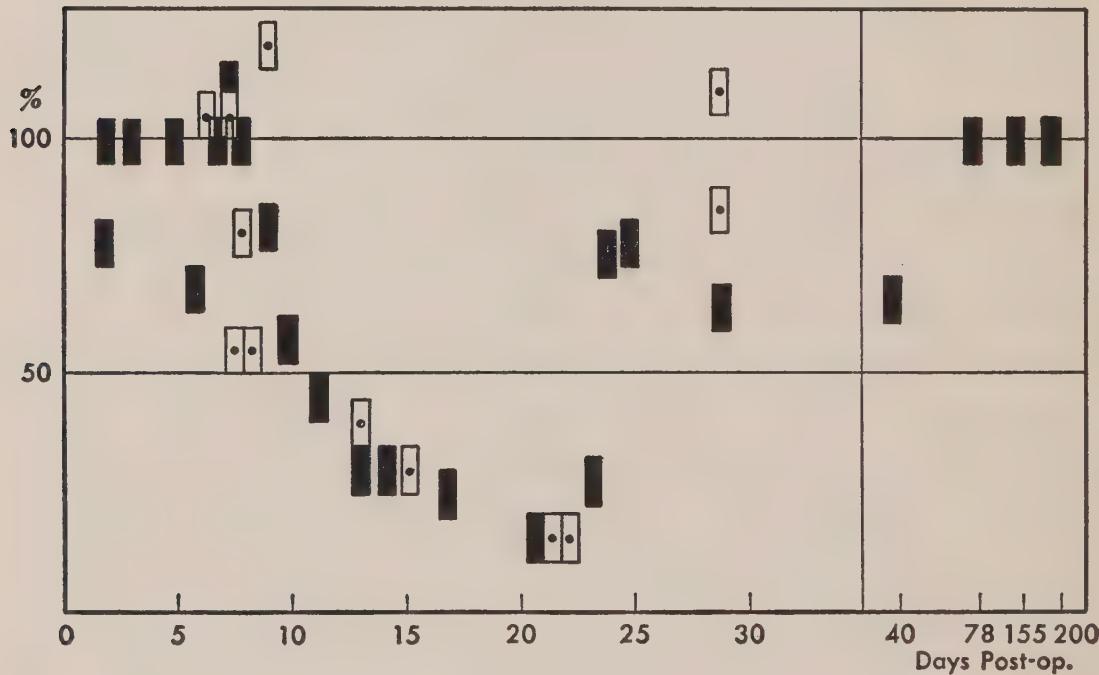


FIG. 62. Time course of deficiency of respiratory discharge from phrenic nerve following peripheral section. Each rectangle represents a single experiment. Ordinates: amplitude of respiratory discharge from the previously sectioned phrenic, expressed as per cent of that from the opposite, acutely severed, control phrenic. Abscissae: days after section of the nerve. Solid rectangles: test phrenic cut near diaphragm; clear rectangles: test phrenic cut at level of first rib. (Acheson, Lee, and Morison, 1942.)

level of the first rib. Acheson, Lee, and Morison studied comparatively some of the nerves with previous low cuts, at the time when the discharge was very slight, together with the corresponding opposite controls, after excising the nerves and transferring them to a moist chamber. The excitability of the cut nerves was not grossly different from that of the controls; the conduction velocity was usually slowed in the severed nerves; the amplitude of the maximal A spike potential was unchanged.

The time course of the phenomenon is similar to that described for retrograde degeneration by Nicholson (1924) and by Bucy (1928). Acheson and his collaborators accordingly examined microscopically the cervical segments 4, 5, and 6 of the spinal cord in several animals in which the deficiency of respiratory discharge was well marked (20 to 70 per cent of the controls). Serial Nissl preparations showed little or no retrograde degeneration in any of the cords from the animals studied, eight in which the phrenic had been cut close to the diaphragm and one in which the section had been made at the level of the first rib. Bodian and Cajal preparations also showed little change either in the boutons terminaux or in the neurofibrillae. Since the degree of functional impairment is not influenced by the level of the cut, whereas in retrograde degeneration the nearer the section to the cell body the more marked the anatomical change, and since the phrenic cell bodies may show little or no morphological deterioration when the functional impairment is at its peak, it may be inferred that retrograde degeneration is not the cause of the deficiency in the respiratory discharge.

The deficiency is not due to failure of conduction in the phrenic axons, since, except for the conduction velocity, the properties of these axons are normal. It is tempting to assume that the phenomenon described by Acheson, Lee, and Morison denotes the gradual development of an "infrasensitivity" of the phrenic motoneurons to the action of the impinging nerve impulses from the respiratory tract as

a consequence of the severance of the motor axons. As shown in Table II, Rosenblueth, Klopp, and Simeone (1938) observed crossed diaphragmatic contractions in animals with a phrenic nerve cut two to five weeks previously, after contralateral spinal semisection (rabbits 2, 3, 4, and 5). The development of the deficient phrenic output, therefore, does not prevent the appearance of the crossed phrenic discharges. The time course of the two phenomena is quite different: the "sensitization" revealed by the crossed diaphragmatic contractions is prompt; the "desensitization" demonstrated by the deficient respiratory discharge of cut phrenics develops gradually.

## CHAPTER XIX

### A LAW OF DENERVATION

It has been shown in Part II that smooth muscle, whether normally stimulated (Chapters III and IV) or inhibited (Chapter V) by sympathetic nerves, or similarly stimulated or inhibited by parasympathetic influences (Chapter VI) becomes more excitable to diverse chemical agents after destruction of the ultimate innervating neurons. The same condition results in melanophores (Chapter VII), in glands subject to sympathetic impulses, adrenergic or cholinergic (Chapter VIII), in the heart (Chapter IX), in skeletal muscles (Chapter X), in the peripheral neurons of autonomic ganglia (Chapter XI), in partially denervated spinal neurons (Chapter XII), and probably also in coördinating centers (Chapter XIII).

It was shown moreover that degeneration of the penultimate neurons that innervate smooth muscle, glands, and striated muscle results also in a supersensitivity of these structures to chemical agents.

This evidence led Cannon to formulate in 1939 the following law. *When in a series of efferent neurons a unit is destroyed, an increased irritability to chemical agents develops in the isolated structure or structures, the effects being maximal in the part directly denervated.*

It is of historical interest that, in 1880, Claude Bernard, on the basis of relatively few observations, expressed the opinion that "the excitability of all tissues seems to augment when they are separated from the nervous influence which dominates them"—a remarkable instance of perspicacity. As another early partial formulation of the law may be cited the following statement made by Elliott in 1905. "This, then, is true for all the muscles thrown into contraction by

adrenalin, that after decentralisation (*i.e.*, degenerative section of the preganglionic sympathetic nerves), and still more clearly after denervation (degenerative section of the postganglionic sympathetic nerves), they contract in the presence of adrenalin alike with greater irritability and persistence. The corresponding lesions of the inhibitor muscle-nerve system result also in a more prolonged inhibition by adrenalin; but perhaps not in greater irritability." Elliott's statement, quoted earlier in this monograph, has been corroborated in all its parts, except in the last cautious hypothesis: the inhibitory actions of adrenaline are not only prolonged but also increased after denervation.

In Part III of this monograph evidence is presented which indicates that decentralized or partially denervated smooth muscles (Chapter XIV), adrenal medullae (Chapter XIV), sympathetic ganglia (Chapter XV), striated muscles (Chapters I and XV) and spinal neurons (Chapter XVI), become supersensitive to the action of nerve impulses impinging on them, whether this action be excitatory (in all the structures mentioned) or inhibitory (in spinal neurons).

This evidence, although not as comprehensive as that reviewed in Part II, justifies the extension of the law of denervation, as suggested by Cannon, Rosenblueth, and García Ramos in 1945. The generalized law may be stated as follows. *When in a functional chain of neurons one of the elements is severed, the ensuing total or partial denervation of some of the subsequent elements in the chain causes a supersensitivity of all the distal elements, including those not denervated, and effectors if present, to the excitatory or inhibitory action of chemical agents and nerve impulses; the supersensitivity is greater for the links which immediately follow the cut neurons and decreases progressively for more distant elements.*

## CHAPTER XX

### THEORIES OF SENSITIZATION: THE DETERMINISM

In attempts to explain the phenomenon of the paradoxical pupillary response many ideas were suggested during the decades when it remained a mystery. We now know that these ideas have no basis in fact. Furthermore, even if they had been appropriate for that particular phenomenon, they would not have applied to other phenomena of sensitization. If possible, a theory would be desirable that can cover all the instances of supersensitivity from denervation which have been described in the foregoing chapters.

The problem of sensitization by severance of nerves involves two questions. Why does a structure become supersensitive when its direct or remote innervation is destroyed? What are the changes in the structure which determine an increased sensitivity? The first question is concerned with the influences which nerves exert on the tissues to which they distribute, and with the mechanisms whereby these influences are exerted; the second refers to the structural and functional conditions upon which depend the responses of excitable cells. Clearly the two questions are independent. There are thus two problems to be solved, and hence two theories are needed: this chapter deals with the first of these problems.

When a nervous pathway is severed there is in most cases relative or absolute inactivity of distal elements in the pathway—precisely the elements which become supersensitive. It is thus conceivable that inactivity, and the consequent metabolic changes, may be the cause of the development of supersensitivity. The rôle of the nerves in

the phenomenon would be then relatively secondary. It is possible, however, that inactivity is not the source of the sensitization, but that nerves exert some influence on the innervated structures, the removal of which leads to supersensitivity—i.e., this influence would restrain the responsiveness of the reacting elements. This possibility should be considered, even though, as pointed out on p. 91, there is some evidence which suggests that the sensitivity of tissues to the effects of chemical agents is enhanced, instead of being depressed, when the tissues are first innervated in the course of ontogenetic development.

With the facts available a general discussion of this problem would not be profitable; it is preferable to consider specific instances. As pertinent data on striated muscles the following may be mentioned. Severance of the motor nerve fibers leads usually to an atrophy and degeneration of the denervated muscles, but not always, for Arloing and Chantre reported in 1898 that the sphincter ani of the dog did not show any evidence of degeneration one year after section of its nerves. This degeneration, which also takes place in other denervated structures (e.g., the salivary glands), has led to the concept of a trophic influence exerted by the nerves—the axons would control directly the metabolism of the structures which they innervate, not indirectly by regulating the activity of these structures. For the acceptance of this trophic influence the possibility that degeneration may result from inactivity should be dismissed. A comparative study of denervated muscles and muscles inactivated by other procedures is thus important.

As already mentioned (p. 165), the isolation of some segments of the spinal cord by section of the corresponding dorsal roots and transection above and below the deafferented segment may result in prolonged (several months) inactivity of the motoneurons included in the isolated portion. In these circumstances, therefore, some striated muscles no longer receive motor nerve impulses, as they do normally. Section of a motor nerve, likewise, interrupts the normal

stream of impulses to the corresponding muscle. But while the two conditions are alike in that no motor nerve impulses reach a given muscle, they differ in that no nerves make contact with the muscle fibers soon after section of the nerve, whereas the motoneurons and motor axons remain in functional connection with the muscle in the isolated-cord preparation.

Like the denervated striated muscles, those innervated but rendered inactive show a gradual atrophy (see Lipschütz and Audova, 1921). Eccles (1941 b) found that the average weight of denervated cat muscles was 64 per cent (fresh weight) and 62 per cent (dry weight) of the normal controls, three weeks after section of the nerves. The corresponding figures for muscles innervated but not used for the same time were 63 (fresh) and 61 (dry). Thus, the degree of atrophy was approximately the same for the two groups of muscles. On the other hand, the two atrophies differ in several respects, as follows. First, Chor and Dolkart (1936) and Tower (1937 b) have reported that the atrophy of denervated muscles differs histologically from that found in disused muscles. Again, Tower (1937 b; see also Tower, Howe, and Bodian, 1941; Reid, 1941; and Eccles, 1941 b) found that whereas denervation leads to the development of fibrillary activity (see Chapter XVII), disuse does not result in fibrillation. Finally, the atrophy which results from denervation differs from that ensuing from disuse when the effects of electrical stimulation are compared in the two. Eccles (1941 b) showed that although daily stimulation of innervated and otherwise not used *gastrocnemius* and *soleus* muscles of cats relieves their atrophy only slightly, a similar daily stimulation of *tibialis anticus* and of *extensor digitorum longus*, even if applied for only 10 seconds, prevents the atrophy of these muscles almost entirely. The puzzle presented by these differences between muscles will not be considered further; the important fact for the present argument is that in some muscles disuse atrophy can be largely canceled by daily stimulation. The influence of periodic

stimulation on the progress of atrophy after denervation has been studied extensively. Contradictory results have been reported: retardation, no change, or acceleration of the rate of atrophy. Recent studies (Solandt, DeLury, and Hunter, 1943; Grodins, Osborne, Johnson, Arana, and Ivy, 1944) favor the view that daily stimulation at the rate of about 25 per second diminishes the rate of atrophy of denervated *gastrocnemius* and *soleus* muscles of rats.

Since the denervated rat muscles mentioned are those which Eccles found, in cats, only moderately benefited by daily stimulations when in disuse, no decisive comparison can be made of the effects of stimulation on the atrophies of denervation and disuse, respectively. The data cited suggest, however, that disuse atrophy may be largely prevented by stimulation, in some muscles, while denervation atrophy is only relatively delayed by stimulation, in all muscles. If this suggestion should be confirmed by future studies, then this difference between the two groups of muscles would be strong evidence for the view that those in disuse do not degenerate as readily and deeply as do those that are denervated, because the former are under the trophic influence of their intact, though inactive, attached nerves.

The three differences mentioned between the atrophy of denervation and that of disuse support the view that nerves exert on the structures to which they distribute other influences than those which result from the delivery of nerve impulses and the consequent liberation of chemical mediators. The bearing of the previous argument on the problem of sensitization will now be considered. Neither Tower nor Eccles reported on the sensitivity to acetylcholine or other substances of the muscles in disuse which they studied. Immobilization of joints may or may not lead to muscular atrophies, depending on the position of the joints (see Thomsen and Luco, 1944). Solandt, Partridge, and Hunter (1943) have reported the development of a supersensitivity to intra-arterially injected acetylcholine in rat *gastrocnemius*-*soleus* muscles which atrophied as a consequence of

skeletal fixation. It would be important to learn the changes of sensitivity which take place in immobilized muscles when they do not atrophy. Although the observations of Solandt and his collaborators may be considered as supporting the hypothesis that inactivity is an important factor in the determination of supersensitivity in denervated muscles, the following data oppose that hypothesis. When fibrillation begins, some days after denervation, the supersensitivity of striated muscles does not decrease, but continues developing (see p. 98), yet fibrillation implies activity, even though probably moderate. Again, semisection of the spinal cord causes only a relatively slight degree of inactivity of the muscles controlled by the motoneurons in segments ipsilateral and caudad to the level of the section, yet clear sensitization to acetylcholine takes place (see Figure 31 and p. 104). Certainly in some other tissues than striated muscle, inactivity is not the cause of the supersensitivity which develops after denervation. Thus, sympathetic denervation of the heart leads to only a slight slowing of its average rate, yet the pacemaker becomes more susceptible to the action of adrenaline (Figure 28). Also, sympathetic denervation of the submaxillary gland does not abolish the secretion due to the remaining control of the chorda tympani, and nevertheless, the gland responds more readily than normally to adrenaline (Figure 24), acetylcholine (Figure 25), and pilocarpine (Figure 26). Finally, as an example of a different sort, the pilomotors of the cat will be considered. These muscles only become active in special circumstances, e.g., when the animal is exposed to cold or when it becomes emotionally excited. A cat may not have any pilomotor erection for months, especially if isolated, fed, protected, and kept warm in the laboratory. Yet the muscles do not develop supersensitivity while innervated, and become supersensitive to adrenaline when the sympathetic chains are excised (see p. 46).

If inactivity is not the factor or is not the sole factor which causes supersensitivity after denervation, then the

inference seems unavoidable that a nervous influence that restrains the responsiveness of innervated elements is removed upon severance of the nerves. The chemical mediators liberated by the nerve impulses in innervated structures might be deemed responsible for the restraining influence. Cannon and Bacq (1931) suggested that the increase in sensitivity of smooth muscle after denervation may be due to a storage of sympathin or of its precursor within the denervated cells. Again, Wolff and Cattell (1937) favored the view that supersensitivity may denote an increase of some substance (or "state"). And Heinbecker (1937) also supported the hypothesis of an increased storage of "epinephrine or of a substance on which it acts" within the cells. These suggestions do not accord with the data which indicate that both the adrenergic and the cholinergic mediators are produced by the nerves, not by the reacting structures (see for references Rosenblueth, 1949), and hence that they should disappear after denervation. The storage theory is likewise opposed by the fact that supersensitivity is not specific for the natural transmitter, but occurs also for many other substances.

In opposition to the theories mentioned above, Bacq (1933) suggested that the sympathin formed upon the arrival of nerve impulses to effectors supplied by adrenergic nerve fibers exerts a "toxic," restraining action on the sensitivity of the innervated cells; supersensitivity after denervation would then be the sign of the suppression of this toxic influence. Some observations made by Simeone in 1938 (*b*) have a bearing on this hypothesis. In a group of eight cats he removed the superior cervical ganglion on one side. After supersensitivity of the corresponding nictitating membrane had developed he either injected subcutaneously 2 mgm. of adrenaline 3 times a day for 5 days, or else injected the hormone intravenously for 30 to 90 minutes with a continuous rate of 0.1 to 0.25 cc. of a 1:50,000 solution per 15 seconds. In all the eight cats either of these procedures resulted in a slight but definite decrease in the

magnitude of the contractions of the denervated membrane to subsequent test doses of adrenaline. This relative desensitization might support the view that supersensitivity had developed because the membrane had been released by the denervation from the influence of periodically delivered sympathin. Simeone found, however, that while the denervated membrane was partially desensitized by prolonged administration of adrenaline, the normal control membrane was invariably supersensitized by the same administration. The conflicting results recorded from the two membranes remain unexplained, but it is not likely that the adrenergic fibers to the membrane hold its sensitivity in check because of a "tonic" release of sympathin. A consideration of the pilomotors is again apposite. When denervated they become sensitized to adrenaline (Elliott, 1905), although manifestly they do not belong to the class of muscles that receive tonic impulses regularly. Experiments corresponding to those of Simeone on the membrane have not been performed on striated muscles, i.e., the influence of repeated administration of acetylcholine on the development of supersensitivity in this tissue after denervation has not been studied. Altschul (1943) has reported that daily intramuscular injections of prostigmine followed by a subcutaneous injection of acetylcholine (0.25 to 0.65 mgm.) do not affect the atrophy of denervated muscles in rabbits and only retard it slightly in cats. These doses of acetylcholine are probably too small to cause significant effects. Altschul did not use atropine in his experiments because of the erroneous notion that this drug prevents the action of the chemical mediator. The technique which he followed did not allow any observations on the sensitivity of the denervated muscles.

Clearly many more observations will be needed in order to decide whether the chemical mediators of nerve impulses not only control the activity of the structures where they are liberated, but also independently influence the sensitivity of these structures to their own effects and to those of other agents. If future experiments should indicate that neither

the relative or total inactivity nor the suppression of the release of chemical mediators, which result after severance of nervous pathways, is the important factor for the development of supersensitivity, then experiments will have to be devised to test the possible rôle of the trophic, non-nerve-impulse influence of nerves on cells that is revealed by the observations on striated muscles. The determinism of the supersensitivity which follows immediate or remote denervation remains at present obscure. All that we have attempted here is to state the problem in terms which make it amenable to an experimental approach.

## CHAPTER XXI

### THEORIES OF SENSITIZATION: THE MECHANISM

As pointed out in Chapter II, different phenomena are covered by the term supersensitivity: *a*) the duration of the responses may be prolonged (see Figure 3); *b*) the susceptibility of the tissue may be increased (see Figure 4 and p. 17); *c*) the reactivity of the effector may be augmented (see Figure 5 and p. 17); *d*) the excitability of the structure may be enhanced (see p. 16). It is probable that these changes are brought about by different mechanisms. In the supersensitivity which follows denervation an increased reactivity has not been found so far; the mechanism of this phenomenon, therefore, need not be considered here. Increased duration of effects, however, has been reported after denervation; and increased susceptibility and excitability are the outstanding aspects of the supersensitivity resulting from severance of nerves, in autonomic and somatic effectors, respectively. This chapter will be devoted to a discussion of mechanisms which possibly underlie these changes.

A prolongation of action or of response may be due to a decreased rate of destruction of the stimulating substance. Thus, as is well known, acetylcholine in normal circumstances is swiftly destroyed by a cholinesterase. It is likely, furthermore, that after a cholinergic nerve has degenerated, the cholinesterase content of the tissue supplied by that nerve decreases significantly. For example, in the superior cervical ganglion Brücke (1937) found a marked decrease of cholinesterase after preganglionic denervation. In agreement with this report Couteaux and Nachmansohn (1940) showed that during the time when the preganglionic endings disappear, the amount of the enzyme present in the ganglion

decreases by about 60 per cent. A lessened amount of cholinesterase in denervated sympathetic ganglia can account for the greater than normal efficacy of acetylcholine (see Figures 32, 33, 34, and 35); the decrease of the concentration of the enzyme in some cells might also explain the increased responses of partially denervated ganglia when activated by the acetylcholine released from the remaining preganglionic fibers (see Figures 46 and 47). Again, in striated muscles Feng and Ting reported in 1938 that the cholinesterase concentration of the end plates decreases by about 30 per cent after section of the motor nerves. These results were confirmed by Meng and by Couteaux and Nachmansohn in 1940. The diminished concentration of the enzyme may account for the prolonged responses evoked by acetylcholine in these muscles (Figure 29) and also for the diffusion of the mediator from blood vessels to the muscle fibers in the Philipeaux-Vulpian and allied phenomena.

With regard to the central nervous system, Martini and Torda (1938) have reported that the cholinesterase normally present in the spinal cord of rats is considerably reduced in concentration in the isolated portion, ten days after a transection in the lower thoracic region. In harmony with this report, Nachmansohn and Hoff (1944) observed that the concentration of cholinesterase in the grey matter of the 6th lumbar segment of the spinal cord, in cats, decreases, both in the ventral and the dorsal horns, by about 10 to 20 per cent upon unilateral deafferentation, and by about 30 per cent upon bilateral section and degeneration of the dorsal roots. Martini and Torda's report suggests that the supersensitivity to acetylcholine of partially denervated spinal neurons (Figures 37 and 38) may be due to a lessened cholinesterase content. The applicability of this hypothesis to the greater efficacy of afferent nerve impulses in eliciting reflex discharges from chronically semisected than from normal cords (see Figures 50 to 54, and also p. 155) depends on whether or not these reflexes involve acetylcholine

transmission. Although there is abundant circumstantial evidence of chemical mediation of nerve impulses by acetylcholine in the brain and spinal cord (see Feldberg, 1945, for a recent review of the topic), definitive proof of this mediation has not been established.

Whether denervation of effectors supplied normally by adrenergic nerve fibers results in a decreased rate of destruction of adrenaline or sympathin has not been determined. A slower destruction of the mediator, at least in some tissues, is suggested by the prolongation of the responses to adrenaline of the denervated nictitating membrane, when compared with those of the normal membrane. With the data on hand it would not be profitable to indulge in speculation on this problem, however, for the mechanism of destruction of adrenaline in the organism has not been elucidated so far. In 1937 Blaschko, Richter, and Schlossmann found that adrenaline is an appropriate substrate of amine oxidase *in vitro*. On this basis, and because amine oxidase is present in vertebrates, they suggested that the hormone might be the chief substrate of the enzyme *in vivo*. Blaschko (1941) points out, however, that this view is unlikely, for in some invertebrates which contain chromaffine tissue and adrenaline the oxidase is not present. It is possible that phenolases may be involved in the enzymic destruction of adrenaline *in vivo* (Blaschko and Schlossmann, 1940).

The foregoing argument shows that more data will have to be acquired before it will be legitimate to accept as a general law that in denervated structures the destruction of the chemical mediators delivered to them by the supplying nerves is retarded. Even if this law should be firmly established it would only explain some but not all the features of the supersensitivity of tissues deprived of their innervation. As was emphasized repeatedly in earlier chapters, this supersensitivity is not specific for the action of the normal mediator of the nerve impulses which the denervated structure receives, but becomes also manifest

when other stimulating chemical agents are tested. Clearly, the supersensitivity of denervated striated muscle to nicotine (Heidenhain, 1883; Langley, 1905), to potassium (Frank, Nothmann, and Guttmann, 1923), and to several quaternary ammonium compounds (Dale and Gasser, 1926), is not due to a decrease of the cholinesterase present in the muscle. Again, the supersensitivity of the denervated nictitating membrane to acetylcholine, pilocarpine, eserine, and histamine (Rosenblueth, 1932 *a*), as well as that to calcium and potassium salts (Bacq and Rosenblueth, 1934), cannot reasonably be attributed to a decrease of amine oxidase or of phenolases. While the law mentioned above would explain the prolongation of the effects of acetylcholine or adrenaline, a more general theory is required to account for the increased susceptibility of denervated or decentralized effectors to a wide variety of chemical agents.

In 1936 Cannon and Rosenblueth suggested that denervation results in supersensitivity because the membrane of the cells partially or totally deprived of nervous influences becomes more readily permeable than normally. This condition would permit an easier passage outward of substances in the cells and inward of substances outside. As a consequence an effective chemical agent in smaller concentration than usual would be able to enter the cells and affect their intrinsic mechanisms. What is the evidence in support of this theory?

If denervated striated muscle were more permeable than normal muscle, a change of its chemical composition might be expected, because some substances might become concentrated and others depleted. After reviewing carefully all the evidence on the subject, Tower concluded in 1939 that the only substances found in disproportionate amounts in the interstitial tissues of denervated muscles are calcium, and perhaps simple fat in some cases; and the only substances disproportionately reduced in the muscle tissues proper are glycogen and phosphocreatine. Tower remarks that the

excess of calcium outside the muscle fibers may have significant consequences.

It is important to emphasize that the potassium content of the denervated muscle fibers is normal (Hines and Knowlton, 1933, 1937). Lee (1939, 1940) has shown that in certain experimental conditions the sensitivity of skeletal muscles to acetylcholine varies inversely as the concentration of potassium within the fibers. From this evidence he has proposed that the supersensitivity of denervated muscles is due to a loss of potassium. Again, as already mentioned (see p. 161), Magladery and Solandt (1942) have suggested that fibrillary activity may result from a persistent action of potassium. Hines and Knowlton's observations do not favor these hypotheses.

Notwithstanding the fact that potassium is found in normal concentration in denervated muscles, these muscles are more permeable to the ion than normally. Thus, Noonan, Fenn, and Haege (1941) have reported that the rate of penetration of radioactive potassium into rat muscles, in one hour, is increased as much as five-fold by stimulation and about two-fold by denervation (i.e., acute section of the sciatic nerve). With due caution, they have emphasized, however, that these rapid rates of penetration may be due exclusively to circulatory changes. Not only is it important to control the circulation in experiments of this type; it is possible that denervation may also render the walls of the capillaries more readily permeable. Thus, in 1926, Gabbe showed, in guinea-pigs, that section of the sympathetic supply makes the capillaries in striated muscle more permeable, as indicated by the readier passage of colloidal dyes through their walls when compared with the control non-operated side.

In the experiments of Lyman, in 1942, the disturbing influence of modified circulation was ruled out as follows. In rats under nembutal anesthesia the dorsal and ventral roots of the lumbar nerves innervating the gastrocnemius

muscle were severed on one side, without injuring the sympathetic nerves. Electrical stimulation of the sciatic proved that the vasoconstrictor fibers remained intact. In testing the permeability of the normal and denervated gastrocnemii to radioactive potassium the animals were anesthetized with nembutal and both hind legs were skinned to above the knee-joint. The skin was then replaced so that the muscles were covered. Then 1 or 2 cc. of a 1.3 per cent solution of sodium-free radioactive potassium chloride was injected intraperitoneally and, after a given period (varying from 2 to 9 minutes), both hind legs were removed above the knee. The gastrocnemii were quickly dissected out and frozen in solid carbon dioxide. In the frozen state each muscle was cut into small pieces and ground with sand. The proteins were precipitated with trichloroacetic acid and the mixture was filtered. The filtrate was evaporated and the radioactivity was measured with an electroscope. Although there was great variation in the amount of potassium taken up by the muscles and also a large variation in the proportion of radioactivity in each pair of muscles within the short period after the injection, the denervated gastrocnemii held, on the average, more than twice as much radioactive potassium as the normal muscles. This larger penetration on the denervated side can be explained as due to increased permeability of the cell membrane.

The experiments of Friedlander, Perlman, and Chaikoff (1941) may also be mentioned. They studied in rats the rate of penetration of radioactive phosphorus as affected by denervation. A striking increase of the capacity to deposit the labeled phospholipid occurred 60 hours after denervation—more than 100 per cent in 10 of 12 animals—in the denervated as compared with the normal muscles. After a longer interval—108 to 336 hours after denervation—the phospholipid activity of the muscles, measured 12 hours after administering the radioactive phosphorus, was increased to as much as 630 per cent, and in terms of increase per gram of muscle it was as high as 750 per cent. The

change in the capacity of the denervated muscles to deposit the labeled phospholipid was demonstrable before appreciable muscular atrophy and was present as long as 19 days after section of the nerve. Although no precautions were taken in these experiments to maintain a normal circulation, the results are consistent with the inference that denervation increases the permeability of striated muscle.

In 1941 Marrazzi and Marrazzi attempted the test of the permeability of the nictitating membrane by measuring its electrical impedance to alternating current. The theoretical basis for this procedure lies in the justified assumption that currents are conveyed by ions in tissues and that the less the number of effective barriers for the migration of ions the less the resistance of the tissue to the passage of current. Accordingly, Marrazzi and Marrazzi dissected and excised from cats the two nictitating membranes, one normal, the other previously denervated by removal of the superior cervical ganglion. The tissues were placed in a moist chamber immersed in a constant temperature bath and the transverse impedance was measured for alternating current of 1,000 cycles per second, with a Wheatstone bridge. The conductance of the denervated tissue was not found consistently higher nor lower than that of the controls in the same animals. Marrazzi and Marrazzi inferred that impedance measurements do not support the concept of the increased ionic permeability of the denervated smooth muscle of the membrane.

This inference is not justified by the observations made. Acheson (1938) showed that the smooth muscle which is associated with and retracts the nictitating membrane of the cat is arranged in two sheets less than one millimeter thick: the inferior and the medial smooth muscles. These sheets of muscle are embedded in the deep layer of fascia of the orbit. Marrazzi and Marrazzi did not state what part of the membrane they studied. Their dissection, however, necessarily included in the excised tissue many other cells than the smooth muscle, since this muscle is less than one milli-

meter thick. It is clear therefore that they measured the transverse impedance of a tissue composed of only a small per cent of smooth muscle and a large per cent of other elements, mainly connective tissue. Their evidence may be dismissed, consequently, as irrelevant to the problem on hand.

Another result which may reasonably be expected if cell membranes are made more permeable by denervation is a faster penetration of dissolved substances into the interior. This phenomenon can best be looked for in cells where the reacting process is slow, ideally in chromatophores. The evidence is clear that the response of denervated chromatophores to chemical stimuli is much more rapid than the response of normally innervated neighboring chromatophores. Smith (1941) observed that adrenaline causes a concentration of the pigment of denervated melanophores at a rate approximately twice that of the normal (see Figure 22). The observations of Parker (1942) on the effects of intermedine are consistent with the results obtained by Smith. When Parker injected a solution of intermedine into catfish with an intermediate tint, he found that a small dose caused the bands of denervated melanophores to darken more rapidly than those on either side that were normally innervated. Later both the cut bands and the rest of the tails were equally dark. If the assumption is granted that the movement of the pigment depends upon the arrival of the effective chemical agents, then the more rapid changes would naturally result from a more rapid entrance of the agent through the cell wall.

The larger penetration of substances into denervated than into normal cells, the more rapid effects of chemical agents on denervated than on normal elements, and the efficacy of doses of chemical substances smaller than the usual on cells which have been denervated, all are consistent with the theory that deprivation of nerve impulses increases the ease of passage of substances through cell walls. It is important to note, however, that increased permeability does

not account for all the phenomena of supersensitivity. To mention only one instance, the greater than normal responses to acetylcholine of denervated striated muscles and sympathetic ganglia cannot reasonably be attributed to increased permeability, for the effects of this agent on these structures, in setting up propagated impulses, are probably exerted on the surface; they do not require penetration. The final conclusions emerge that supersensitivity evoked by denervation involves probably a plurality of causes and a plurality of mechanisms, and that both the causes and the mechanisms may differ in different structures and with the different modes of denervation: ultimate, penultimate, or even more distant from the cells considered.

## CHAPTER XXII

### SOME IMPLICATIONS OF THE LAW OF DENERVATION

It was pointed out in Chapter IV that it is preferable to sever preganglionic rather than postganglionic fibers when disconnecting an autonomic effector from the central nervous system in human surgery. This is an important practical corollary of the law of denervation. In the present chapter will be considered other interesting implications of the law, more theoretical in nature and scope.

*The Functional Conservation of Isolated Organs.* The development of supersensitivity to the action of circulating chemical agents in organs disconnected from the nervous system by section of their nerve supply has obvious important conservative consequences. Although these organs no longer receive the nerve impulses that elicit their activity in normal circumstances, they may still respond in certain conditions and thus share in some of the general reactions of the organism. This vicarious chemical control is especially important and significant in tissues which receive a sympathetic adrenergic nerve supply. As pointed out by Cannon (1928, 1930), the sympathetic nervous system is organized so that it can elicit widespread activity in the body in conditions of stress. Thus, during asphyxia, exposure to cold, hemorrhage, hypoglycemia, and emotional excitement, there is hyperactivity of this division of the autonomic nervous system. This hyperactivity not only includes the arrival of nerve impulses over adrenergic nerve fibers to smooth muscles and glands throughout the organism, but involves also the delivery of impulses, via the splanchnic nerves, to the adrenal medullae, and hence it causes the

secretion of adrenaline into the blood stream. In normal conditions, this output of adrenaline reinforces the effects of nerve impulses from the postganglionic adrenergic fibers on the sundry effectors controlled by the sympathetic nervous system. When the postganglionic sympathetic fibers to a given structure have been severed, or when merely the connections of the corresponding sympathetic ganglia with the central nervous system have been interrupted, the denervated or decentralized structures will no longer receive nerve impulses during the several emergencies mentioned above; they will, however, be exposed to the action of the adrenaline present in the blood stream in those circumstances, and since these structures will have become sensitized to the influence of the hormone, as a consequence of their previous denervation, they will respond much as they would have if their nervous control had been intact. Indeed, as was emphasized on p. 44, their reactions may be undesirably greater than normal, because of their supersensitivity.

While denervated effectors can thus participate in general organismic reactions when the interrupted nerve pathway includes ultimate adrenergic neurons, it is improbable in most cases that effectors controlled by cholinergic ultimate neurons can similarly share in adaptive activity. As was stressed repeatedly, acetylcholine is as a rule promptly hydrolyzed in mammalian blood and tissues, and is in consequence rapidly inactivated. The acetylcholine which may diffuse into the blood when cholinergic fibers become active elsewhere in the body will therefore usually not reach denervated structures in its active form. Two exceptions were noted to this general statement in foregoing chapters. The Philipeaux-Vulpian phenomenon and the other phenomena related to it were attributed to a diffusion of acetylcholine from the neighboring blood vessels to the denervated striated muscle fibers; thus somatic effectors may become active by devious nervous influences even though deprived of their direct nervous control. Again, the

experiments of Bender, cited on p. 100, bear testimony to the possibility that denervated striated muscles may share in some general organismic reactions in some species because of acetylcholine released into the blood stream by innervated structures.

*Spinal Shock.* The observations of Cannon, Rosenblueth, and García Ramos on the results of spinal semisections, illustrated in Figures 50 to 55, have a bearing on the problem of spinal shock. The expression "spinal shock" is not merely descriptive, but implies an interpretation. The term "shock" suggests that the section of the spinal cord actively depresses or inhibits the segments of the cord caudad to the cut level. Most of the theories which have been proposed to account for or explain spinal shock have been influenced by this tacit suggestion, i.e., depression or inhibition has been assumed to develop as a consequence of the section of the cord. As Sherrington pointed out in 1906, however, the search for the depressing factor has been barren. Harreveld (1940) has recently proposed a variant to the theories which attribute shock to an inhibitory process. He suggests that there is in the cord an "inhibitory spinal mechanism" which is normally checked by influences from higher centers. Spinal section would eliminate these higher checking influences and the intrinsic inhibitory mechanism would become ostensible. Harreveld based his hypothesis on the fact that asphyxia increases the reflex activity of the spinal cord. He assumed that asphyxia should exert a depressing influence on nervous activities; the appearance of increased reflexes would then denote the suppression by asphyxia of an inhibitory system. There is no *a priori* reason, however, for the assumption that asphyxia can only depress nervous activities; and even if that assumption were granted, the increased reflexes might be due to a greater depressing action of asphyxia on the inhibitory effects of afferent nerve impulses than on their excitatory synaptic influences. It is pertinent to recall that Sherrington's careful observations and analysis led him to discard inhibition as a factor important in shock.

Phenomenologically, the spinal shock produced by an experimental complete transection of the cord develops as follows. After the initial stimulating effects induced by the excitation of nerve tracts consequent to the trauma have subsided, fewer and weaker reflexes than normal can be elicited from the isolated segments. Thereafter a gradual increase of reflex activity is seen, with a time course and a final result that vary considerably in different species. This gradual increase of reflex activity is usually designated by the word "recovery," a term which again implies an interpretation, for it suggests tacitly that the increase of activity is a restoration toward the normal conditions. The higher in the phylogenetic scale the animal, the fewer the reflexes obtained shortly after the transection, the slower the gradual subsequent increase of reflex activity, and the lower the degree of reflex reactions which obtains in the final steady conditions.

An entirely different interpretation of the phenomenon from that implied by the term shock is possible and legitimate. It may be assumed that the segments of the cord isolated from the higher centers by the section are in a normal condition a few seconds or minutes after making the cut. If the reflexes obtainable at that time are not as intense or abundant as those seen before transection, the deficiency is due to the suppression of facilitating higher influences. In other words, in an intact animal the reactions considered as spinal reflexes involve the activity of neurons not only in the spinal cord but also in other higher centers. After a transection these reactions are restricted exclusively to elements in the cord. This interpretation is similar to that suggested by Sherrington and his collaborators (see Sherrington, 1898 and 1906; Creed *et al.*, 1938). The fact that the degree of reflex activity obtainable after transection decreases as the phylogenetic scale is ascended is in agreement with all the data grouped together under the expression "principle of encephalization." In higher animals more and more functions mediated in lower forms mainly or exclusively by lower centers become more and more

encephalized, i.e., the control of the higher centers over these functions becomes increasingly predominant. In man, where encephalization is supreme, the number of spinal reflexes elicitable after spinal transection is minimal; this paucity of reflex activity reveals that in normal humans the so-called spinal reflexes are only secondarily spinal; they require facilitation from neurons in other higher regions of the nervous system.

If this interpretation of the initial scarcity of reflex responses from isolated regions of the cord is accepted, the question arises of the mechanism of the subsequent gradual enrichment of the reflex activity. The law of denervation offers a plausible explanation for this gradual improvement. Transection of the cord leads to the degeneration of all the long descending pathways. This degeneration denerves partially or totally many spinal neurons. Supersensitivity to the effects of nervous impulses will not only develop in these denervated nervous elements, but may also become manifest in other cells, normally innervated by the now denervated neurons. This sensitization leads to progressively greater efficacy of the afferent nerve impulses in conveying the direct or indirect, positive or negative influences which they may exert on the motoneurons. In other words, much as a partial denervation of the cellular elements in the superior cervical ganglion brings about supersensitivity and consequently renders liminal the effects of preganglionic fibers which are normally subliminal, similarly in the spinal cord partial denervation may render liminal some effects of afferent nerve impulses which are normally subliminal and which only elicit efferent nerve impulses in the normal conditions when facilitated by higher nervous influences.

According to this theory so-called spinal shock does not exist *qua* shock. Spinal section does not depress the aboral segments for any significant period of time; it isolates these segments but does not alter their excitability. So-called restoration of spinal function *qua* restoration is again nonexistent. The isolated segments do not recover toward the

normal conditions but become abnormally hyperexcitable. The reason for this hyperexcitability is the denervation of some spinal neurons.

*The Problem of Central Reorganization after Nervous Lesions.* The phenomenon to be considered under this heading may be described schematically as follows. Damage of the central nervous system leads to a deficit of function. This deficit is maximal immediately or shortly after the damage has occurred. Thereafter, there is often a gradual improvement of the impaired function or functions. This schematic statement is worded deliberately with the purpose of emphasizing the phenomenological similarity of the present contingency with spinal shock. As in the case of shock, the main problem to be discussed is the improvement of function which follows damage.

Many theories have been proposed to account for this improvement. The theories may be grouped under two main headings: (a) those which attribute the recovery to "plasticity" of the nervous system, i.e., to almost unrestricted interchangeability of nervous pathways and influences; (b) those which explain improvement or recovery as due to a process of learning or relearning, i.e., to the functional opening of new vicarious pathways determined by training. These statements about the theories in question are broad and vague, but no broader or more vague than the theories themselves.

The complexity of the central nervous system is such that it is likely that no single explanation may cover all the facts designated by the expression central nervous reorganization. The discussion will be limited, therefore, to the recovery of function as it has been observed in a specific instance, namely, after a spinal semisection in mammals. Immediately after the semisection there is a gross impairment of function in the cat. This impairment affects primarily posture and locomotion. When the animal attempts to walk, both hind legs, and especially that homonymous to the semisection, do not share appropriately in the coöordinated

activity necessary for walking. The leg on the operated side is usually held in extension, dragging at times, or else is moved excessively forward, but it never supports or propels as normally. The postural deficiencies are obvious whether the animal be lying or sitting. The leg on the semisectioned side more often than not is in awkward abnormal positions. Other deficiencies may be noted: the placing and hopping reactions are absent on the operated side; while the animal can scratch with the hind limb on the non-operated side it does not scratch with the opposite leg; the flexor reflex, although operative if the foot on the cut side is pinched or stimulated electrically, does not have its normal protective consequences—that foot and leg may develop cuts, abrasions, edema, infections, etc., because that limb is not appropriately withdrawn in response to noxious stimulation.

As time elapses after the operation, there is a gradual remarkable attenuation of the deficiencies mentioned. This gradual change has been repeatedly studied. Good reviews of the early observations may be found in Schiff's (1858-59) and in Mott's (1892) reports. Weiss (1879) observed that three to four weeks after spinal semisection, a dog could run so effectively that it was difficult to perceive which side had been sectioned. Similar results have been published by Marshall (1895) for the cat and by Mott (1892) and Schäfer (1899) for the monkey. Osawa (1882) and Mott agree with Schiff's conclusion that not only does motility become greatly restored with time but sensations are significantly recovered.

Osawa made other important observations, as follows. In dogs several weeks after an initial semisection a second semisection was performed on the opposite side, two or three segments away from the first one. Even after this severe injury a dog could walk a little eight days after the second operation, and six weeks later it could run without difficulty and jump into its cage at the height of 85 cm. It could attempt to remove clamps which had been attached to one

of its hind legs, thus showing an ability to localize sensory stimuli. In a few instances, Osawa made a third section of the cord. Paralysis appeared anew, but one of the operated animals showed astonishing recovery. After six weeks it could stand on all four legs. Two weeks later it could walk a little. Danitch (1924) performed, in rabbits, experiments similar to those of Osawa. After the first semisection a Brown-Séquard syndrome developed, and disappeared in 2 to 4 days. The second semisection produced the same effects on the opposite side; the recovery was slower—about 10 days. After the third semisection the animals behaved at first as if completely transected; a marked recovery was apparent, however, after 16 to 18 days had elapsed.

In commenting on these observations, Bethe and Fischer (1931) reached the conclusion that in vertebrates no section of central tracts leads to a lasting cessation of function; as long as indirect connections remain between separated parts, the damage is repaired, generally in such a way as to produce a "purposeful coördination." This conclusion is not justified by the data. Thus, although Osawa reported a considerable restoration of sensation, Sherrington (1900) points out that after two semisections even strong faradization of the sciatic nerves fails to elicit any pain signs from the animals. Again, Osawa claimed that rubbing the skin on either side of the neck above the spinal sections in his operated dogs elicited scratching by the corresponding hind leg. Sherrington (1900; see also Sherrington and Laslett, 1903), however, could not confirm this observation in a series of carefully studied dogs. He could only see the scratch reflex on the operated side when the stimuli moved the skin below the level of the semisection; indeed, in those circumstances scratch reflexes could be obtained in animals with a complete transection of the cord. He concluded that the scratch reflex is strictly ipsilateral. Results similar to those of Sherrington were obtained in cats by Mándoki and Obrador in 1947. Here, then, is a function lost irretrievably after semisection of the cord. Finally, the respiratory

hemiplegia which results from a semisection between C<sub>1</sub> and C<sub>4</sub> only disappears in some species in the corresponding hemidiaphragm if the opposite phrenic is blocked or cut, and never is canceled in the intercostal muscles (see p. 170). The degree of recovery of posture and motility after semi-sections is considerable, however, and challenges an explanation.

In an attempt to gain some insight into the mechanism of the recovery, Mándoki and Obrador studied several reactions of chronically semisected cats, comparing them with those found in acutely semisected animals. The placing and hopping reactions, absent on the side of the cut in the acute controls, progressively reappeared in the chronic preparations, but even 5 or 6 weeks after the operation they were sluggish and abnormal, especially the placing reaction; this deficiency could be noted even when walking had improved vastly. Intersegmentary reflexes of the hind legs upon stimulation of afferent nerves in the front limbs, tested after decerebration, were approximately alike in the chronically operated animals and in the acute controls. Decerebrate rigidity was present in the previously operated cats, in the limbs controlled by motor neurons on the same side below a high semisection, but it was also seen to develop 2 to 4 hours after an acute semisection. The tonic neck reflexes were not significantly different in the two groups of animals.

Neither in the acutely nor in the chronically semisected cats could Mándoki and Obrador obtain in the limb or limbs ipsilateral to the semisection any responses to stimulation of either motor cortex. These negative results in cats agree with Sherrington's (1900) statement that in animals with 2 or 3 semisections at different levels even strong faradization of the spinal cord in the cervical region elicits no movement in the hind limbs. In monkeys, on the other hand, Mott (1892) observed that strong cortical stimulation of the leg area contralateral to a previous spinal semisection occasionally caused movements of the limbs on either side

of the animal. In agreement with this report Mettler (1944) found that stimulation of the cortical areas 4 and 6 on the side contralateral to a chronic semisection elicits movements in the extremity or extremities of the side beneath the semisection, but not without concomitant movement of the companion limbs on the uncut side.

The observations of Mándoki and Obrador support the view that restoration of function is not due to indiscriminate "plasticity" of nerve connections but that it denotes the functional opening of preëxisting specific pathways. It may be assumed that if coördinated locomotion is recovered by an animal with a spinal semisection, this recovery is due to the existence of appropriate devious nervous connections, even though in normal circumstances these detours are not employed and only the direct routes come into play. Conversely, it may be assumed that the non-reappearance of the scratch reflex or of the respiratory activity of the intercostal muscles, after spinal semisection at the corresponding levels, is due to the absence of the appropriate specific detours.

The main problem of recovery of function after injury to half a spinal segment is then that of the mechanism by which specific normally non-functioning nervous connections become functional in the course of time. Three instances are known of functional opening of new pathways in the central nervous system, and two of these have been already discussed in foregoing pages. These instances are: (a) the crossed phrenic phenomenon; (b) the establishment of conditioned reflexes; and (c) the conversion of normally subliminal connections into a liminal pathway because of supersensitivity consequent to denervation.

The opening of the crossed respiratory spinal pathway is prompt (see Figures 57 and 59) and the effectiveness of the crossed impulses is not significantly modified with time if a phrenic nerve has been cut (see p. 176). It does not appear probable, therefore, that the obscure mechanism which determines the opening of the crossed respiratory route

should play any part in the phenomena of readjustment and reorganization of the central nervous system in the case under consideration. Incidentally, it may be repeated here that the crossed phrenic phenomenon does not take place in all mammals; its absence in some species does not support the concept of indiscriminate plasticity but reinforces the hypothesis of the specificity of the pathways involved in specific functions.

The possibility of establishing conditioned reflexes mediated primarily or exclusively by the spinal cord has not been explored. Few responses can be conditioned in the absence of the cerebral cortex (see Pavlov, 1927; Culler and Mettler, 1934). It is improbable, therefore, that learning, i.e., conditioning, is an important factor in the recovery of motility of semisected animals.

Since spinal semisection has been shown to lead to the supersensitivity of the partially denervated neurons to the influence of impinging nerve impulses, it appears likely that this supersensitivity is one of the important mechanisms for the opening of the vicarious pathways indicated by the restoration of function in the chronically semisected animals, i.e., that it is an important factor in so-called reorganization of the central nervous system after injuries.

*The Problem of "Irritative Foci" in the Central Nervous System.* Neurological observers often encounter excessive or abnormal central nervous function which originates at a localizable site and which they attribute to an "irritative" focus. Jacksonian epilepsy, as a specific example, is usually attributed to the hyperirritability of some cortical cells, caused by the existence of a localized injury to the cortex. This interpretation is corroborated by the finding of the injured region when the cortex is exposed upon operation and by the disappearance of the symptoms when that region is removed. Jackson (1931) attributed the epileptic seizures to an abrupt and excessive local discharge from some highly unstable part of the cerebral hemisphere. He emphasized

that the unstable elements should be in the boundaries of the damaged region.

Observations on the tonic-clonic responses of the cerebral cortex in monkeys, i.e., on experimental epilepsy, carried out by Rosenblueth and Cannon in 1942 are in agreement with Jackson's views. The tonic-clonic responses seen in the animal experiments denote physiological activity in a normal cortex elicited by an abnormal degree of stimulation (electrical in these cases). Normal stimuli in physiological conditions might become abnormally intense if the "threshold" of some cortical elements were abnormally low. This statement is a rewording in modern terminology of Jackson's hypothesis with regard to the source of the epileptic seizures which bear his name. The question arises as to how or why a localized injury to the cerebral cortex leads to the development of an unusually high excitability in neighboring nervous elements.

It is not improbable, as Cannon (1939) suggested, that tumors and other lesions of the motor area of the cortex may induce instability of the surrounding cortical cells by destroying their connections with other cortical elements, that is, by denervating them partially, and consequently rendering them supersensitive to the influences of chemical agents or of nerve impulses in the axons which still innervate them. This hypothesis does not account for the cessation of the epileptic seizures when the damaged cortical area is surgically removed. Nevertheless, it is plausible that other clinical manifestations attributed to irritative foci may be produced by this mechanism. The applicability of the law of denervation to these instances will be decided by future experiments.



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