CONTENTS

CHARLES WILSON GREENE
1866 - 1947

Dr. Greene had a long and distinguished career in American physiology. He was born in Mill Town, Indiana, August 12, 1866. He attended De Pauw University for a short time then received his AB from Stanford where he was a member of the first graduating class in 1892 and received his AM in 1893. He received his Ph.D. degree from Hopkins in 1898. He was instructor in physiology at Stanford from 1893 to 1898 and assistant professor from 1898 to 1900. He became professor of physiology and pharmacology at the School of Medicine, University of Missouri where he organized the physiological department and established a laboratory of experimental pharmacology, the first in the Mississippi Valley. He served as professor from 1900 to 1936 and became emeritus professor in 1937. He was instructor at the Marine Biological Laboratory, Woods Hole in 1896, 1897 and 1900. He was special investigator, Bureau of Fisheries in 1901, 1902, 1904 and 1906 to 1917. He was a major in the Sanitary Corps of the Army in World War I working in aviation medicine. He edited Kirke's Handbook of Physiology in 1922 and a pharmacology handbook in 1914.

He became a member of the American Physiological Society in 1899, became secretary in 1916 and for nine years continued to serve in that capacity. It was a period of expansion and growth and the duties and responsibilities of the secretary increased each year. He met all the situations with tact and good judgment. Many of the secretarial devices which now insure the smooth operation of a large organization were introduced by him. For his carefully edited and bound volumes of the Council and Society minutes he received a special vote of thanks. In 1934-35 he served as the fourteenth president of the Society. Throughout his long connection with the Society Dr. Greene was constantly relied upon by the Council and officers for advice and helped initiate and carry out all kinds of administrative policies. He prepared the second part of the Society's Semi-Centennial History.

As an investigator for the Bureau of Fisheries he conducted and published numerous field studies on the physiology of the Pacific salmon. During World War I he studied the physiological problems of altitude responses in aviation. Out of this assignment grew a number of scientific papers on anoxia. He also published many papers on cardiovascular physiology - cardiac nerve reactions of the coronary blood vessels; influence of inorganic salts on cardiac tissues; pharmacological reactions of the mammalian heart; changes in the human heart in oxygen want. Dr. Greene's interests in physiology were wide and varied. They included studies on the respiratory gas percentages in anesthesia; reaction of organs at the asphyxial crisis; chemical composition of muscle tissue; storage of proteins in the body; chemistry of animal tissues in inanition.

Material from the History of the American Physiological Society and other sources supplied by Dr. J. O. Davis, current Professor of Physiology at Missouri.

After his retirement from teaching in 1936 Dr. Greene made many contributions to community life through activities in social and humanitarian organizations in Columbia and the state of Missouri. It was through a program which he inspired and initiated that hot lunches were served in Columbia schools and summer playgrounds were opened. Dr. Greene did outstanding work in nutrition and relief studies in Columbia for many years through his work in the Kiwanis Club.

Dr. Greene proposed and initiated the Medical School Foundation at Missouri. This Foundation made funds available to medical students. An oil painting of Dr. Greene, presented by his students, hangs in the library of the School of Medicine.

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ABSTRACTS FOR FEDERATION MEETING

The last couple of years the American Physiological Society has had the ruling of eliminating from oral presentation every n^{th} paper to reduce the number to approximately 850. Since the biochemists are not meeting with us in Chicago this year it leaves a few extra rooms. We received 900 abstracts this year so decided not to eliminate any from oral presentation. This large number means that there may be some unavoidable conflicts since there are more sessions on a particular general subject (such as cardiovascular physiology) than spaces in morning and afternoon times. There are also some sessions with dual titles, but every one will have an opportunity to give his paper.

There were a few abstracts received too late to be considered. Most of them were mailed too late, but two of them were mailed by ordinary mail in what appeared to be plenty of time but were evidently lost in the mail somewhere and not received at APS in Bethesda until after January 1. Approximately 500 abstracts were received on the deadline day, December 17. This, and the fact that some abstracts get lost when sent by ordinary mail, again emphasizes the fact that abstracts should be sent early and by airmail, special delivery.

TRAVEL TO MUNICH

The announcement in "The Physiologist" Vol. 13. No.4, pp. 357 and 358 brought numerous suggestions as to routings and duration of proposed trips to the XXVth International Congress of Physiological Sciences in Munich.

Heritage Travel, Inc. of Cambridge, Mass. offers as a result of these suggestions, the following travel alternatives:

Group NY-1	New York-Munich Munich-New York	July 15 August 2	$305 per Person
Group NY-2	New York-Munich Munich-New York	July 17 August 1	$335 per Person
Group NY-3	New York-Munich Munich-New York	July 22 August 12	$305 per Person
Group NY-4	New York-Munich Munich-New York	July 23 August 15	$335 per Person
Group B-1	Boston-Munich Munich-Boston	July 15 August 2	$298 per Person
Group B-2	Boston-Munich Munich-Boston	July 17 August 1	$328 per Person
Group B-3	Boston-Munich Munich-Boston	July 22 August 12	$298 per Person
Group B-4	Boston-Munich Munich-Boston	July 24 August 7	$328 per Person
Group CH-1	Chicago-Munich Munich-Chicago	July 22 August 12	$375 per Person
Group CH-2	Chicago-Munich Munich-Chicago	July 17 August 1	$405 per Person
Group LA-1	Los Angeles-Munich Munich-Los Angeles	July 22 August 12	$457 per Person
Group LA-2	Los Angeles-Munich Munich-Los Angeles	July 17 August 1	$487 per Person

Groups will travel on scheduled flights of Pan American World Airways and Lufthansa German Airlines, which is the official airline for the Munich Congress. All participants who will be assisted financially by US Government grants will travel on Pan American World Airways.

For those who wish to travel just to attend the Munich Congress, a CHARTER flight will be offered on a Boeing 707 of World Airlines, US

Flag Supplemental Carrier. The charter is scheduled to depart New York on Friday, July 23 and return to New York on Monday, August 2. The cost of participation will be in the vicinity of $225. In case the charter flight would not be filled, a special group on Pan American would be offered based on supplement up to the group cost of $305. This special group would depart on Thursday, July 22 and return on Monday, August 2.

You will find as a loose leaf enclosure an application form stating in details all the conditions. Please complete either the paragraph relating to group flights or the paragraph relating to charter flight and return to Heritage Travel.

Should any of the prospective participants in the Congress desire information about alternative methods of traveling to the Congress and being accommodated in Munich, please write to Heritage Travel which will willingly answer all individual queries.

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ENDOCRINOLOGY SYMPOSIUM

An international symposium on endocrine polypeptide hormones and their secreting cells will be held at the Royal Postgraduate Medical School in London, July 19-22, 1971. The program will include sessions on the chemistry of polypeptide hormones, hormones affecting bone (calcitonin, vitamin D and parathyroid hormone), and the function and structure of the endocrine polypeptide secreting cells. In addition there will be papers on cyclic AMP, new concepts (including the naturetic hormone), gut hormones, and APUD cell neoplasms. Further information can be obtained by writing Dr. Giraud V. Foster, Endocrinology 1971, Royal Postgraduate Medical School, Ducane Road, London W.12 England.

FIFTEENTH BOWDITCH LECTURE*

Crayfish Interneurons

DONALD KENNEDY
Department of Biological Sciences
Stanford University

By way of introduction, I want to acknowledge some historical debts. The rationale behind our work is simple: a way to understand information processing in the nervous system is to investigate the properties of networks formed by specific connections among identified neurons. To identify the neurons, one needs a restricted nervous system. C.Ladd Prosser first put the crayfish into such service in 1934, with a preamble which could hardly be improved on now: "The principal difficulty encountered in studying electrically the interaction between the central neurons is the confusion resulting from the large number of neurons in any given region of the central nervous system. Characteristic of invertebrate nervous systems is their more diffuse nature and the fact that each ganglionic center contains relatively few cells, when compared, for example, with one segment of the spinal cord of the cat."

Prosser demonstrated (1935) that afferent impulses in large tactile fibers excited interneurons in abdominal ganglia with short delay, and he made a good beginning at charting the central pathways of those interneurons. In a later, equally pioneering effort, C. A. G. Wiersma and his colleagues (Wiersma, 1958; Wiersma and Hughes, 1961; Wiersma and Bush, 1964) identified over one hundred elements that could be isolated repeatedly from specific regions of the central nervous system and responded to the natural stimulation of special and unique fields in the periphery. These remarkable studies clearly established that each interneuron made a highly specific and unique set of central connections with afferent and other elements, and marked the birth of the "identified cell" concept.

A third background element is the discovery that certain interneurons can release temporally and spatially patterned motor output of a highly stereotyped kind. That single cells could do this was suggested strongly by the studies of Wiersma's group (Hughes and Wiersma, 1960b; Wiersma and Ikeda, 1964) on the control of the rhythmic beating of crayfish swimmerets, and proven by single-cell stimulation in our laboratory for interneurons controlling other behaviors (Kennedy, Evoy and Hanawalt, 1966; Larimer and Kennedy, 1969). But such experiments might not have

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* Original research reported in this paper has been supported by grants from the U.S, Public Health Service (NB-02944) and the U.S. Air Force Office of Scientific Research (OSR68-1373). The author happily acknowledges the rewards and pleasures of collaborating with Drs. D. Mellon, K. Takeda, W. H. Evoy, H.L. Fields, A. I. Selverston, M. P. Remler, P.B. Stein, W. J. Davis and R. S. Zucker on various phases of the work described. He is also grateful for the expert technical help, critical advice and encouragement supplied by Joanna Hanawalt over the eight-year period in which these experiments were done.

been done - or believed - without the background provided by Wilson's (1960) demonstration that in at least one case arthropod locomotion was a central rhythm - in other words, derived its complex structure from the connectivity of central elements, not from phasic sensory feedback. This discovery had a profound effect in liberating us from the "reflex view" of these nervous systems. I know that my own thinking about the organization of motor output underwent a profound change as a result of Wilson's influence, which he exerted first through his work and then, more monosynaptically, as a Stanford colleague. Don Wilson's death this past June ended, suddenly and prematurely, a remarkably productive career. I hope you will agree that both the timing and the subject matter make it appropriate for me to dedicate this lecture to him.

In what follows, I shall discuss both the input - or "stimulus-filtering" - properties of crayfish interneurons, and the output - or "pattern-generating" - properties. Neurophysiologists who work with single units have, by and large, attacked the central nervous system either by following a sensory pathway down or by following a motor pathway up. I sometimes think of the central nervous system as a wilderness occupied by two groups of explorers. Each group knows where it is (or believes it does), but has no inkling whatever about the location of the other. It is believed that interesting territory lies between the watersheds each has been following; but since their paths into the wilderness have been so separate, neither group can locate the divide. If we found some levels at which we could deal simultaneously with both input and output properties, we might begin to recognize some of the features of the divide, and so to deal more explicitly with the problem of neuronal hierarchies. I propose to discuss some identified interneurons from this point of view, and to draw at the end a simple picture - incomplete in many important ways - of the hierarchical relationships among them.

First, it is clear that not all interneurons are situated equally close to the sensory and motor "sides" of the system. In Figure 1a, a cross section through a crayfish abdominal connective at segment 3, several identified axon profiles are shown. At various times A.I. Selverston and M.P. Remler in our laboratory have injected each of these cells with the fluorescent dye Procion Yellow, so that their pattern of branching can be reconstructed (Stretton and Kravitz, 1968; Remler, Selverston and Kennedy, 1968; Selverston and Kennedy, 1969). A brief account of their anatomy and physiology will provide a useful beginning.

The medial and lateral giant fibers (MG and LG) have clearly defined motor functions. Both excite flexor motor neurons in the abdomen (Wiersma, 1947), though they connect selectively and have distinctive behavioral actions (Larimer et al., unpublished). LG axons are segmental; they have a cell body and dendritic apparatus in each abdominal ganglion (see Figure 2) and the axons in adjacent connectives are electrically coupled to one another to provide a through-conducting pathway. The MG axons, by contrast, are continuous and unbranched except in the brain, where their cell bodies are located (Horiuchi, Hayashi and Takahashi, 1966). The connections of both axons with segmental phasic motor neurons are monosynaptic, and probably electrical (see below). The branches of the LG cells in abdominal ganglia probably mediate

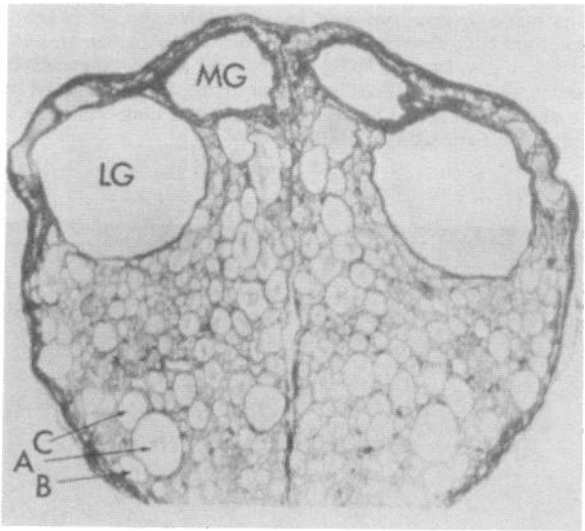

Fig.1. Cross section of the ventral nerve cord, just rostral to the fourth abdominal ganglion, stained with Masson's trichrome. The axon profiles labelled in the left connective are discussed in the text.

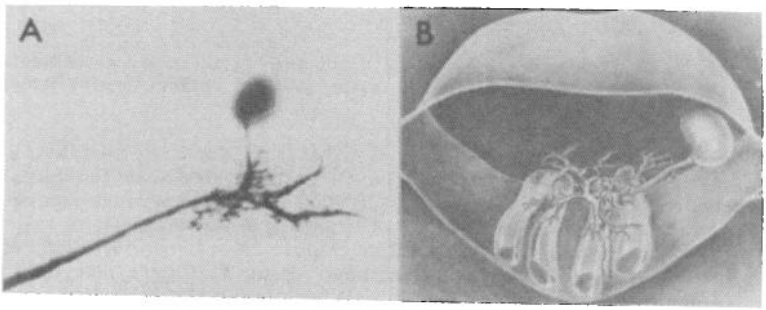

Fig.2. A. Flexor motor neuron F-3, photographed in the fluorescence microscope from a whole-mount in which the axon had been injected with Procion Yellow (Selverston and Kennedy, 1969). B. Reconstruction of F-3 and its connections with the giant fibers, made from serial sections by the method described in Selverston and Kennedy (1969).

input carried by segmental afferents (Krasne, 1969); but the output junctions made by both types of axons involve the unbranched axis cylinder (Kennedy, Selverston and Remler, 1969; for an extension of this principle see Davis, 1970). A typical set of junctions between giant axons and a fast flexor motor neuron, reconstructed by dye injection, is shown in Figure 3. Both LG and MG neurons have very high thresholds for natural stimulation.

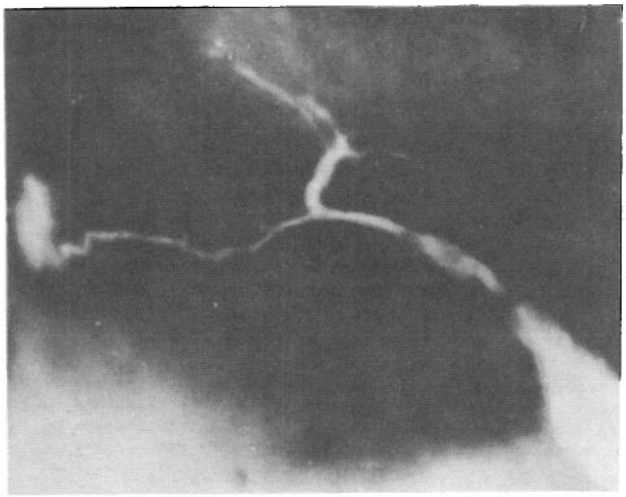

Fig.3. Whole-mount of the lateral giant axon in the third abdominal ganglion, injected with Procion Yellow (Remler, Selverston and Kennedy, 1968).

The interneurons labeled A, B and C in Figure 1, by contrast, are easily excited by stimulation of tactile hairs located along the dorsal abdominal segments. A, the largest non-giant neuron in the system, produces a fairly localized halo of branches in the neuropile of one half of the sixth abdominal ganglion (Figure 4a). It receives input only ipsilaterally, and only in that segment. B and C (Figure 4b, c, d) ramify in several ganglia; one has its cell body in the sixth ganglion, and that of the other has not yet been found. Both cells can be excited in several different segments, with the result that shifting sites of impulse origin - and collision of impulses - can be directly demonstrated (Hughes and Wiersma, 1960a; Kennedy and Mellon, 1964b). Neither A, B nor C produce any motor effect when stimulated.

The synaptic relationships of these identified cells will be discussed later. Some conclusion can already be reached, however, from the preliminary data already given. First, a crude form of hierarchical order

is already apparent: some cells are difficult to excite by sensory stimuli, but deliver powerful motor output when stimulated, whereas others respond well to natural stimulation but are behaviorally ineffectual when active by themselves. Second, a central neuron apparently needs to branch in order to receive input, but not to deliver output. Finally, in all the cases so far known (seven) the cell bodies of interneurons are located on the opposite side of the nervous system with respect to the position of their axons. The vertebrates, it would appear, were not the first bilaterally symmetrical animals to make central decussation a general principle.

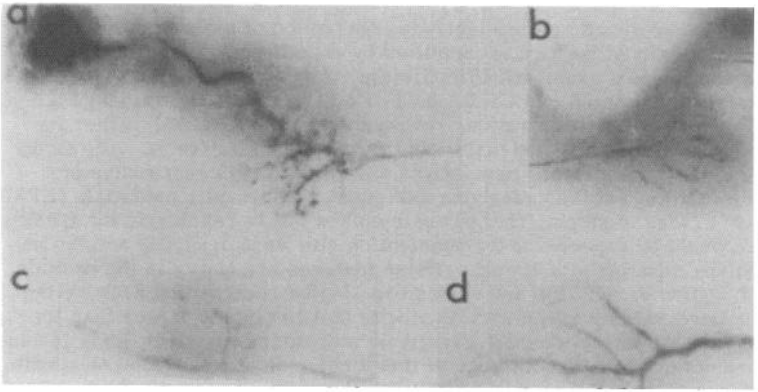

Fig.4. Whole-mounts of interneurons injected with Procion Yellow. A. Interneuron A, sixth abdominal ganglion (M.P. Remler); B. Interneuron B, sixth ganglion; C. Interneuron B, fifth ganglion; D. Interneuron C, sixth ganglion (A.I. Selverston).

The point concerning hierarchy can be expanded. In three different series of experiments, we have tried to correlate the input to central neurons with their motor effects. The most extensive of these involved cells active in producing postural abdominal movements (Kennedy and Hanawalt, unpublished; for a preliminary account see Kennedy, 1968). A shorter series concerned neurons activated by receptors in soft cuticle (Pabst and Kennedy, 1968), and in the most recent effort Davis and I have tested command fibers for swimmeret movement in the lobster. In each case, the aim was to find units that when stimulated singly would produce a defined motor output, and which could also be excited by natural stimulation. I shall describe our rather limited successes below; for present purposes, the failures are more significant. Whichever way one began, there was a low probability that a given neuron would do both things. The most effective command fibers seldom could be activated from the periphery; conversely, the units with clearly defined receptive fields and low thresholds for natural stimulation almost never produced motor output. Indeed, we now suspect that the exceptions

are interneurons that participate in rather short reflex chains resembling, in many ways, the vertebrate flexor reflexes.

In any event, these results support the view that there may be a number of levels of central elements in this system, some relatively near the motor side and others closer to the input end. From previous experiments, it had become clear that the multisegmental interneurons showed especially complex synaptic interactions (Kennedy and Mellon, 1964a, b): we hypothesized that interneurons receiving input in a single segment might turn out to have the lowest hierarchical position. For that reason we looked at interneuron A in some detail; the experiments were done in collaboration with Allen Selverston.

We have made microelectrode penetration of the dendritic areas of interneuron A; the cell is identified by repetitive antidromic stimulation of the isolated axon, and orthodromic volleys can be initiated by electrical stimulation of roots 1, 2, 3, 4 or 5 of the sixth abdominal ganglion. Typically, such penetrations reveal spikes of equivalent, rather low amplitude for anti- and orthodromic activation (Figure 5). The firing level for orthodromic impulses is variable for different routes of stimulation and the underlying excitatory postsynaptic potentials (EPSP's) are graded in steps. Orthodromic spikes fail to repolarize the EPSP's, as would be expected if the penetration site were dendritic and the impulse-initiating site axonal. These features are shown in the records of Figure 6. EPSP's and spikes can also be recorded by drawing the isolated axon up into a suction pipette that is slightly larger than the axon's diameter, near the ganglionic margin. Consistent firing levels are obtained with recordings of this kind, indicating that the electrode is recording subthreshold activity as though it were at the spike-initiating zone.

When natural stimuli are delivered to the tactile hairs on the ipsilateral telson and uropods, unitary EPSP's of a variety of sizes are produced in interneuron A; these may be analyzed by either of the recording methods described above. The size of unitary EPSP's covers about a tenfold range in most preparations. Usually, two or three of the largest ones would be sufficient to discharge A if they occurred simultaneously. When the cell is activated by electrical stimulation of a root, three or four increments of EPSP amplitude usually occur below spike threshold.

A more detailed analysis of the peripheral connections to A has been made by recording unitary EPSP's while monitoring afferent activity in a single nerve branch. For this purpose, we have used a semi-isolated preparation. The sixth abdominal and the 5-6 connective are left attached to the telson and uropod of one side by one or more roots; the appendages are pinned out dorsal side up and the nervous system rotated, so that the ventral side of the connective is available by dissection in transmitted light. A's axon is isolated for recording, and suction electrodes are placed proximally and distally on one of the roots so that afferent impulses may be identified and their conduction velocity measured. The receptive field of the root under study is explored carefully under the dissecting microscope; the position of each sensory hair is mapped, and the responses

to bending each one are recorded. Afterward, the axon of A is drawn into a suction electrode, and unitary EPSP's are recorded as the mapped hairs are stimulated in sequences. This procedure yields a receptive-field map that is quite different from those obtained by stimulating peripherally and recording impulse activity in a central neuron. The traditional map is a kind of approximation; it defines a center of activation and sets a border that is arbitrary, though functional. The maps we have made are, at least in theory, connection maps.

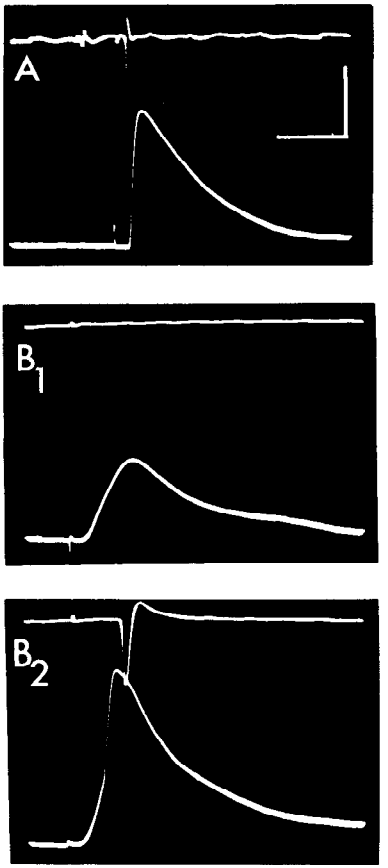

Fig.5. Responses recorded intracellularly in dendrite of interneuron A (lower traces). A. Antidromic stimulation; upper trace, electrode on 5-6 connective. B. Orthodromic stimulation; upper traces, electrode on axon of interneuron A in 4-5 connective. B_1, subthreshold volley in root 4 of sixth ganglion; B_2, suprathreshold. Calibrations: 10mV, 10 msec.

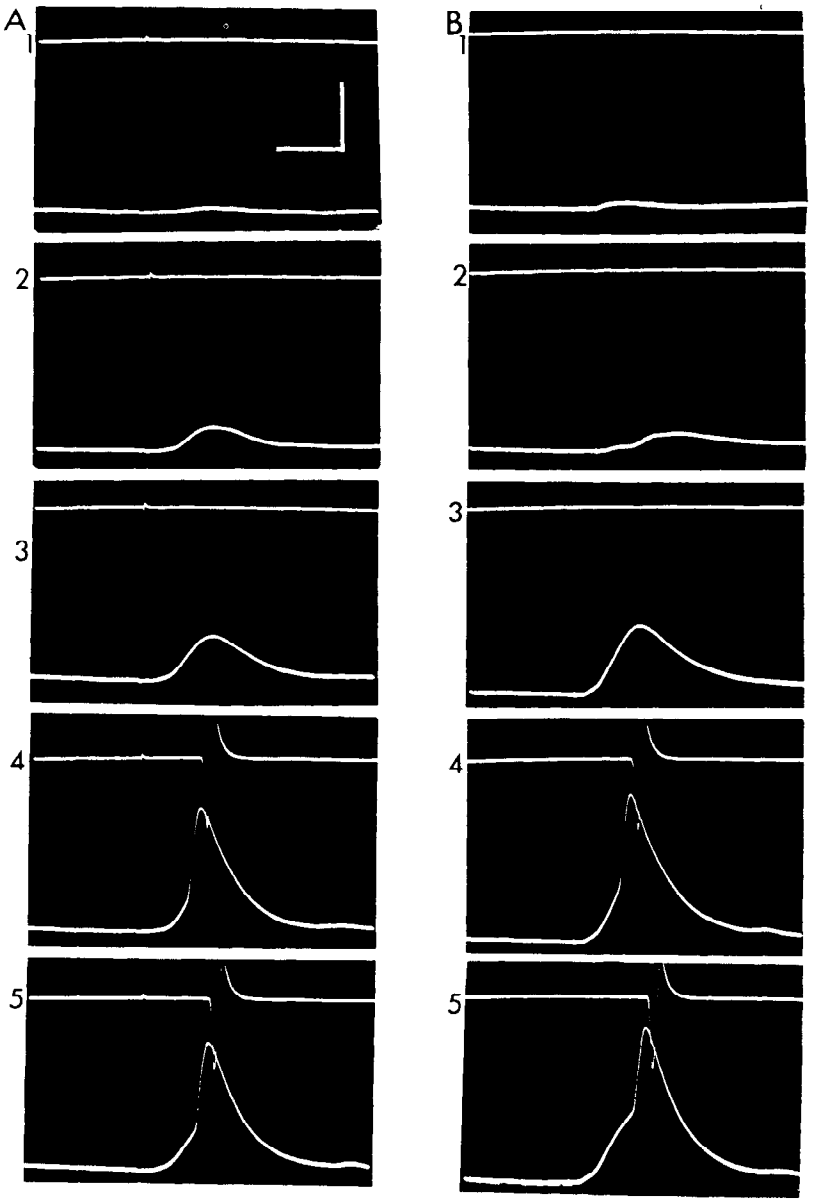

Fig.6. Responses recorded intracellularly in dendrite of interneuron A (lower traces) to stimulation of two different sixth-ganglion roots. The upper traces are extracellular records from the axon of A isolated in the 4-5 connective. The sweeps selected for both A and B series show all-or-none increments in the amplitude of EPSP's; note that the firing level in B is higher than that in A. Calibrations: 10 mV, 10 msec.

The distribution of sensory hairs within the fourth-root innervation field is shown in Figure 7. The map shows the proximal left telson of a single individual. The position of particular hairs varies, but the following features are quite general. Two to four anterior hairs, labeled "P" in the figure, are singly innervated by very large phasic axons. Proximal hairs in the S and T rows are similar, while members of the L and (sometimes) B groups are dually innervated, resembling the hairs on the branchiostegite described by Mellon (1963). Proximal hairs are innervated by the largest axons, distal ones by the smallest. Typically, the entire receptive field contains about 30 hairs, of which no more than a dozen produce unitary EPSP's in A.

What features govern the pattern of connection between these afferent fibers and the internauron? Do axons from certain hairs make rigorously specified connections with A? Is there any relationship between the location of a hair or the conduction velocity of its afferent axon and the efficacy of the central connection it makes? Figure 8 shows the results of an experiment in which the size of EPSP produced by afferents from given hair was mapped upon the receptive field. The largest filled circles represent hairs responsible for the upper third of the amplitude range for unitary EPSP's, middle-sized circles stand for the middle third, and small ones for the lowest third. Open circles indicate hairs that had identifiable afferents in the root, but no synaptic connection with A. This experiment, and a few others, are especially good ones in that axonal recordings could be made over a fairly long period from A, and even the smaller EPSP's were readily distinguishable. It is clear that some relatively distal, therefore slowly conducting, afferents can evoke large unitary EPSP's, and that effective and ineffective axons can have neighboring peripheral origins.

A receptive-field map composed from the results of eight experiments is given in Figure 9. Here the data have been mapped on an enlarged grid, the squares of which have been filled in proportion to the number of times they contain hairs with axons innervating the interneuron. Individual occurrences have been weighted for synaptic efficacy on a 3:2:1 basis, as in the preceding map. This is probably the fairest approximation of the extent to which receptors contribute to excitation on an area basis, though it is admittedly an approximation. The map shows that the faster-conducting, proximal and paramedial axons make the strongest contributions. It leaves open, however, the question of whether this contribution is due to a higher probability of connection or, instead, to a greater average synaptic efficacy. Figure 10 demonstrates that the latter is not the case. It plots conduction velocity of the afferent fibers in the eight experiments against the average size of the unitary EPSP's they produce; the fastest conduction velocity and the largest EPSP in each experiment have been given a value of 1.0 to normalize the results. No correlation whatever between axon diameter and synaptic efficacy can be discerned from the scatter-plot, and we must conclude that the size (or proximal origin) of a sensory neuron determines only its probability of connection.

Other differences between peripheral sites are seen from the records of Figure 8. EPSP's from neighboring hairs, even when they are of

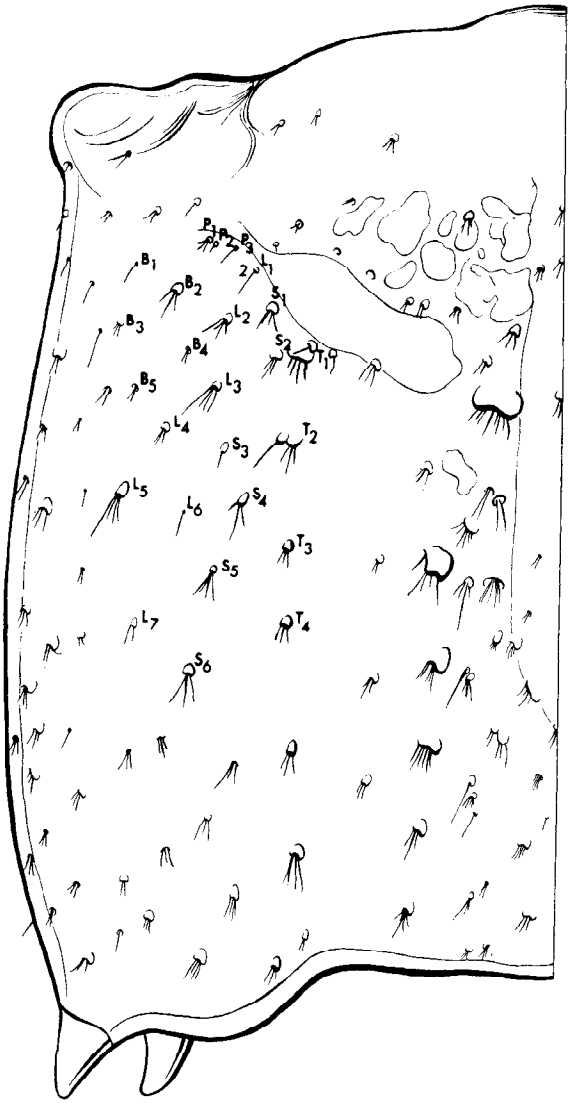

Fig.7. Map of the exoskeletal hairs on the left half of the telson. For discussion, see text.

approximately the same initial amplitude, may differ consistently in some temporal property. The third and fourth hairs on the right, for example, differ in that the EPSP's from the more proximal one show facilitation at short intervals, whereas those from the other exhibit mild antifacilitation. The uppermost hair on the left shows a much more dramatic antifacilitation, such that even at modest frequencies the fifth or sixth afferent impulse in a train produces a vanishingly small synaptic event in A. Such differences in recovery cycles provide a simple basis for changes in receptive field structure with repeated stimulation.

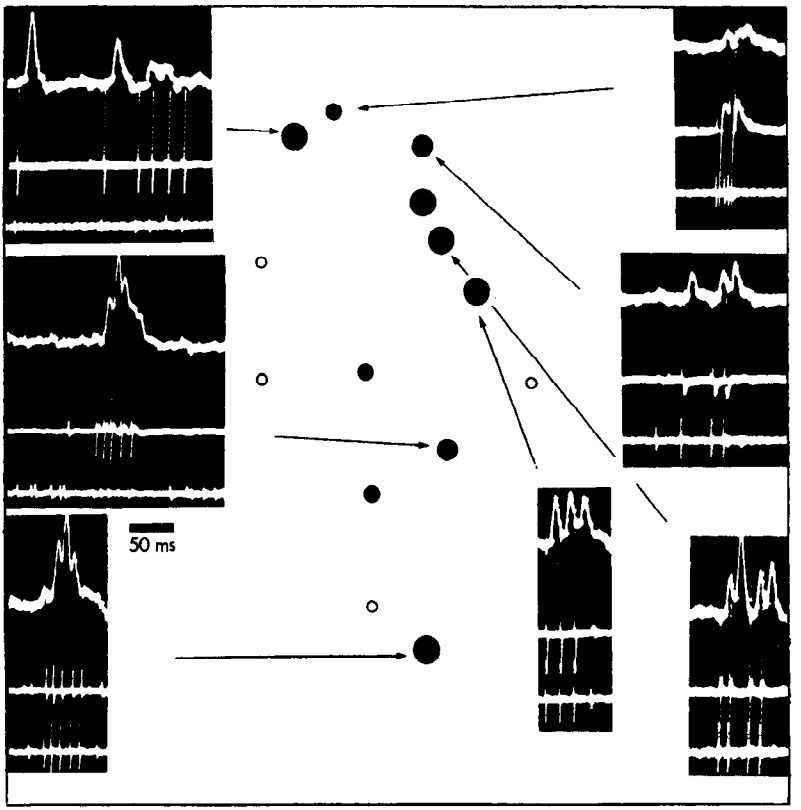

Fig.8. Responses recorded by a suction electrode from the axon of interneuron A near the margin of the sixth ganglion (see text), upper traces, to stimulation of single hairs mapped by the circles. The unitary EPSP's recorded in the upper traces can be associated with impulses in primary afferent fibers recorded by extracellular electrodes on the fourth root of the sixth ganglion; the middle trace in each record is from a proximal site, the bottom one from a distal site. Empty circles represent hairs that produced impulses in afferent fibers that were without effect in interneuron A. The sizes of the filled circles correspond to the relative amplitudes of unitary EPSP's produced by those hairs.

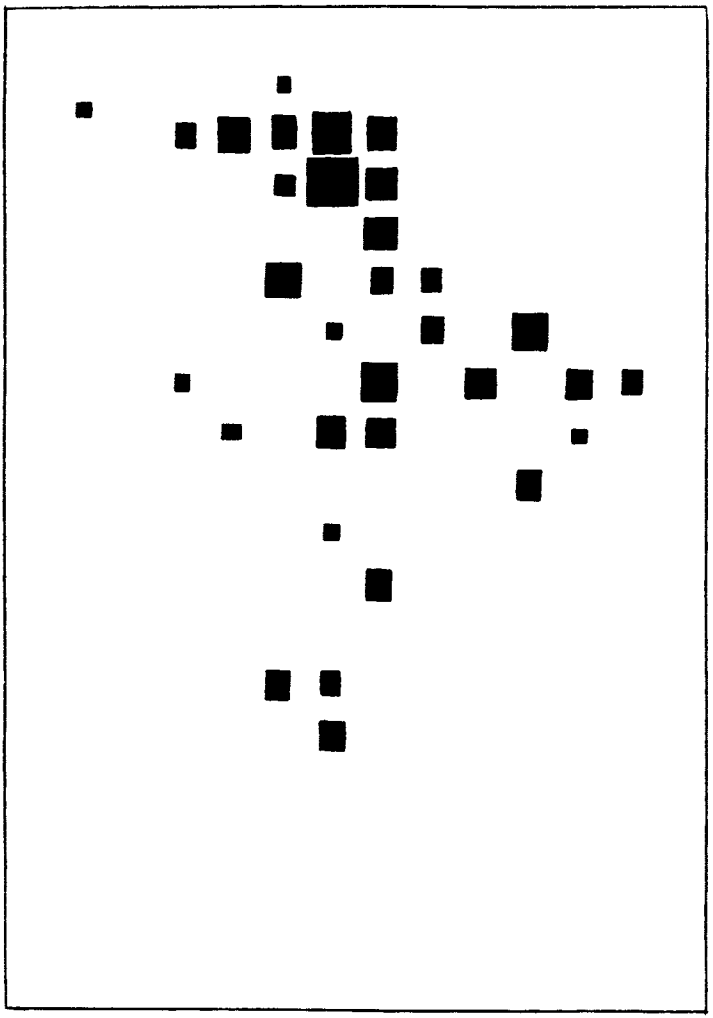

Fig.9. Receptive field map drawn from grids like the one shown in Fig.7. The size of the squares is proportional to the frequency of innervation of interneuron A by afferents from hairs falling within that grid square, and to the efficacy of the connection (see text).

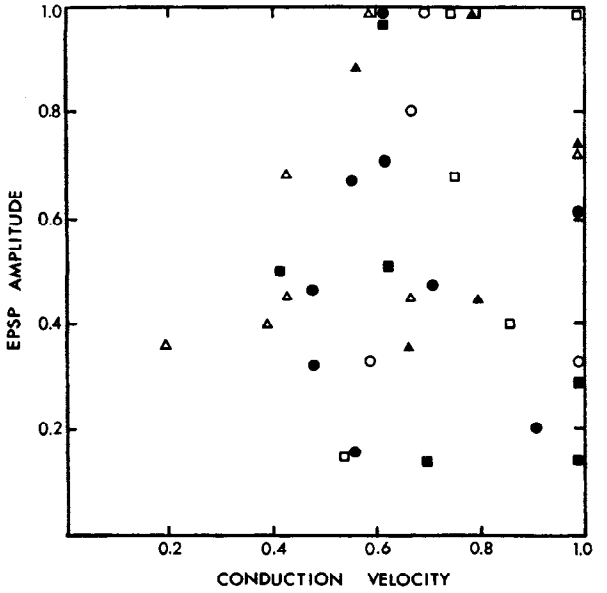

Fig.10. Scatterplot of the relation between conduction velocity of afferent fibers and the relative efficacy of their connections. For discussion, see text.

Interneuron A appears to be the simplest kind of primary central element. It receives exclusively excitatory input from a single class of receptor in a single segment; these terminate upon a globe of dendritic processes that apparently act only upon one axonal impulse-initiating site. The absence of inhibitory input and of complex spatial interactions already differentiates A from many other crayfish interneurons (Hughes and Wiersma, 1960; Kennedy and Mellon, 1964b). Because these differences suggested the possibility of hierarchical relationships between A and other interneurons (like B and C) having broader and more complex receptive fields including that of A, we have investigated interactions between them.

The multisegmental interneurons characteristically have branches in each of several ganglia, as shown in Figure 3b, c, d; not only do impulses arise separately at these different sites, but the latter interact in other ways as well. Even more important, stimuli delivered to a given root produce synaptic excitation in adjacent ganglia. For example, depolarizing after-potentials following a spike initiated in one place affect the excitability of distant synaptic sites. There is a pathway of unknown kind that distributes excitation up and down the cord to affect the same interneuron in nearby segments. These two findings (Kennedy and Mellon, 1964) are demonstrated for interneuron B in Figure 11.

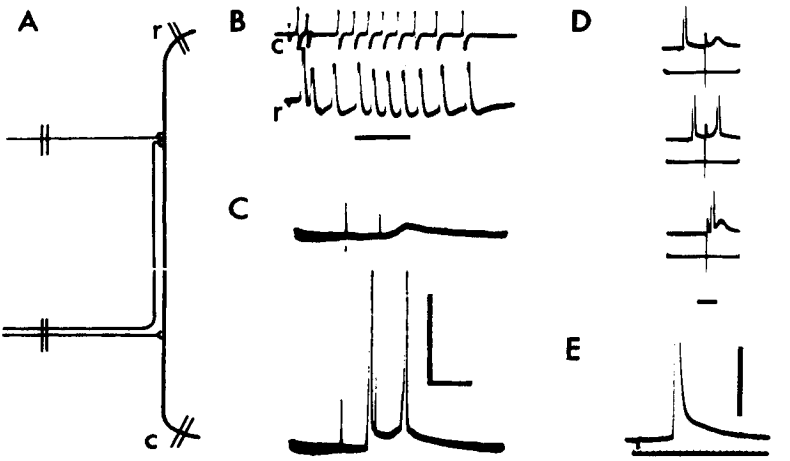

Fig.11. Organization of input to multisegmental interneurons. A, map of connections to interneuron B in abdominal segments 3 and 4; r and c represent rostral and caudal recording sites, respectively. B, simultaneously recorded impulse trains at r and c to a strong shock to the second root of the caudal segment in A. Note that the site of impulse initiation shifts from the caudal to the rostral ganglion for the third impulse in the train, indicating the rostrally projecting pathway shown in A. C, effect of depolarizing after-potentials upon subsequent subthreshold EPSP's. The upper and lower records are just below and just above threshold for a direct stimulus to the axon at c; following it is a direct root stimulus delivered at constant intensity to afferents entering the ganglion in which the recording microelectrode is located. The following EPSP is brought above threshold for spike initiation by the depolarizing after-potential due to the direct spike. D. A similar experiment, in which the interval between the preceding direct stimulus and the subsequent orthodromic volley is reduced. E. Record from a multisegmental interneuron at higher gain to show depolarizing after-potential. Time calibrations: 25 msec in B, 10 msec elsewhere. Voltage calibrations (for intracellular records only): 50 mV.

The central intersegmental pathway could in principle consist either of sensory axons that turn up and down the cord, or of interneurons. Tactile afferents are known to ascend and descend the cord (Wiersma and Hughes, 1961). Typically, interneurons B and C respond to strong second-root volleys with a firing pattern that consists of an early and then a late discharge; the pattern is accounted for in part by shifts in the locus of impulse initiation (Kennedy and Mellon, 1964a); there are usually two components in the response even when impulse origin is restricted to a single ganglion. The discharge is generated by a complex, prolonged depolarization that has been shown to consist of all-or-none responses in branches as well as summated EPSP's (Takeda and Kennedy, 1965). The question is whether the later phases of the compound EPSP represent polysynaptic input via such primary interneurons as A.

Recent experiments by Robert Zucker in our laboratory indicate that they do. Figure 12 shows responses of interneuron B to stimulation of primary afferent fibers and of other interneurons, including A. There is a consistent small depolarization produced in B by each impulse in A; the synaptic delay is vanishingly small. Similar connections are made by A upon other, unidentified interneurons, and conversely, other primary interneurons contribute excitation to B. In each case we have been able to analyze so far, the connections from lower- to higher-order interneurons involve brief postsynaptic potentials that repeat stably at high frequency and arise essentially without synaptic delay. We have concluded that they represent electrical connections. They are irreciprocal: A excites B, but B is known not to excite A.

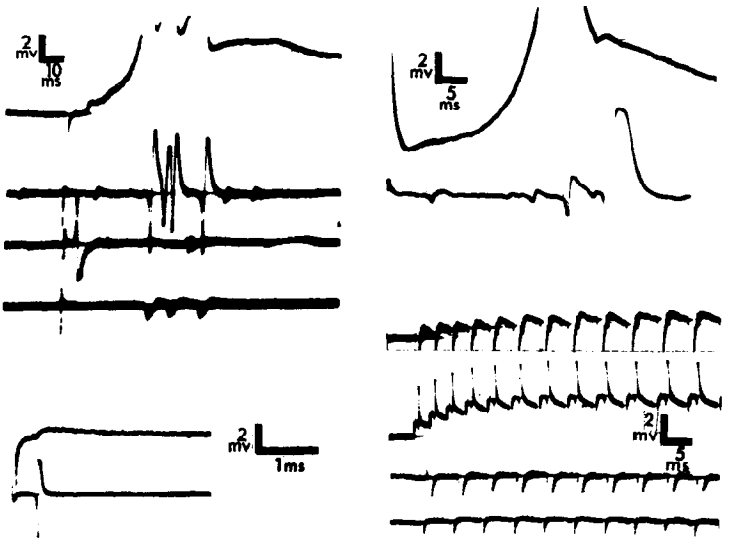

Fig.12. Inputs to interneuron B; intracellular recordings in the fourth ganglion (upper traces). Upper left, response to a single shock to the second root. The second trace is a monitor in the 3-4 connective; the third is a second-root monitor displaying the afferent volley; the fourth is a suction electrode recording from the isolated axon of B in the 4-5 connective. Lower left, response of B to an impulse in A; the isolated axon of A is monitored in the lower trace. Upper right, influence of another interneuron on B. A second-root volley was delivered at the beginning of the sweep; the lower trace is a monitor on a central connective. The intracellularly recorded impulse in B corresponds to the smaller of the two late discharges in the connective. The larger spike (due to another multisegmental interneuron) produced a small EPSP in B. Lower right, responses to stimulating A at high frequency (200 hz). Traces at lower left; the bottom record is at subthreshold intensity for A, the top is suprathreshold.

component drops out because its own first stage (e.g., the input from primary afferent fibers to interneuron A) is chemical and exhibits antifacilitation.

A word should be said about the junctions between the LG fibers and segmental fast flexor motor neurons. Until recently we have not been sure whether these were electrical or chemical in nature, since the somatic recording sites available to date have not given one a very close look at subthreshold synaptic events (Takeda and Kennedy, 1964; Kennedy, Selverston and Remler, 1969). Zucker now has evidence, from penetrations of motor neuron branches near the LG axon that the underlying PSP repeats at high frequencies and shows virtually no delay. If this connection is confirmed to be electrical, it will explain a perplexing phenomenon. LG impulses excite flexor motor neurons, but produce IPSP's in the peripheral inhibitory neurons that innervate the same muscles. The IPSP's have a somewhat longer latency than the concurrently generated EPSP's in the motor neurons, and we wondered whether they might depend upon a polysynaptic pathway. It now appears likely that the LG axons function as double-action interneurons, supplying electrical excitation to one population of follower cells and chemical inhibition to another. (It is already known that the inhibitory neurons themselves do something of the same sort. Bilaterally paired inhibitory neurons, which of course produce chemical IPSP's in skeletal muscles, are excitatorily coupled by electrical cross-connections (Otsuka, Potter and Kravitz, 1967; Evoy, Kennedy and Wilson, 1967).)

The foregoing description suggests some principles for hierarchical organization. It says that an element can be defined as to level: each neuron receives input only from levels below, and supplies output only to levels above. There are so far few interconnections between elements at a given level, and no "feedback" connections. There is no restriction about how many levels a connection may span, as long as it is in the right direction. Thus primary sensory fibers may connect with primary, secondary, or tertiary interneurons; and each class of interneurons receives input from all classes below it. The resulting cascade means that higher-order interneurons are infinitely more complex than primary ones. Cell A, for example, receives input in only one half of a single segment. All of it is excitatory, and all of it is mediated by chemical EPSP's from primary afferent fibers. By contrast the lateral giant receives a great variety of input from three different levels of elements, some of it excitatory and some inhibitory. A final consequence of this arrangement is that the same afferent elements may be supplying excitation to several classes of central neurons according to the input requirements of the latter. The same small population of tactile afferents (and, we suppose, the same individual fibers) will supply electrical excitation to the lateral giant and chemical excitation to primary interneurons like A.

In fact, this account of interneuronal connections describes a real reflex. If one taps a well-rested crayfish on the side of the abdomen, the animal gives a rapid tail-flip that usually can be shown to result from a lateral giant impulse (Krasne, 1969). That response comes about through a series of reflex loops of varying length: disynaptic (electrical PSP's from primary afferents to LG, electrical PSP's from LG to motor neurons);

trisynaptic (chemical PSP's from primary afferents to primary interneurons, electrical PSP's from primary interneurons to LG, etc.); and still higher-order pathways like the demonstrated one involving A→B/C→LG. The α component is normally inadequate to discharge the lateral giant axon, and so the reflex pathway depends critically upon the state of the temporally sensitive first-stage synapses between primary afferents and primary interneurons - upon which all long-loop excitation depends. That, presumably, is why the pathway shows such dramatic habituation in behavioral experiments (Krasne and Woodsmall, 1969).

In studies on "motor" interneurons, we have devoted most of our attention to those that control tonic, postural outputs instead of those that, like the giant fibers, mediate the phasic responses of the more specialized twitch muscles. A number of interneurons have been shown to produce widespread, coordinated output to the postural muscles of the abdominal segments (Evoy and Kennedy, 1967). Typically these elements affect several segments at once; they thus control the activity of one or two hundred motor neurons. We looked first at command fibers for postural flexion or extension in the abdomen. For each type of movement, there appeared to be several elements, occurring in consistent locations in the abdominal connectives. They differed from one another in several ways. First, they were not all equally effective, suggesting that they - like the sensory interneurons - might be arranged in hierarchical fashion. Second, they showed some selectivity about which motor neurons they activated. In a few instances, there is an apparent rationale for that selectivity. Some extensor command fibers, for example, activate only motor neurons that innervate "working" muscles, while others also innervate the fibers of the muscle receptor organs. Activity in the latter set therefore produce movements in which there is parallel contraction of working and receptor muscles, so that the command compensates for load (Fields, Evoy and Kennedy, 1967). Finally, there are important differences in the spatial distribution of motor output. Certain command fibers will produce strong contraction in rostral segments, others in more caudal segments; the proposal that these identified neurons produce unique geometries of movement has been verified by cinematographic recording of the results of single-cell stimulation, as well as by monitoring motor output to the appropriate muscles. Thus "motor" interneurons show the same sort of differentiation we find in "sensory" interneurons: individual cells are uniquely specified by a set of connections, and therefore can be mapped in terms of their relationship with the periphery. In this case the connections are efferent instead of afferent.

A more dramatic instance may be found of the principle, first stated by Hughlings Jackson, that the central nervous system controls movements and not muscles. Command fibers that control output to the muscles of the terminal abdominal appendages, which are capable of movements in three dimensions, operate on the muscles as a group, not in antagonistic pairs. Most such elements, as shown in Figure 14, affect the discharge to a number of different muscles; conversely, a given pair of muscles may be excited jointly by some commands, affected differently by others, and treated as antagonists by still others.

sequence like the one I just described. Paul Stein, in our laboratory, has found a pathway consisting of a relatively few fibers that maintains the phase relationships between adjacent segments in the swimmeret rhythm. If this pathway is interrupted, the output is produced separately in the two segments and lacks the characteristic posterior/anterior phase relation. That the connecting elements may be collaterals of the oscillator units is suggested by Stein's finding that the isolated intersegmental fibers, when stimulated to produce an antidromic burst, reset the motor output rhythm in the ganglion from which they come. Yet they are not themselves the branches of motor neurons, because they do not show impulse correspondence and root stimulation does not discharge them.

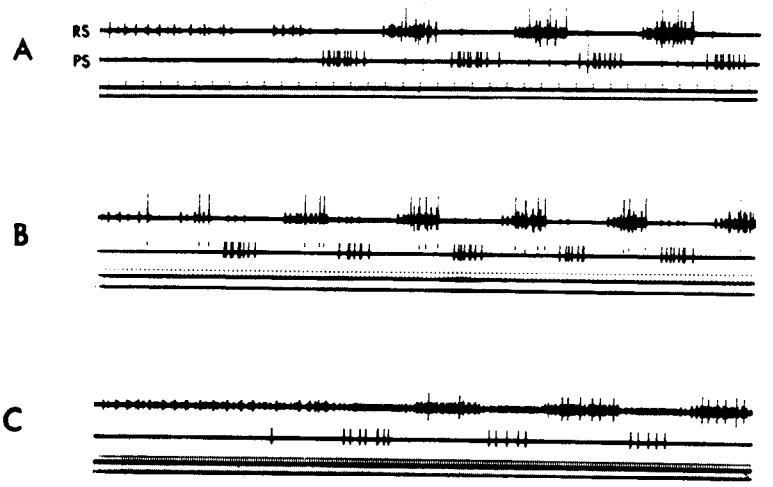

Fig.16. Influence of command interneurons on the rhythmic motor output of lobster swimmeret muscles. Three different command fibers are shown; they were isolated serially in the same preparation. Traces, from top to bottom: RS, returnstroke motor neurons; PS, powerstroke motor neurons; stimulus marker; time marker (10 msec). In each case, the stimulation begins at the beginning of the record. The command fibers in A and B resemble one another except in dynamic output range: that in A produces nearly the same oscillation frequency and burst strength at only one-fourth the stimulation frequency. The interneuron stimulated in C failed to excite the largest of the returnstroke motor neurons at all, but - in contrast to A and B - had a very powerful effect upon the returnstroke inhibitor (the small unit discharging concurrently with the powerstroke).

The main tasks for the future, then, concern the elements that we have so far been unable to identify in the control hierarchy. The neurons and connections that pattern temporal sequences of motor output are still unknown, though certain special instances of patterning can be attributed to motor neurons themselves (Gillary and Kennedy, 1969). An equally important objective will be to determine what elements activate command

interneurons - and in what combinations, if any - to generate specific behavioral instructions.

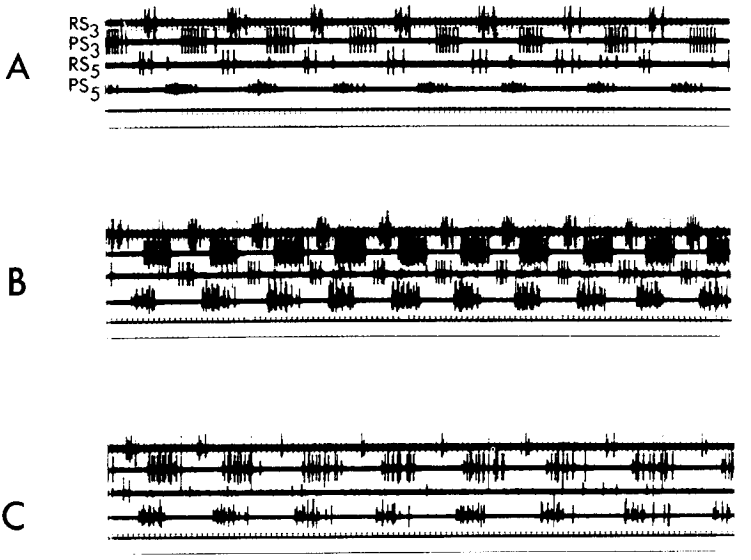

Fig.17. As in Figure 16, except that the four traces recording motor output represent the returnstroke and powerstroke motor neurons in segments 3 and 5. Two different command fibers were isolated simultaneously and placed on different stimulating electrodes; they are stimulated separately in A and C, simultaneously in B. Note that the command fiber in A selects but a single unit in the two powerstroke nerves, whereas that in C excites several, and that in C the influence upon returnstroke discharge is almost absent. A also shows a relatively greater outflow in the anterior segment. When the two command fibers are stimulated together, their effects show addition, both in terms of frequency and recruitment within a burst and in terms of "oscillator" frequency.

What we have already learned, however, leads us to propose some similarities between command systems just discussed and the pattern of reflex connections among identified interneurons that mediates "escape" responses. First, there is solid evidence for hierarchies of interneurons: we know that motor neurons are driven by a special category of premotor element, which in turn can be switched on by activity in command fibers; and we know that the latter, for the most part, are already several stages removed from the primary afferents. Second, we are quite sure that the chains of action are of very varied lengths. In some cases, direct motor commands may be issued by interneurons that are unmistakably driven by the periphery, while usually the pathway is much more tortuous. To turn the example around, LG axons in the "escape" system and the "oscillator" neurons in the swimmeret

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A MUSCLE APPARATUS FOR STUDENTS

By
ROBERT M. REINKING, PAUL C. JOHNSON,
RAPHAEL P. GRUENER, and DOUGLAS G. STUART*

It is conventional for freshman medical students to study muscle under isometric conditions. This report describes the construction and performance of an apparatus that simplifies execution of such measurements and in addition permits a muscle to be arranged for isotonic recording. Observations can then be made on the load-velocity of shortening relationship and on the visco-elastic properties of muscle or its mechanical analogues. The apparatus is simple to construct and easy to operate. Results obtained are sufficiently accurate to illustrate the major principles of muscle mechanics.

METHODS

The apparatus is shown in Figs. 1 and 2. It consists of a steel base plate upon which various components are assembled. The in situ muscle or a spring-dashpot analogue is mounted on a vise bolted to the base plate. The vise controls static length of the muscle or analogue. Steel posts are threaded into the base plate for mounting length and tension transducers. A brake-pulley-weight pan assembly can also be mounted for applying a rapid stretch to the muscle. The apparatus is used with a conventional electronic recorder. Most components of this apparatus are used in various other configurations for several experiments (e.g. intracellular recording of electro-physiological activity, human respiratory activity). This versatility reduces cost and storage requirements.

Description of components:

I. An anodized steel base plate measuring 24" x 12" x 1/2". The plate is tapped with 3/8" - 18 threads to receive a vise and two 10" x 3/4" steel posts.

II. A commercially available vise (4" travel) bolted to the base plate. A millimeter rule is attached to the stationary jaw for measurement of static length changes.

III. An aluminum mounting plate measuring 6" x 4" x 1/4" tapped with #8-32 threads to receive 3" x 1/4" steel posts which support the stimulating and recording electrodes. The frog leg is mounted by gripping the femur and tibia with firm alligator clips screwed to the plate. The clamps are arranged such that the gastrocnemius can be stretched in the horizontal plane. This arrangement is shown in Fig. 2. Alternatively a muscle analogue is attached to the mounting plate as shown in Fig. 1.

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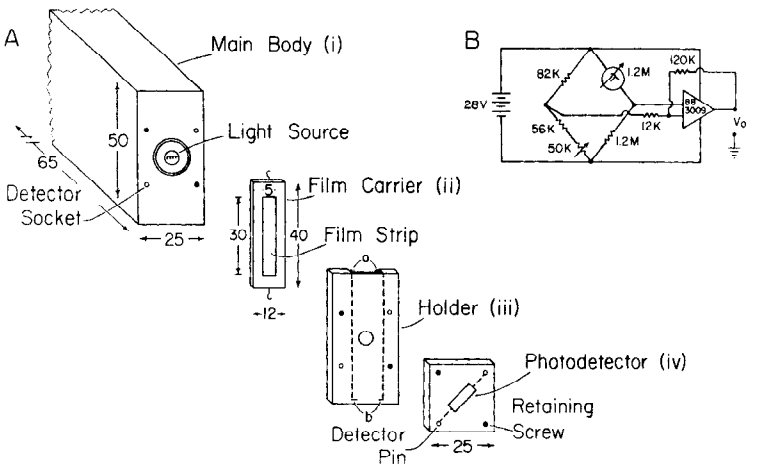

Fig.3. Mechanical (A) and electronic (B) schemas of a photoelectric length transducer which operates by varying the light intensity transmitted through a linear-density film strip. Variations are detected by a photoelectric device (Mithras Co., Model SPC-5, Cambridge, Mass.). The device is incorporated into a bridge arrangement shown in B, and its output is then amplified (Burr-Brown, Model 3009) and fed into a conventional recorder. The transducer (A) is made of four sections: i), an aluminum case main body containing all wiring and the light source; ii), a 450 mg magnesium film carrier containing a strip of linear-density film made from a neutral density filter wedge (Kodak). Hooks at each end of the carrier serve for muscle and weight pan attachments; iii), an aluminum holder for the film carrier. This holder permits the carrier a 10 mm travel which is limited by two sets of stop screws (a and b). These prevent disengagement of the film carrier and permit measurement of both pre- and after-loaded muscle contractions; iv), a photodetector which is embedded in an opaque plastic block mounted by retaining screws which fit through sections (i), (iii) and (iv). The insulated detector pins traverse sections (iv) and (iii) to contact the detector sockets on the main body. All dimensions are given in mm.

RESULTS AND DISCUSSION

The exercise on muscle begins with the apparatus in the isometric mode (Fig. 2). During the exercise the apparatus is changed to the isotonic mode (Fig. 1).

Isometric mode:

A frog is pithed, the sciatic nerve exposed for electrical stimulation and the left gastrocnemius detached from its insertion and freed to points

of origin on the condyles of the femur. The left leg is gripped to the mounting plate as shown in Fig. 2. The muscle tendon is firmly attached by cotton ligature to a strain ring mounted horizontally. Prior to this attachment the ring is mounted vertically and calibrated by hanging weights from an extension hook. Reference muscle length is measured with the previously described millimeter rule and varied in accordance with experimental protocol by moving the vise. Figure 2 also shows the arrangement for Canberra-type stimulating and recording electrodes. The muscle electrode can be used for direct electrical stimulation or for recording electromyographic activity.

With the apparatus in this configuration the nerve-muscle preparation may be used to record: tension development as a function of stimulus frequency and intensity; electromyographic potentials and tension during single twitches, summated responses, tetanic activity, onset of fatigue and neuromuscular blockade; and, the length-tension diagram. While some or all of these procedures are conventional in the student laboratory, the presently described techniques greatly simplify and speed the execution of these measurements by medical students.

Isotonic mode:

The apparatus may be rapidly changed to the isotonic mode by disconnecting the strain ring from its holder and tying one end directly to the muscle tendon. The other end of the strain ring is then connected by ligature to the brake-pulley-length transducer-weight pan assembly. This arrangement is shown in Fig. 1 with an analogue rather than a muscle attached to the mounting plate. In both cases the length transducer is calibrated by moving the mounting plate measured distances.

Load-velocity relations. The ligature connecting the strain ring to the length transducer is passed over the brake and pulley. To generate load-velocity curves, length is recorded while delivering supramaximal shocks to the muscle. Weights of increasing magnitude are added to the pan. The muscle is pre-loaded when the moving slide of the length transducer is positioned such that its movement is not restricted when weights are added to the pan. For after-loaded measurements, the slide is positioned to rest against a metal pin. The pin acts as a stop and prevents movement of the slide when weights are added to the pan. In this configuration the only load on the muscle is the slide of the length transducer (0.45 gms) and the minimal ligature-pulley friction. Typical results for pre- and after-loaded states are shown in Fig. 4.

Visco-elastic properties. The ligature connecting the strain ring to the length transducer is threaded through the jaws of the brake and looped over and around the friction pulley. The system is now ready for measurement of the rate of extension of the muscle with sudden application of tension. This dynamic stress-strain curve is especially useful in considering the viscoelastic properties of muscle. To generate the curve, the brake is applied to the ligature. An appropriate weight is placed on the pan. While recording length and tension, the brake is abruptly released. Tension increases almost instantaneously while a finite time is required for extension of the muscle. Oscillations

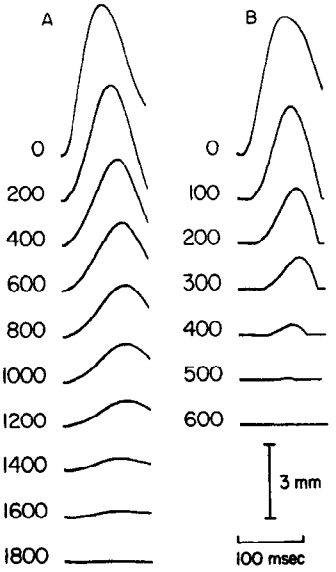

Fig.4. Extent and rate of muscle shortening during supramaximal twitches for pre-loaded (A) and after-loaded (B) frog gastrocnemius as a function of load (in gms). Single shock delivered to muscle at onset of each trace. For pre-loaded state muscle length was initially 50 mm. This length was progressively increased to 68 mm for the 1800 gm load.

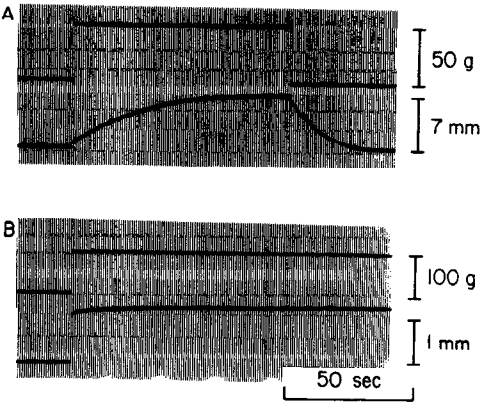

Fig.5. Dynamic stress-strain curve for: A, the spring-dashpot combination shown in Fig.1; and B, frog gastrocnemius. Upper traces show tension change on application of stress and lower traces the corresponding length changes. Stress release also shown for the muscle analogue.

at stress onset may be damped by adjustment of friction on the pulley. Stress release may also be studied by sequentially: applying the brake; removing the weight from the pan; and, abruptly releasing the brake. These procedures may be repeated with mechanical analogues consisting of a spring, a dashpot and a parallel combination of the two. Typical results are shown in Fig. 5.

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SUMMER COURSE IN BIOLOGY OF AGING

The Adult Development and Aging Branch, National Institute of Child Health and Human Development, NIH, in conjunction with various university departments will present the course at the Jackson Laboratory, Bar Harbor, Maine, Sept. 6-17, 1971. The course will be in three parts - The aging process; cellular aging; subcellular and molecular aging. Detailed information may be obtained from Dr. Gabe Maletta, Adult Development and Aging Branch, National Institute of Child Health and Human Development, NIH, Bethesda, Md. 20014.

EIGHTH ANNUAL ROCKY MOUNTAIN BIOENGINEERING SYMPOSIUM

This symposium will be presented in cooperation with the Group on Engineering in Medicine and Biology of the Institute of Electrical and Electronics Engineers at Colorado State University, May 3-5, 1971. Further information can be obtained by writing Rocky Mountain Bioengineering Symposium, Inc. P. O. Box 59, USAF Academy, Colorado 80840.

STATUS OF COMPARATIVE PHYSIOLOGY

The Physiology Training Committee of the National Institute of General Medical Sciences periodically surveys the status of various physiological subspecialties, to see how well research training is proceeding and to recommend ways to strengthen our national effort. This year the committee considers Comparative Physiology.

Scope of Special Organism Physiology and of General Physiology

Physiology is a broad biological subdivision concerned with the description and understanding of the functioning of organisms in terms of underlying anatomical structures and the chemical and physical processes which occur in or among them. There is an appropriate special physiology for every organism, including man. For example, no animal has a circulatory system exactly like that of man, but there are certain aspects of the heart function and peripheral circulation which are common to many species. Special organismic physiology emphasizes those features which are more or less unique for the particular organism under study.

However, physiological processes are based upon physico-chemical phenomena occurring in the constituent cells or smaller substructures such as mitochondria and cell membranes. Most of these processes have counterparts in many, or even most, organisms. This is because all life is related through evolution from common stems, because major basic processes and structures evolved early, serving as elemental building blocks from which living systems are constructed. In accepting evolution we assume the progressive development of physiological processes from common stems, so that major systems, specific organs, cells and subcellular molecular systems are related throughout animal life. It follows that there are general principles underlying any physiological process which are universally applicable. These principles provide the basis for understanding not just one but many systems, and they form the core of teaching about the operation of organs. They provide the basis for ultimate understanding of processes at cellular and molecular levels, and are the subject matter of the discipline termed General Physiology. Together they form a relatively cohesive subject. Since the processes are physico-chemical ones, the principles draw heavily upon established physics, chemistry and mathematics and this allies General Physiology with these disciplines. Both directly and indirectly, General Physiology contributes enormously to providing the scientific basis for medical practice.

Scope of Comparative Physiology

Comparative Physiology seeks to understand the diversity of physiological processes throughout the range of kinds of animals in which they

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*Submitted by the Physiology Training Committee, NIGMS with special acknowledgment to Drs. G. Hoyle, C. L. Prosser, B. Schmidt-Nielsen, and W. Van der Kloot.

occur. Comparative Physiology is much more than the study of function in non-familiar laboratory animals and it is sometimes considered as synonymous with Physiological Zoology or Zoophysiology. Comparative Physiology is unique in using kind of organism as one experimental variable. From this unique approach, new biological generalizations emerge that could not be reached by either Special Organism Physiology or General Physiology. Five broad areas of the application of Comparative Physiology to human welfare are outlined as follows:

1. The place of man in evolution. Comparative Physiology contributes to the understanding of man in terms of his evolution. The physiology of man is the result of a long evolutionary history and knowledge of man puts much of human physiology into a meaningful biological perpspective. Every successful species, including man, is uniquely adapted, by virtue of natural selection, to its particular environment and every species has characters which are derived from its evolutionary ancestors. If all of those characteristics which uniquely suit a species to its ecological niche and geographic range were understood, a true physiological definition of the species would result. Clinicians use empirical descriptions of normal man and they diagnose deviations from normality. The comparative and general physiologist goes further by providing explanations of the processes which underlie the clinicians' descriptions.

An example of the contribution of the evolutionary approach at the organ level may be taken from the kidney. The mammalian kidney initially eliminates most small molecules from the blood by glomerular filtration, and later by an intricate series of active transport mechanisms laboriously recovers the greater part of the filtrate. To understand the kidney one must realize that it was originally evolved in animals living in fresh water or dilute sea water where the major problem was to bail out the large volumes of water that entered their bodies through the permeable surfaces of their gills. Tubular reabsorption of solutes gradually evolved in different degrees according to need. Also, primitive aquatic ancestors excreted their nitrogenous wastes as ammonia but mammals evolved from water-living reptiles in which urea was probably a major nitrogenous excretory product. Terrestrial reptiles, evolving from primitive stem reptiles, adapted to the shortage of water by synthesizing and excreting uric acid. Because of the low solubility of this solute, it can be excreted by the kidneys in solid form with a minimum of water, even though the kidney cannot concentrate the urine to above the osmotic concentration of the blood. Mammals, on the other hand, retained urea as a metabolic end-product and developed instead a kidney that can produce urine with a osmotic concentration much higher than that in the blood (20 times more concentrated in desert rodents). This capacity of the mammalian kidney is based on the countercurrent principle together with active transport of ions and hormonal regulation of tubular permeability. The extensive comparative studies on the evolution of the vertebrate kidney, particularly as initiated by Homer Smith, have led to an understanding of the complex organization and function of the human kidney.

Examples of the evolutionary approach may also be taken from the study of macromolecules. Here comparative physiology reveals those

molecular features which are unique to man and those which are in common with other organisms because of common ancestry. For example, heme proteins evolved very early, probably in the period of pre-organismic or chemical evolution. Some of these compounds, the cytochromes, changed very little in their function, and by ascertaining the number of differences in amino acid sequences some measures of evolutionary affinity can be obtained for various kinds of organisms. Another group of heme proteins, the hemoglobins, appeared independently several times and in the vertebrates a variety of related hemoglobins is found. The oxygen-carrying capacity is modified in an adaptive fashion by the protein structure and the meaning of a multiple of hemoglobins forms is thus elucidated.

2. Environmental Physiology. Comparative physiology emphasizes the interactions between organisms and their environments. Currently, it is increasingly evident that if man is to survive he must understand and modify his interactions with his environment; he must maintain and yet use effectively his ecological resources. Since all organisms are specifically adapted to their natural environments, an understanding of such adaptations is essential for better utilization of the earth, as in food production. Certain kinds of organisms are better suited than others to particular environments, such as the deserts, the oceans, estuaries, the polar areas. By learning these adaptations, man may better manipulate and use the resources of all environments. A related practical application is to ascertain the physiological consequences of environmental disturbances. Physiological tests are the most sensitive indicators of pollution, and comparative physiologists can make important contributions toward relevant improvement of environmental quality.

Physiological variation in respect to a particular environmental stress is determined in two ways - genetically determined variation and environmentally induced. For example the limits of cold and heat tolerance are set genetically for a given kind of animal but within those limits the tolerance of a particular individual can be altered by acclimation or immediate experience. There is evidence that animals which inhabit very constant environments have less lability or capacity for acclimation than do animals which live in fluctuating environments. The capacity to adapt to a new environment must, therefore, be considered when man tries to extend his agriculture to deserts, to the tundra, to estuaries. It is possible that some native ungulates are better adapted and might yield more protein in our western plains than the cattle which civilized man introduced. The feedback interactions between physical factors in the environment and organisms, including man, are poorly understood. To increase man's food supply, much study of the physiology of organisms in specific environments is needed.

Food production has been limited by competing pests and their success depends on their unique adaptations. Examples are the spread of lampreys in the Great Lakes and the spread of specific insects on certain plant species. Control of these pests requires an understanding of their unique adaptations. Similarly some species (e.g. trout) are especially sensitive to pollution as by chemicals or hot water. Among those species which survive stressful environments, the enzyme systems of metabolism often

differ from corresponding enzyme systems in more sensitive species.

Finally, man's tolerance of new environments is limited and as man spends more time under the sea, in the arctic, in outer space, it becomes increasingly important to understand his physiological limitations and the extent of possible modification of these. Understanding of the physiology of tolerance of environmental extremes is aided by comparative studies - rodents and camels in the desert, hibernators in hypothermia, alpine residents in conditions of low oxygen.

3. Extrapolation from animals to man. Comparative physiology provides the basis for extrapolation from one kind of an animal to another. Modern medicine, particularly as regulated in the United States, demands preliminary tests and experiments on animals as a basis of practical application of therapy to man. The validity of the extrapolation from infrahuman species to man can be measured only by the comparative approach. Many examples of generalizations based on inadequate knowledge of test animals or tissues could be cited. In hormone therapy the differences in mode of reproduction and in sensitivity to estrogens between primates and rodents and even among species of rodents are important. Species differences in sensitivities to drugs are illustrated by the need for different doses of anesthetics. In basal or standard metabolism man has his place on a curve of mammals in which metabolism is exponentially related to body weight. Marked species and tissue differences in radiosensitivity have led to disagreement as to "safe" levels of ionizing radiation. Visceral smooth muscles show extreme diversity from organ to organ within one animal and the corresponding smooth muscles may be very different in different species; there is no generalized or typical smooth muscle. Far too often it has been assumed that because a natural product, e.g. acetylcholine or norepinephrine, acts in a given way in one animal, it will act similarly in another.

Domestic animals with spontaneous diseases provide useful models for diseases of man; strains can be bred for a disease, for example, hereditary heart disease in dogs.

Different kinds of animals have solved their physiological problems by analogous organs which may function differently. For example, the heart of most arthropods is neurogenic, and acetylcholine is excitatory to it rather than inhibitory as in the myogenic vertebrate heart. Study of the ganglionic pacemaker of the arthropod heart is therefore useful for neurophysiology rather than cardiac physiology. Comparative physiology is essential to determine when an extrapolation is appropriate.

4. Physiology of economically important animals. An important part of Comparative Physiology is the study of special kinds of animals which are of importance to man.

Veterinary medicine has many problems which differ from those of human medicine. Some of these are unique to ruminant animals - sheep, cattle, goats. The enlargement of the ruminant stomach, its specialized compartments, and the large microbial population in it represent an evolutionary adaptation to special nutritional conditions. One important

function of this complex organ is to regenerate proteins. The regeneration cycle begins with the movement of endogenous urea through the relatively impermeable rumen wall into the microbial culture of the rumen. Bacterial urease hydrolyzes the urea, the resulting ammonia is used in bacterial synthesis of amino acids which are then incorporated into bacterial proteins. These proteins are digested and absorbed lower in the digestive tract. A special mechanism which uses bacterial urease facilitates urea transfer across the epithelial barrier. In addition to having importance for veterinary medicine, the rumen serves as a model for the study of nitrogen cycling in man. It is known that in man a significant amount of endogenous urea (about 20 percent) formed daily passes into the gastrointestinal tract, is hydrolyzed and the resultant ammonia is absorbed and reconverted to urea. Studies on ruminants may lead to better understanding of this cycle which is important in nitrogen metabolism.

Insect Physiology is a large branch of Comparative Physiology which may be expected to lead to improved methods of natural control of insect pests, and hence to improved food supplies and reduction insect borne disease. It is of utmost importance to discover methods of insect control as substitutes for persistent insecticides such as DDT. An example of such potential control is the discovery of a factor present in certain North American trees which acts in a similar manner to insect juvenile hormone. Both the hormone and the product of the trees is the methyl ester of a previously unknown fatty acid. The material from the trees was discovered accidentally when a Czechoslovakian entomologist was unable to grow the European bug Pyrrhocoris apteris in America. The insects did not mature normally and the cause was traced to the source of paper towelling used in the rearing cages. Since a number of pests of cotton plants are included in the family Pyrrocoridae, paper of an appropriate kind (or better the appropriate ester) can be used as an insecticide. The North American evergreen trees must have discovered how to defend themselves from attacks by comparable bugs scores of millions of years ago, by producing this exact chemical! A whole new field of investigations of chemical by-products of plants has thereby been opened and the possibilities of insect control by natural products established.

Another important area of Comparative Physiology deals with the physiology and biochemistry of parasites. The control of various parasites, particularly helminths and protozoans, and, in fact, the understanding of the nature of parasitism in general depend on knowing their physiology. The metabolic pathways of parasites, particularly under anaerobic conditions, are markedly different from the pathways used in most aerobic animals. Intestinal worms, for example, produce a variety of products in glycolysis and need not use the common electron transport chain. Also their nutrition is highly simplified; they appear to have lost or not to use some of the enzymes which are important in their hosts. Thus parasites provide useful models for enzyme system analysis; also control of diseases caused by parasites can be rational only when their physiology and biochemistry are understood.

Another area of the physiology of special groups which is of increasing practical importance is fish physiology. The culture of fish is a growing method for providing animal proteins in many parts of the world. Nutritionally, fish have somewhat different requirements from mammals. Also marked species differences exist in growth rates and environmental tolerances. It is probable that many comparative physiologists will be needed as fish become more important as a food source.

5. Discovery of unique physiological preparations. Perhaps the greatest contribution of Comparative Physiology to medicine has been to provide biological preparations which permit the solution of critical problems in cellular physiology. Examples will be given from neurophysiology, sensory physiology and muscle physiology.

The history of discoveries of the nature of axon conduction and of synaptic transmission suggests that most major advances have depended upon discovery of uniquely suitable preparations. The modern era in excitable membrane physiology started with the introduction of the giant axons of squid to neurophysiological research. These single nerve fibers can be up to half a millimeter in diameter, large enough for insertion of two sizeable electrodes. Introduction of two electrodes permitted clamping of the membrane at desired voltages and then following the ionic currents resulting from controlled displacement of the membrane potential. The entire theory of conductance changes for specific ions as the basis of the nerve impulse came from studies on these giant axons of squid and today it remains necessary for most work in this field to be done at marine laboratories. Discovery of the squid giant fiber system was an important contribution of a comparative physiologist. Extrapolation to nerve conduction in man is justified.

Various invertebrate central nervous systems which contain relatively few neurons as compared to mammalian brains are proving to be valuable for discovery of basic principles of interneuronic coupling. For example, central ganglia of gastropod molluscs have clusters of large nerve cells which are so regular in their arrangement that a neurone that can be penetrated with a microelectrode in one mollusc can also be impaled in another animal. It has been found that one identified neurone can elicit opposite physiological actions, excitation or inhibition by the same transmitter, in two kinds of cells which it innervates. These remarkable cells can be monitored continually for a few days and in their spontaneous activity some cells have a built-in circadean rhythm. These gastropod neurones offer promise for the study of long-lasting changes in the neural networks as a result of multiple stimuli, i.e. a sort of learning process. In other experiments on these ganglia single interneurones have been stimulated electrically and motor responses which involve the coordination of many muscles have been elicited. Even more complex behaviors have been evoked by localized stimulation in the "brain" of certain insects. While much remains to be learned about the circuitry of the large neurones even more exciting discoveries await techniques for study of the many small neurones of the neuropile. The basic cellular principles of neuronal interaction found in these simple nervous systems provide important leads for mammalian neurophysiology.

The nervous system of the leech has provided valuable information as to the function of glia, and provided a clear example of electrical coupling by low resistance connections between two neurons. The first electrical synapse to be discovered was in crayfish ganglia. Now many examples of electronic coupling between cells have been found.

Remarkable progress in understanding the transduction of mechanical deformation to sensory impulses was made possible by crustacean stretch receptors. These receptors are rather like a vertebrate muscle spindle in function but they are not buried in muscle, but are isolated as bridges between two segments of the exoskeleton. Therefore they are easily dissected free from an animal. As is typical of the arthropods, the sensory neuron's cell body lies out in the periphery; the cell body can be penetrated with a microelectrode. Using this preparation, comparative neurophysiologists were able to learn many of the details of the mechanism that transduces mechanical movements into nerve impulses. Stretch of the dendrites produces a depolarization. The depolarization spreads electrotonically to the trigger zone of the neuron and sets up an action potential Since the action potential is not conducted back into the dendrites, the generated potentials persist as long as the receptor is stretched. This preparation has provided many of the basic ideas now extended to other sensory systems.

Another example of accessibility is also widely known to sensory physiologists - the eye of the horseshoe crab, Limulus. This eye is typical of the compound type: it is made up of a large number of identical subunits. A single nerve fiber leaves each of the subunits and runs to the brain. The optic nerve can be easily split into small bundles of fibers and it is rather easy to record from a single visual sense unit. It is also possible to insert microelectrodes into the cell body of the neuron that runs into the optic nerve. This preparation has played an important role in our understanding of visual mechanisms and of sensory physiology in general. Here the advances have not only been in our understanding of how light energy is transduced into nerve impulses, but there have also been important generalizations about the organization of the entire system. A system of lateral inhibition was discovered; the illumination of one sensory element produces an inhibition of the neighboring sensory cells. This phenomenon may be widely used in sensory systems as a way of sharpening input to emphasize boundaries.

The comparative approach using cells of large size has also profited muscle physiology. Giant muscle fibers that are especially suitable for experimentation are obtained from large barnacles. The fibers are roughly 2 mm in diameter and about 5 cm long, more than 20 times larger than any vertebrate fibers. They can be cannulated, so substances can be directly injected into their interior. At the same time the membrane potential can be controlled and the contraction measured. The use of these fibers is permitting the direct study of the intracellular events associated with contraction - the action potential leading to the release of calcium ions that switch on the contractile machinery. Such fibers could play a unique role in illuminating the, as yet, unknown steps in the control and mechanism of contraction.

Recently an unique in vitro method for determining the release of calcium has been devised by putting together two pieces of comparative physiology in order to measure from moment to moment the calcium ion concentration in a muscle fiber in the range from 10^{-8} M to 10^{-6} M. The jellyfish, Aequorea, releases a bioluminescent protein when it is mechanically stimulated. When this protein reacts with calcium in the sea water the system glows. The protein begins to emit light in the presence of 10^{-8} M calcium and the light intensity increases as the calcium concentration rises, reaching a maximum at about 10^{-6} M. The purified protein is injected into a single giant muscle fiber which is then placed close to a photomultiplier tube. When the fiber is at rest no light is detected, but when the fiber is stimulated light is emitted. This means that the calcium ion concentration within the sarcoplasm is rising. Light emission is then followed by the development of tension, owing to the reaction between actomyosin, ATP and calcium. Another method for estimating calcium ion concentration in a muscle fiber is by the murexide reaction. These studies have proven that calcium is released within the living fiber as a result of membrane depolarization and they provide a method for quantitatively studying the coupling between membrane potential and calcium release.

Conclusions

The preceding sections mention under five general headings some of the ways in which Comparative Physiology contributes to human welfare in general and to medicine in particular. Comparative Physiology is more a viewpoint, a philosophical approach than a sharply limited discipline. Viewed in this way, Comparative Physiology fully justifies support from NIH. A comparative physiologist must have unusual breadth of training; he must be grounded not only in traditional physiology, biophysics and biochemistry but also in zoology, ecology and genetics. Comparative physiologists at present tend to work in departments of biology rather than in medical physiology departments. This is unfortunate because Comparative Physiology is now embracing some of the most exciting areas in biological science. Many problems in comparative physiology have been studied little or not at all, so the future opportunities exceed the substantial achievements of the past.

At the present time a vigorous effort is being carried out in the United States in many aspects of comparative physiology. Some of the training programs already supported by the NIGMS have a comparative orientation and the number of such programs should be expanded as funds and meritorious applications increase. There also is some representation of comparative physiology in broadly-based, or in mammalian-oriented training programs. Physiologists who are responsible for organizing training programs should carefully consider the part played by comparative studies in a modern curriculum and the advantages of including some comparative research within Physiology Departments.

In a medical school department within a university setting a comparative physiologist can also serve as an important link with research in Departments of Biology and Zoology. There seems to be a general tendency for biological sciences to subdivide itself into smaller and

smaller subgroupings, so developments like comparative physiology that help to bring researchers together are especially worthwhile.

In a time of intense public scrutiny of the justifications for supporting scientific research and training, comparative physiology requires special explanations. As was mentioned before, even those members of the public who enthusiastically support medical research may be dumbfounded at the titles of some of the projects in comparative physiology. Therefore it is important to keep the interested public informed about the ideas and the applications coming from comparative physiology. When surveying physiology training and research as a whole it becomes clear that certain areas such as comparative physiology offer most impressive challenges and opportunities for the future.

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SYMPOSIUM ON OTOPHYSIOLOGY

An International Symposium on Otophysiology will be held at the University of Michigan, Ann Arbor, on May 20-22, 1971. The Symposium will be sponsored by the S. S. Kresge Foundation and the Deafness Research Foundation, and by the Departments of Otorhinolaryngology and Postgraduate Medicine and the Kresge Hearing Research Institute, University of Michigan Medical School. For further information write to Merle Lawrence, Ph. D., Director, Kresge Hearing Research Institute, 1301 East Ann Street, Ann Arbor, Michigan 48104.

SENIOR PHYSIOLOGISTS
REPLIES TO BIRTHDAY GREETINGS

When members reach their 80th birthday they receive from one of the Committee on Senior Physiologists a greeting on the Beaumont House Card. Many express their appreciation. Excerpts from recent replies follow:

Charles D. Snyder, (1871) our oldest member, replied to Hal Davis:

"Thank you for writing me the news of my election to the order of Senior Physiologists. They are indeed a distinguished body of scientists - and I'm proud to be one of them."

Dennis Jackson, (1878) replied to Bruce Dill:

"I am grateful for your reminder that time moves on - and often we have so little to show for it. If I live four more days (Sept. 3) I shall be 92 and I am still painting, I believe a little better than last year. These pictures are for an exhibit in New York City in October. In the meantime I am working as hard as I can on some research that I felt I absolutely must finish. The most difficult task with that now is how and where to get my paper satisfactorily published exactly as I write it, and within my lifetime."

Thorne Carpenter, (1878) replied to Hal Davis:

"Thank you very much for the greeting card which arrived on my 92nd birthday. It is wonderful to be remembered, after nearly 25 years of retirement. I am glad to write that I am quite well. Aside from domestic activities, I spend a good deal of my time reading the various scientific publications which I receive as an emeritus member of several societies. Among these periodicals I read The Physiologist usually from cover to cover. I really lead a quiet, lazy life."

Bruce Dill wrote to Thorne during the year asking for reference to analyses of atmospheric air. Thorne replied giving several references including one to his paper. "The constancy of the atmosphere with regard to carbon dioxide and oxygen", J. Am. Chem. Soc. 59: 358-360, 1937. His modification of the Haldane apparatus gave CO_2 to $\pm$ 0.002%. The accepted values then were O_2, 20.940% and CO_2, 0.03%. It would be interesting to know if his apparatus used today with his skill would bear out the reported increase in recent decades of atmospheric CO_2.

J. H. McClendon, (1880) wrote Ladd Prosser:

"Since I am 90, and too deaf, blind, and crippled to attend meetings, I hope you will pardon my writing. Steggerda was one of my graduate students, give him my love. Your Past President's Address stirs up old memories of why I became a physiologist.....I got malaria in Mexico and Dr. Turpin, who had never been to medical school gave me strychnine every day for 100 days. He robbed me of money I intended to use to study medicine. I retreated to my animals and got B.S., M.S.

and Ph. D. degrees in zoology and botany. I found lies in my history book and learned from archeologists studying Maya astronomy that Eratosthenes about 300 B. C. found that the difference in the angle of the sun between Alexandria and Cyene (Aswan) was 1/50 of 360 degrees and the distance was 5000 Olympic Stadia, so that the circumference of the earth is 50 times 5000 Stadia, a fact unknown to Columbus. I got hold of a translation of Paul Bert's Physiology and with the aid of the Encyclopedia Brittanica and years of study found that Erasistratus about 300 B. C. showed that the brain is the thinking machine and the heart is a pump and pumps aerated blood to the brain."

Dayton Edwards, (1882) replied to Hi Essex:

"Thank you very much for the attractive card and your personal message for my birthday. These milestones seem to roll around every twelve months but in the meantime, I don't get any younger. In younger days, I had the good fortune of spending a few months in the Lab of William Einthoven in Leiden. He was then past 70 but it was his practice to ascend the steep Dutch stairs two steps at a stride. It was nerve wracking to watch but a good lesson to watch; Never give up! Finally, may I congratulate you on your beautiful handwriting; its perfectly formed letters and undulating lines. No sign of tremor or incoordination. Again my hearty thanks and all good wishes."

Walter Alvarez, (1884) replied to Ladd Prosser:

"Thank you and your associates for the great pleasure you have given me in publishing in The Physiologist for May notes on what your older retired members are doing. It has been such a joy to me to hear about some of my old friends, so many I knew well including W. R. Hess of Switzerland. It is such a joy to see that many of the men can still go into a laboratory and have fun; men like my old friend Philip Bard, whom I have known ever since he was a boy.....I am doing as my friend Hi Essex says all of us should do, and that is keep hard at work, even at age 85.....Incidentally, the man who got me into the American Physiological Society is that wonderful man George Whipple. The last I heard from him he was alive and active at 91 or 92, and so he is older than I am."

Alvalyn Woodward, (1884) replied to Bruce Dill:

"Thank you for your birthday greetings, both this year and last. I hope you will be as healthy and happy on your next birthday as I have been on my recent one. Last year at this time I was on a delightful trip to Alaska and the Canadian Rockies. This seems to be the year for small trips, to mountains and sea-shore, and for many quests."

Henry Laurens, (1885) replied to Bruce Dill:

"I must sincerely thank you for your note of birthday congratulations. It was extremely good of you to remember this old man."

Carlos Monge, b. Dec. 13, 1884; died Feb. 15, 1970. Bruce Dill writes of Monge: "My friend since we first met in 1935, Carlos Monge was elected to honorary membership in the Society in 1952. His illustrious career included training in the London School of Tropical Medicine and the University of Paris prior to his appointment as Professor in the Faculty of Medicine in 1919 in the most ancient University in the Americas, the University of San Marcos. He has written authoritative articles and books beginning with a clinical paper in 1911: Algunos apuntes sobre la hematologia de enfermedad de Carion. He described "chronic mountain sickness" now known as Monge's disease; it affects many natives of the Andes. One of his books best known in this country was published by the Johns Hopkins Press in 1948: Acclimatization in the Andes: Historical Confirmation of Climatic Aggression in the Development of Andean Man. Professor Monge's achievements were manifold - in clinical medicine, clinical investigation, physiology and sociology all as related to high altitude. His eminence in medicine and science was matched by his character and leadership. He was regarded highly by all regimes in Peru, surviving political change with acumen. He is mourned by his former students many of whom are eminent in science. The first two I knew were E. S. G. Barron who was a member of our high altitude party of 1935 and Alberto Hurtado - internationally famous."

Edward Boyden, (1886) replied to Bruce Dill:

"How nice of you to have written to me on my birthday! It was a very happy one, full of surprises. In the morning, while I was at work, one of the graduate students tapped on the door and said, 'There's someone to see you in the Seminar Room.' I went in, and there were a dozen graduate students sitting around a birthday cake and grinning like Cheshire cats. In the afternoon, the procedure was repeated by the Department Staff. How lucky can one be!"

Marian Osterhout, (1888) replied to Hal Davis:

'It was very kind of you and the American Physiological Society to remember me on my birthday. I wish to thank you and the Society for the very throughtful message. I have been away from New York almost two years before I moved into my new apartment in the city. I am so used to living here that I even feel at home with air and water pollution, crimes, and noise. I seem to get lonely without them. I have spent several summers at Woods Hole recently so that I am in touch with the work done at M. B. L. Their work is very interesting and stimulating. I have gone over the archives of my late husband very carefully and placed them in the library of the American Philosophical Society in Philadelphia."

Helen Graham, (1890) replied to Hal Davis:

" Thank you and the Committee on Senior Physiologists, and the American Physiological Society for the birthday greeting last month - it should have been acknowledged long ago, for it was much appreciated - but was it deserved? Senior I certainly am, eminent I certainly am not,

and maybe I'm not a physiologist either, though I like to think I am. With gratitude for your thought of me."

Mrs. Richard Ashman (1890) wrote Hi Essex that her husband, born Aug. 13, 1890 died Feb. 28, 1970 after five years of invalidism. He was mentally active until shortly before his death. Richard's reply to last year's inquiry was quoted in part in The Physiologist for May, 1970.

Farewell to Percy Dawson, May 19, 1873 - September 27, 1970.

Bruce Dill writes that readers of news items from Senior Physiologists will be saddened to learn of Percy Dawson's death: He wrote me about his autobiography on August 10, 1970:

"Before me lies the manuscript of ca. 14,000 pages; these ar typed in triplicate and beside them ar roughly 1,000 pages not yet typed. This collection constitutes what has been called a 1st draft of my autobiography. During the riting of this material, especially the last part of it, I hav been helpt by others and they wd. be unwilling to content themselves with a disorderly, incomplete ms upon which they hav labored with the expectation of becoming, mor or less, notorious as authors, and since this loquatious overflow of mine has been promist to the archivists of the state and university library of Wisconsin, which has a copy of it, the problem is obvious. - What sage suggestion hav yu?"

I offered to seek for a publisher but he replied on Sept. 4 in a letter dictated to his assistant Pat Hamm:

"I believe myself incompetent to consider matters regarding my manuscript and including the material of your letter of the 25th of August. I have put all matters regarding the manuscript in hands which I deem to be competent, namely, those of my typist, Mrs. Judith Lutge. All my manuscripts are given to her in my recent will. I hope that you will consult her in these matters. You will find her cooperative, devoted, and reliable."

Word of his death came from Judith Lutge and Pat Hamm who can be reached at Percy's address: 1072 Portola Ave., Los Altos, Calif. 94022. They are continuing to work toward the completion of his autobiography.

Some details of Percy's unique career other than those in American Men of Science were recorded by Dill and Wassermen, Gerontologist 4: 136-140, 1964. Rather than recount them there follows an excerpt read at burial from a humanist burial service he wrote in 1920:

> "Hav we not all lernd to associate with deth
> certain great truths which hav been the themes
> of poets and the consolation of all? Let us re-
> call sum of these. When a companion disappears
> from our circle, that circle closes in ther is a
> quickening of the usual attachments of the mem-
> bers of that circle. We see become mor manifest