IX. SUMMARY

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It is not entirely clear to me why I was asked to summarize and close this program. As the lone American in this distinguished international galaxy and as one of the few physiologists amidst the pharmacological cohorts, the Committee may have wished me to serve as devil's advocate for an electrophysiological approach to neural functioning, or it may have proceeded on the (apparently justified) assumption, that if I were foolish enough to undertake such a summarizing job at the earlier symposium in this area, some eight years ago, I might be sufficiently foolhardy to try it again. In any event, I assure you that I have been under considerable stress for the past couple of days and that the urinary catechol level, not to mention that of hydroxy-steroids, would please Dr. von Euler.

There are several ways in which my assignment might be discharged, each with its own danger. I might give you my impressionistic reactions, which would perhaps be art rather than science; or propound extensive generalizations, which might merely repeat former pronouncements; or strongly advocate electrophysiological as opposed to neurohumoral mechanisms, which would hardly win friends in this gathering; or contribute a number of particular comments, thus belatedly imposing upon you some of our own work and ideas. So I shall attempt all four!

First, then, some impressions. By and large we seem to be straining at the very limit of available methods and factual results. This accounts for the somewhat greater than ordinary disagreement as to what is actually so, and the even greater disagreement as to what may be concluded from the things which are agreed to be so. The main advances have been in those areas where new agents have been applied to the problem—whether a new histochemical method, such as those for cholinesterase; a new pharmacological agent, such as the methoniums; a new instrument, such as the impaling microelectrode, or what not; as indeed the most dramatic advances in the symposium already referred to came from the application of the then new agent, DFP. The straining of methods and results, in turn, is perhaps the result of a certain overcommitment to the operational hypotheses guiding the experimentation and thinking in this field. In our eagerness to get some of these things settled, we are perhaps neglecting a bit Claude Bernard's sage advice to, "Entertain theories but be not entertained by them; wear the cloak of imagination but remove it in the laboratory." In any event, I am forcibly reminded of the pink elephant and green snake who entered the saloon and were told by the barkeeper, "You're too early; he isn't here yet."

Perhaps the best support for my feeling that conclusions are outstripping evidence comes from the reactions of several listeners to Dr. Paton's valiant effort in comparing the neuromuscular junction and the autonomic ganglion

synapse. He carefully marshalled several points in which these react alike and others in which they react differently. Many persons were thereby convinced that the two structures are basically alike and may be treated as one in theoretical arguments, whereas others were equally convinced that the two structures are basically different so that findings on one may not be applied to the other. I am reminded of the simple psychological test of showing a couple of keys to a person and eliciting comments about them. Nearly all individuals will make a series of statements either about their likenesses or about their unlikenesses; only the exceptional individual includes both kinds of statements. Paton himself was inclined to succumb to that "Great urge for monism that burns in many scientific breasts", as Whitehead put it; but when one's reactions to a given body of evidence can be guided by an emotional predisposition, it is obvious that the evidence is not yet scientifically firm in either direction.

Incidentally, I fear that my own most enduring reaction to Paton's admirable talk was in response to his vivid characterization of the C-6 man. This chap haunted me last night until I had put down a couple of verses concerning him. The second I shall reserve for those who hear me out this morning, the first is as follows:

When C-6 is about in excess,
A man's organs yield under stress.
In a corset they're tucked,
His gut can't eruct,
And he faces an entropic death.

I turn now, for a moment, from arty impressions to more factual generalizations. As compared with the status revealed at the symposium nearly a decade ago, I do not recognize any revolutionary new ideas or approaches—perhaps a tribute to the wisdom of the founders of this field, of whom Loewi and Dale are still happily participating. Much the same issues that were active than are now being attacked or defended by much the same sorts of arguments. There has, however, been a significant increase in the clarity of formulation of many of these issues and in the quantitation of the evidence pro or con. There has also, it seems to me, been an overall increase in probability of the physiological importance of neurohumoral mechanisms in a number of particular situations. In the 1946 publication I summarized my own views as follows: "With these facts and arguments before us, we must conclude that ACh is not critically involved in nerve conduction, and we must be reserved in assigning it a role in junctional transmission, particularly within the nervous system."

Today, the existence of chemical transmission at autonomic effectors has not even been questioned, at one extreme; and at the other, a neurohumoral mechanism in actual nerve or muscle conduction has been almost as unanimously renounced. At some junctions, however, the evidence today is certainly more in favor of chemical transmission than it was earlier. It is reasonably convincing for the myoneural junction, still definitely moot for the autonomic synapse, and, in my judgment, continues to weigh on the negative side for the central

nervous system—despite the latest bulletin from Canberra. It is also interesting that both Liljestrand and Buchthal presented evidence of a neurohumoral mechanism for receptor transmission, although this was not even considered at the earlier meeting.

This brings me to my role as advocate for electrical transmission, and I am reminded vividly of the conference a summer back, at Cold Spring Harbor, on electrophysiology. There, in one report after another—many using our relatively new microelectrode—resting and action potentials were recorded from all sorts of units, from fibers, neurones, receptor cells of ear and eye, membranes. Moreover, the phenomena of excitation, summation, inhibition, facilitation, and transmission across several kinds of junction were accounted for in terms of potentials and currents with some approach to quantitation. I see only one or two in this room who attended that conference; perhaps evidence of a somewhat dangerous schizophrenia in this field.

The arguments and counter arguments presented here often seemed to me, in contrast, to lack the desiderata of simplicity and coherence. When one worker, as Hebb, reports good function remaining after all cholinesterase has been inhibited, or another, as Douglas, can find no ACh liberated with physiological activity, it is, of course, always possible to insist, as did Walop, on undetected traces. But the burden of proof is then shifted sharply to the proponents of an ACh mechanism. When DFP acts differently on different spinal reflexes, and when, moreover, it acts differently from other anticholinesterases or from ACh, as found by Holmstedt, it is correct to point out, as did McIntyre, that, since very different fibers and connections are involved in different reflexes, identity of action is not to be expected. But the positive argument is surely now, so to speak, on the defensive; the more so, since in similar experiments, Holmstedt found good consistency in the drug effect on the vestibular system, and since different anticholinesterases have long been known to have different effects—at myoneural junctions and, in our hands, on the spontaneous electrical beats of the isolated frog cerebrum.

Parenthetically, Nickerson's argument—that, since DFP blocks a nerve without depolarizing it, the block is not due to ACh accumulation—is also not logically convincing. This would be true only if the whole action of ACh were to depolarize. This, however, is far from proven; indeed, just at the endplate, where the high concentration of ChE should destroy ACh most rapidly, the depolarization is especially prolonged.

In general, the strongest arguments for the functional role of the ACh system were the extremely high concentration of ChE at critical regions and the characteristic and uniform action of the anti-ChE drugs. When function persists with most, if not all, of the ChE gone and when the drugs have widely different physiological effects, it is, indeed, possible to argue away the discrepancies. The position can fairly be caricatured then, however, by saying, to mutilate an old favorite, "If we had some ham, we could have some ham and eggs, if we had some eggs. We have no eggs, therefore all we have is ham."

On the positive side, we should not forget that the electrical currents *must*

be involved in conduction of the nerve impulse. No liberation or diffusion of chemicals could account for the ability of an impulse to jump over a millimeter of inactivable nerve fiber in a fraction of a millisecond; and other facts, that conduction velocity slows with increasing electrical resistance of the external medium and that block can be produced or removed by opening or closing an external circuit, likewise demand the existence of eddy currents in conduction. Moreover, many, if not all, of the phenomena of junctional transmission are neatly accounted for by the properties of eddy currents and the geometry of the junctional region—even to good quantitative agreement between the intensity and duration characteristics of the physiological currents and of the junctional transmission process.

It is a real tribute to the stature of Dr. Eccles that so many have cited his saltation from his earlier conviction that eddy currents are the transmitter to his present one that chemical transmitters are involved at neural synapses. It would be more scientific, however, to look at the facts which led Eccles to change his opinion and to judge these on their own worth. His conversion resulted from his finding, with an intracellular electrode in a central neurone, that inhibition was associated with an increase in the membrane polarization. Such hyperpolarization, he believed, could not be produced physically by any type of external current and must therefore be attributed to a chemical mediator. In the intervening year or two, others have pointed out several reasonable ways in which eddy currents could produce a recorded hyperpolarization, so the chemical explanation is therefore not logically compelling, whether or not it be a useful working hypothesis.

Actually, it remains generally accepted that irritability does vary with membrane potential, as in the technically impressive experiments on neurones that McIntyre mentioned. In the case of frog sartorius muscle, when the membrane potential is reduced to 57 millivolts, by any means we have tried, a conducted response is set up. Parenthetically, and clearly related to the effects of depolarization reported by Zaimis, at membrane potentials maintained below this level (by high potassium or by cathodal polarization) an electrical stimulus produces a vigorous local contracture but no propagated contraction.

Finally, a few miscellaneous questions may serve to point up reasonable doubts concerning the enthusiastic adoption of a universal neurohumoral interpretation. Any number of physiological effects have been reported from the administration of ACh or functionally related substances; but in how many of these is the dose used so excessive as to make the positive finding rather meaningless? Conversely, if other substances were examined as minutely as ACh has been, might they not seem equally active and ubiquitous? In almost every case where sought, functional changes have been found in relation to ATP, K, -SH, thiamine, quaternaries other than ACh, and so on and on. I can not forget that, in my scientific youth, lactic acid was *the* cause of muscle contraction. Later CrP and then ATP evoked shortening. Now we have turned up the disturbing fact that ATP acts, causes muscle twitching, only when placed outside the fiber, not when injected into it; and the related one that cathodal

contracture of muscle is associated with a *decreased* turn-over of creatine phosphate!

A third question, to which I am convinced the answer is "Yes", is, "Do the anti-cholinesterase drugs have other significant actions besides inhibiting this enzyme?" Eserine protects against DFP poisoning and, even when DFP has presumably inhibited all ChE, eserine administration still has pharmacological effects. As Burn brought out for the heart and Holmstedt for the cord, eserine and prostigmine act differently, and the actions of "Darmstoff" are also different. Further, by direct biochemical studies, we showed the anti-esterase drugs to inhibit the dehydrogenases at low concentrations and, recently, to decouple oxidative phosphorylation.

Yet another question, "Does ACh act only on the cell membrane or may it act in a variety of ways unrelated to conduction or propagation?" has already been touched upon. Several possible roles in metabolism have been suggested over the past years, and the findings here reported by Burgen on the intracellular location of the ACh system, as well as those of Burn on the role of this system in the cardiac response, strongly support its playing some role in the cell economy in addition to any membrane effect. In much the same way, Vogt's evidence indicates that, while sympathin may act on blood vessels in the nervous system, it also has some other independent action or actions.

A final question, "Dare one conclude from the presence of a large amount of substance that it is of particular importance?" This has been pointed up by several essayists and discussants, including von Euler, West, Goldstein, Hebb, and Blaschko, who repeatedly emphasized that substances accumulate in quantity only when they do *not* react rapidly. Such reasoning may be less true when applied to enzymes, in which case it becomes significant that cholinesterase is high in the cell bodies of neurones rather than in the synaptic regions. One is reminded also of the growing evidence that the posterior pituitary hormones are in fact formed in hypothalamic nuclei and merely stored in the posterior lobe. Incidentally, and along the lines of Vogt's finding of sympathin changes with orthosympathetic activity, we have seen a marked change in the CrP/P in the hypothalamus and the pituitary, but not in other brain regions, after adrenalectomy in the rat.

This brings me to the fourth way of summarizing, by particular comments. Perhaps they will turn out to be rather more general than particular, after all. The broad problem with which we are grappling is that of the shift from rest to activity of a physiological system, or the reverse shift back to rest, or the ways in which this shift is altered or blocked. Somewhat differently stated, the biological system changes from one state, in which certain substances are in certain places and are there undergoing certain rates of change in amount or position, to another state in which these parameters are significantly different. In the phenomena of conduction and transmission, and in contrast to others such as secretion, the time-space sequence is of especial importance and the distances involved may be large.

For the successful spread of excitation, region A activates region B. Therefore,

some agent must move, and the question is whether nonspecific ions or specific particles are the more important in any particular instance. Even this dichotomy is somewhat artificial, for chemical changes can start currents and electrical changes can start chemical reactions. In conduction phenomena, where the distance between A and B is great, electric currents must be the main link between exciting and responding regions; over the shorter distances involved in transmission, either currents or specific chemicals could be the primary agent. Our problem then is to answer, for the response to stimulation, "What happens, where, and when?" Later we will also need to answer, "How much, and how does it come about?"

The difficulties arise in passing from such abstract generalizations to real concrete cases. Different stimuli, or even the same stimulis, may lead to different responses in different cases. Obviously, the states before and after excitation are not identical for cell, junction, fiber, etc.; if they were, all stimuli, drugs and other operators, would act alike—which they clearly do not. But even the same system may behave quite differently from one species to another —as emphasized by the results of Hebb and of Zaimis on monkey, cat, frog, and chick. Further, even in one tissue in one species, different subgroups may behave differently—witness the responses of different muscles to the methonium drugs, as described by Zaimis; the different populations of cells in a single autonomic ganglion, outlined by Shaw; the different responses of various regions of the central nervous system to the anti-esterase drugs, seen clearly in the different behavior of cord and vestibular system already noted and also in the ability of succinate to counteract mescaline hallucinations but not to restore cord function abolished in hypoglycemia; and the difference between neurones and glia in ChE type or amount, emphasized by Bülbring for neurones and glia and by Feldberg for different types of nerve cells and fibers. Finally, perhaps an even finer discrimination resides in the state of the system, aside from any anatomical difference from cell to cell. Thus, the mere repetition of drug administration leads to markedly different responses of the same system—emphasized for norepinephrine in shock by MacIntosh, for C-10 in monkeys by Zaimis, for ACh on touch receptors after C-10 by Douglas, and for ACh on the endplate response, reported some time ago by Buchthal. Perhaps the vastly different responses to different doses of the same agent also belong in this category. In any event, it is perfectly clear that there are differences in the chain of events from case to case in moving from one physiological state to another.

It may be of some help to consider further in just what ways individual cases may differ. The "chemical" and the "electrical" mechanisms turn out to fit, perhaps surprisingly, into the same rubrics. Differences can be primarily in the agent or in the receptor; and, for the agent, in kind, amount, or distribution. The kind of agent might be an ion (K, Na, etc.) or a "chemical", of the acetylcholine or epinephrine type or some other type. Epinephrine and norepinephrine are now well recognized among the sympathins, but further members may well yet turn up. Acetylcholine is now being supplemented by propionylcholine and,

as Whittaker just reported, by other higher esters of choline as well. Still other tertiary and quaternary amines are obviously involved in neural function, at least, and are formed reversibly in considerable quantities by stimulation of the cortex—as Geiger has recently shown.

Even with the same agent, differences in amount may excite or block, as emphasized by Daly and Douglas and as reported for ACh on skin pain by Buchthal and on the cat optic system by Marrazzi. We have similarly found that one gamma or less of epinephrine or norepinephrine may markedly facilitate leg movement on stimulation of the cat motor cortex, whereas 10 gamma or more is likely to give a prompt and lasting suppression. But the amount of agent reaching the receptor, as a concentration function of time, is itself complicated by the factors of release, spread, and destruction. Thus, ACh release is greatly augmented in the presence of calcium and is presumably greatly decreased by progressive fatigue, with exhaustion either of a store or a precursor. Spread is similarly influenced by diffusion conditions and, far more, by the ability to penetrate particular barriers; in turn determined by the permeability of membranes and the like. And destruction, for example by ChE in the case of ACh or by amine oxidase in the case of sympathins, needs no further comment.

Finally, the distribution of the agent and the actual geometry of the entire system may play sufficiently important roles to be mentioned, apart from kind and amount of agent. Certainly the effects of current in a volume conductor will be vastly different on long branched dendrites and on small compact end-plates, and chemical agents might also act quite differently. The distribution of materials inside or outside of membranes seems to be particularly critical in determining the behavior of a system. ATP placed outside single muscle fibers produces vigorous twitching, as long known; but injected into a fiber we have found it entirely inactive, either in changing membrane potential or producing a mechanical response. Junctions with ChE mainly in the pre-junctional unit behave quite differently from those in which it is mainly in the post-junctional unit, and in any case the enzyme is present not only in the neural membrane but throughout the cytoplasm, as Burgen showed us, and in mitochondria, as we have reported. Indeed, the mitochondria themselves have a membrane or a reasonable facsimile thereof, have ATP and vigorous oxidases and phosphorylases and so an energy source, and respond with metabolic changes comparable to those of activity when subjected to simple electric currents or pulses. Similarly, in the adrenal medulla granules, Blaschko has pointed out the tremendous concentration of epinephrine and the probability that the methylation is carried on in the mitochondria with the aid of ATP and oxidative phosphorylations. Moreover, the amine oxidase is in these particulates so that epinephrine and norepinephrine must enter cells, at least to be oxidized, and probably have metabolic actions as well as any surface effect.

Turning now from the agent to the receptor, it may be useful to distinguish between environmental effects, membrane properties, and the machinery interposed between receptor activation and final physiological response. The external environment or the internal environment of a receptor may strongly

condition its response to a given transmitter. The influences of ions on ACh and ATP action are well known, as is now the influence of cortisone on norepinephrine action, particularly on capillary vessels. Electric currents can profoundly influence irritability and response of neurones, and changes in ionic environment can in turn greatly affect these current influences. Again, the responding receptor element, presumably the membrane, may differ from cell to cell or even from patch to patch on a given cell. Feldberg recognized and aired this problem in connection with central excitation and inhibition, but certainly did not resolve it. Along the same lines is Marrazzi's evidence that the anticholinesterases, even in lethal doses, block junctional transmission in ganglia while leaving conduction intact in the fibers. Finally, even with an identical receptor in an initially identical environment, the subsequent links between its response and the final physiological response of the tissue may be quite different. Certainly, in the case of muscle, the important steps between a fall in membrane potential and the actual shortening of contractile elements are at present completely unknown.

It may be of some interest to exemplify the many ways in which excitation, inhibition, or block could be produced, and I shall attempt this for the particular case of block of the nerve-muscle response, assuming for this discussion that acetylcholine is in fact "the myoneural transmittor". I can suggest at once some ten different mechanisms for block, so that stimulation of a motor nerve does not result in a contraction. First, the impulse may never reach the nerve terminal, dying out in the fine peripheral twigs—as certainly can occur in inhibition in the central nervous system. Second, even though the impulse reaches the terminals the transmittor may not be liberated—it may no longer be present, due to fatigue, or excessive magnesium may be present (Buchthal). Or, conversely, it may, third, be liberated in excess and so jam the works—as long ago urged by Rosenblueth for the myoneural junction, and as supported in this symposium for ganglia by Feldberg, for skin by Buchthal and for central nervous system by Marrazzi. Or, fourth, the ACh, although liberated in normal amount, is destroyed prematurely—presumably by excessive quantity or availability of esterase present. Fifth, although normal amounts of ACh are released at the proper sites, they are unable to reach the receptor in normal fashion. I do not recall what hypertonic sucrose does to myoneural conduction, but I expect it could produce block by shrinking the structures and producing an actual separation of nerve terminals and muscle receptors. Sixth is the situation in which ACh reaches the receptor normally enough but is unable to produce the usual effect on it. This is presumably involved in the action of curare and perhaps of quinidine. Seventh, perhaps only a variation of the above but a more general case, the receptor is stabilized so that even a proper response to the transmittor is not sufficient to produce full activation. This might correspond to the nondepolarizing block emphasized by Foldes and reminds one that methyl fluoroacetate blocks nerve by greatly increasing threshold, without altering membrane potential, while iodoacetic acid blocks by progressively lowering membrane potential. Or, eighth, the receptor might respond to ACh in the normal manner,

say by becoming highly permeable to ions, and yet fail to depolarize because an insufficient population of ions is available to carry the necessary currents. Block in the absence of sodium or potassium might be of this type. Again related, but actually different, would be, ninth, the failure of the fully activated receptor to generate local currents sufficient to initiate the further steps of a response. Thus propagation in the muscle fiber will not occur if the external resistance is made excessively high, by immersing the fiber in oil or in air. Tenth and last, a perfectly good membrane activation may sweep down the muscle fiber and yet fail to produce shortening because the intervening steps are jammed. Local contractures in the myofibrils might be an example of this sort.

When these many stages in the transmission of excitation from nerve to muscle at the relatively simple myoneural junction are thus picked out, and some ways (more could easily be introduced) in which the system can be interrupted are noted, it does not seem probable that any one agent or system will be responsible for all types of excitation or that its exact opposite will be responsible for all types of inhibition. Different agents, different receptors, different impulses, different geometry, and different membrane properties and environment will insure that successful overriding generalizations remain few and that the particulars of each case will long depend on experimental demonstration rather than on logical inference. And in support of this, perhaps somewhat gloomy, conclusion, I offer those of you who have patiently stayed through the normal lunch period the second verse of the C-6 man, promised half an hour earlier.

The charge on his cells is depressed,
ACh seems the end of the quest.
But when error is blocked
And the full truth unlocked
We may still find we've been second guessed.