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THE ARGUMENT FOR CHEMICAL MEDIATION OF NERVE IMPULSES[1]

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WHEN an impulse travels along a nerve it is attended by a quick rise of negative electrical potential, followed by a quick fall. The duration of this "spike potential" includes the absolutely refractory and the relatively refractory periods of nerve function. The fall is not immediately to zero, but is checked by a slower, negative after-potential. And that in turn is followed by a longer, positive after-potential. The changes are more rapid in fibers of large diameter than in small fibers; the spike lasts only 0.4 msec. in the fastest fibers (*e.g.*, those supplying skeletal muscles) and thereupon the nerve can be stimulated again.[2] As elsewhere in the body, such electrical phenomena are signs of physicochemical or chemical processes which accompany functional activity. In nerve there must be to a large extent a restoration of the resting state, when an impulse has passed, before another impulse can traverse the same course. Associated with the electrical phenomena of nervous activity is a use of oxygen, an output of carbon dioxide and a display of heat. Since a nerve soon ceases to transmit impulses in the absence of oxygen, it is reasonable to assume that the increased metabolism, demonstrable when a nerve functions, indicates that chemical work is involved. According to evi-

[1] The annual Alpha Omega Alpha address, given on October 13, 1939, at the celebration of the fiftieth anniversary of the founding of the Medical School of the University of Minnesota.

[2] H. S. Gasser, Harvey Lectures (Baltimore), 32: 169–174, 1937.

dence furnished by Schmitt and Gasser,[3] the negative after-potential depends on active oxidation. It is depressed by asphyxia, and after asphyxia it is much augmented by conditions which favor oxidation. And when this potential is augmented the rate of recovery is hastened. It is highly probable, therefore, that even in the subsidence of the spike potential—for in fibers of fast conduction the negative wave "is first seen at its maximal value"[4]—an oxidative restorative process occurs, essential for returning to the nerve the ability to act again.

The electrical theory. The spike potential, sweeping along a nerve which has been effectively stimulated, causes an electric current to run, in a conductor external to the nerve, from inactive points to the active region, the "action current." It is assumed that a polarized state exists in the surface membrane of each nerve fiber (the outer charges positive); that a stimulus decreases or abolishes the potential difference across the membrane at a node of Ranvier, thus making it permeable and depolarizing it; and that thereupon adjacent nodes of the fiber discharge through the node already discharged so that new nodes in turn become permeable, depolarized and activated, and cause next proximal nodes to do the same. That the circulating local currents resulting from this traveling negative electrical wave can cause stimulation of successive portions of a nerve has been proved by Hodgkin,[5] who has demonstrated that, when an impulse reaches a region blocked by cold or compression, the local currents extend beyond the block and set up there a change of potential with an accompanying change of excitability. Blair and Erlanger[6] have brought supporting evidence by showing that an action current will pass an inert polarized region of the nerve and stimulate the responsive segment beyond. According to the traditional theory of transmission of influence from neurones to other neurones (as in a sympathetic ganglion) or from neurones to the cells of an effector organ (a muscle or gland), that is the sort of process which occurs—it is supposed that the local circuits reach beyond the limits of the active nerve and excite, electrically, the next element in the series.

At the synapse between neurones and effectors there is evidence of a protoplasmic discontinuity. As Bronk and Brink[7] and also Forbes[8] have pointed out, this introduces a physical condition not found in Hodgkin's experiments. As Forbes has remarked, "The structural and presumably the electrical conditions are quite different in an unbroken but inactive axon from those at the synapse. Here, at the termination of the neurone, histology seems to reveal a transverse membrane, which may well act as a short circuit to the action potential. . . . If the membrane theory of nerve conduction holds good, there is every reason to expect such a short-circuiting effect at the termination of the axon." Furthermore, Rosenblueth and I[9,10] have shown that smooth muscle—that of the nictitating membrane—though readily responsive to a single nerve volley, is, after being deprived of its nerves, quite unresponsive to a single electric shock. Similarly, the cells of a gland (the adrenal medulla in our tests), easily activated by impulses delivered by their nerve supply, are, after denervation, affected to only a very slight degree, even when electric shocks are applied so powerful and prolonged as to injure the tissue. Obviously, there are synapses at which the possibility of stimulation by the action current appears to be highly improbable.

The chemical theory. In recent years evidence has accumulated that when an impulse arrives at a nerve ending it sets free a chemical substance—adrenaline at sympathetic synapses, acetylcholine at parasympathetic synapses and at synapses in motor end plates and in sympathetic ganglia—and that these substances rouse in the next distal element its typical reaction—secretion, contraction, relaxation or nervous conduction. Two theories of synaptic transmission have been proposed—the traditional electrical theory and the newer chemical theory. Each theory has its ardent advocates, and a vigorous controversy prevails between them. For purposes of the present discussion these advocates may be called the "electragonists" and the "chemagonists"—"agonist" meaning contestant or combatant!

There is agreement of the agonists on certain points. In order to simplify the consideration of these points, and also the points of difference, I propose to limit the present discussion to synapses in which there is evidence that acetylcholine is involved. The liberation of this substance at parasympathetic synapses and at the synapses of sympathetic ganglia and motor end plates, on the arrival of nerve impulses, is frankly admitted by the electragonists. They also admit that tissues where acetylcholine appears contain a cholinesterase which quickly destroys that highly unstable combination of choline and acetic acid. They admit, further, that acetylcholine, when perfused in very minute amounts through organs in which it is dis-

[3] F. O. Schmitt and H. S. Gasser, *Am. Jour. Physiol.*, 104: 320, 1933.
[4] H. S. Gasser, *loc. cit.*
[5] A. L. Hodgkin, *Jour. Physiol.*, 90: 193, 211, 1937.
[6] E. A. Blair and J. Erlanger, *Am. Jour. Physiol.*, 126: 97, 1939.
[7] D. W. Bronk and F. Brink, *Ann. Rev. Physiol.*, 1: 398, 1939.
[8] A. Forbes, *Jour. Neurophysiol.*, 2: 465, 1939.
[9] A. Rosenblueth and W. B. Cannon, *Am. Jour. Physiol.*, 108: 384, 1934.
[10] W. B. Cannon and A. Rosenblueth, *Am. Jour. Physiol.*, 119: 221, 1937a; *C. r. Soc. de Biol.*, 124: 1262, 1937b.

charged by nerve impulses, will influence these organs as do the impulses themselves. They admit, likewise, that when present in excess acetylcholine has not a stimulating but a paralyzing action. And, finally, they admit that the acetylcholine naturally set free at parasympathetic endings in slowly reacting structures —*e.g.*, in the heart, smooth muscle and glands—is the agent which affects these structures. The main difference now concerns the rôle of this mediator in the transmission of influence from nerves to skeletal muscle cells and from nerves to nerve cells at ganglionic synapses. Electrical stimulation will, indeed, excite both muscle cells and nerve cells; and therefore the action current, if it reaches with effective intensity beyond the junctions, would act as a stimulus. But, if that is the mode of transmission, why the discharge of acetylcholine by the nerve impulse? This is where theories begin to conflict.

In evaluating two antagonistic theories, nothing is to be gained by a recital of the phenomena which both can explain. Deeper insight into the processes concerned in the theories is likely to result from a survey of the phenomena which only one, or neither, can account for. On that basis we may proceed to compare the two theories—electrical and chemical—as explanations of observed facts.

A comparison of the electrical and the chemical theories. First, the analogy between evidence for chemical mediation in the slowly responsive heart and evidence for it in rapidly responsive striate muscle and sympathetic ganglia. In all three structures a perfusate obtained while they are not stimulated contains no demonstrable acetylcholine. In all three, nerve-stimulation causes the appearance of acetylcholine, though eserine may have to be used to prevent its sudden destruction by cholinesterase. In all three, perfusion with acetylcholine induces the typical effects of nerve impulses—inhibition of the heart beat, contraction of skeletal muscle and discharge of ganglion cells. And in all three the effect of acetylcholine can be blocked by drugs, without preventing its liberation at nerve endings—in the heart by atropine, in striate muscle by curare and in the ganglion by nicotine. Under these conditions, with nerve impulses delivered to the tips of the active nerve fibers, as shown by the discharge of acetylcholine there, and with the recipient elements still sensitive to electrical stimulation, the action current is without influence.

The practical identity of evidence for chemical mediation in the three regions raises pertinent questions. If the action current is the true transjunctional stimulus, what is the use of acetylcholine, regularly produced during stimulation? How is the efficacy of minute amounts of acetylcholine, as a stimulus to ganglion cells and muscle fibers, to be accounted for? Why should there be a concentration of cholinesterase in sympathetic ganglia and at the motor end plate (*cf.* Marnay and Nachmansohn, 1937;[11] Feng and Ting, 1938?[12] The electragonists surely face difficulties in answering these questions, difficulties not encountered in the chemical explanation. The orthodox electragonists, moreover, are inconsistent; they agree that acetylcholine is a deputy of nerve impulses at vago-cardiac synapses, but deny it that function for neuromyal synapses. Furthermore, they confront a dilemma—on the one hand, agreement that acetylcholine is a chemical mediator of nerve impulses, and on the other, admission that the wide-spread arrangements for its routine production and prompt destruction at synapses, where it can stimulate, are merely a false and futile show. In attempted avoidance of this dilemma hypothetical suggestions of imaginary functions have been offered, *e.g.*, that acetylcholine may serve to increase excitability at synapses, or to delay the onset of fatigue, or to dilate blood vessels locally, or to exert vague "trophic influences."[13] Perhaps it "may"; but it can demonstrably do things to muscle cells and to sympathetic neurones; it can excite in them their peculiar functions. Confessedly, the argument implied in emphasizing the presence and capacity for action of acetylcholine at synapses is based on the reasonableness of means being adapted to ends. In biology, however, that argument is so generally valid as to be, in special cases, quite respectable.

The electragonists have set up what they regard as a serious obstacle to the chemical theory in stressing the brevity of the latent period of the postsynaptic elements before action occurs. In supporting this criticism Fulton[14] has expressed the judgment that "synaptic transmission is probably brought about directly by the action currents of the axon terminal rather than by a specific humoral agent such as acetylcholine," which, in his opinion, "appears to be a by-product of nerve metabolism." It is true that the synaptic events are very brief. In the neurones of the superior cervical ganglion the latent period of the most rapid responses ranges from 2 to 4 msec.,[15] and in the fibers of some skeletal muscles it can be shorter, 1 to 2 msec. (Eccles, footnote 13). If acetylcholine is the transmitter it must flash forth and excite in that brief time; and since a single nerve volley induces a single response the acetylcholine must be quickly rendered ineffective during the refractory phase of the responding elements (which lasts in neurones and muscle fibers about as long as the latent period) be-

[11] A. Marnay and D. Nachmansohn, *C. r. Soc. de Biol.*, 125: 41, 1937.

[12] T. P. Feng and Y. C. Ting, *Chinese Jour. Physiol.*, 13: 141, 1938.

[13] J. C. Eccles, *Physiol. Rev.*, 17: 538, 1937.

[14] J. F. Fulton, "Physiology of the Nervous System." New York, 1938.

[15] G. L. Brown, *Physiol. Rev.*, 17: 485, 1937.

fore another nerve volley can act again. The time is too short, the electragonists assert, for these chemical changes to occur. It is pertinent to point out that this is an assumption. Too little is known of the speed of chemical processes at synapses to justify categorical limitations. Furthermore, the electragonists neglect the fact that the nerve fiber itself, when it transmits an impulse, must recover before it can transmit another. Evidence indicates, as we have noted, that this recovery is an oxidative chemical process, and in fast mammalian fibers it lasts less than 1.0 msec., *i.e.,* it may be much more rapid than the transmission at ganglionic and muscular synapses, which the electragonists assume to be too rapid to be chemical. Until they can offer better evidence for their assumption they have a frail basis for belittling the speed of intimate molecular changes.

The argument that the delay at synapses is too short for the processes required by the chemical theory can be turned against the electragonists. When an electric current is applied to a nerve, the interval between the instant of shock and the discharge of the nerve impulses ranges between 0.2 and 0.4 msec. The delay between the arrival of the impulse at the superior cervical sympathetic ganglion and the discharge of the ganglion cells is at least 5 times as long as that for the quickest cells and at least 11 times as long for the next quickest.[16] If a weak electric current can activate the nerve fibers almost instantly, and the electric action current activates the ganglionic neurones, as the electragonists argue, why this protracted latent period? The relatively long delay in the ganglion is matched by a similar delay in the motor end plate. Here the electragonists have a real problem. The chemagonists, on the other hand, can readily account for the extra time as due to the requirements of an interposed chemical mediation.

At this point may be mentioned an attempt to bring electrical events in the ganglion into relation to electrical events in the nerve trunk. As shown by Gasser and Erlanger[17] and Graham and Gasser,[18] nerves have a heightened electrical excitability during the negative after-potential and a decreased excitability during the subsequent positive after-potential. These two phases, negative and positive, following the spike potential, can also be recorded from the ganglion after it has been stimulated; and Eccles[19] has claimed that submaximal volleys of nerve impulses delivered to the ganglion during its negative after-potential evoke smaller spikes than the original. These observations, if confirmed, might bring support to the electrical theory. In the present discussion a detailed review of the evidence and arguments against Eccles's contentions is impossible. Suffice it to remark that Rosenblueth and Simeone[20] have recorded variations of the spike potentials of the ganglion, responding to maximal and submaximal stimulation, that were precisely opposite to what the inferences of Eccles would predict—maximal spikes when the ganglion was maximally positive, and noteworthy decrease of the spikes at the peak of the negative phase. Furthermore, they noted that by altering conditions the synaptic delay could be shortened to less than 1 msec. or increased to twice its usual length. Because of the lack of correlation between after-potentials and the responsiveness of the ganglion cells and also because of the large variations in synaptic delay, as well as for other reasons, Rosenblueth and Simeone concluded that the electrical theory, instead of being in agreement with observed facts, is definitely opposed by them.

Evidence for the chemical theory from experiments with curare. The action of curare confronts the electragonists with a perplexing question. As is well known, this drug is capable of paralyzing muscles by interrupting the nervous influence at the neuromuscular junctions. It does not, however, prevent the discharge of acetylcholine at the nerve endings; the nervous action currents, therefore, run their full course. And with small doses of curare the inherent irritability of the paralyzed muscle is not altered.[21] Yet the muscle does not respond to the nerve impulse. The paralyzing effect of curare at the motor end plate offers no difficulty for the chemical theory. What curare does is to reduce the sensitiveness of the responsive element in the plate. In studies on denervated skeletal muscle Rosenblueth and Luco[22] demonstrated that even with doses too small to stop action of the respiratory muscles curare markedly lessens the contractions resulting from uniform injections of acetylcholine. And the same phenomenon is seen in normally innervated skeletal muscle.[23] In short, curare acts as if it raised the threshold to acetylcholine. Then the normal amount of the mediator which is released by the nerve impulse is incapable of causing the muscle to contract. Thus, while a curare paralysis displays a phenomenon hard for the electragonists to explain, it forms a part of the factual support for chemical transmission.

[16] J. C. Eccles, *Jour. Physiol.,* 85: 179, 1935.
[17] H. S. Gasser and J. Erlanger, *Am. Jour. Physiol.,* 94: 247, 1930.
[18] H. T. Graham and H. S. Gasser, *Proc. Soc. Exp. Biol. Med.,* 32: 553, 1934.
[19] J. C. Eccles, *Jour. Physiol.,* 85: 464, 1935; 88: 1, 1936.
[20] A. Rosenblueth and F. A. Simeone, *Am. Jour. Physiol.,* 122: 688, 1938.
[21] H. Grundfest, *Jour. Physiol.,* 76: 95, 1932.
[22] A. Rosenblueth and J. V. Luco, *Am. Jour. Physiol.,* 120: 781, 1937.
[23] G. L. Brown, H. H. Dale and W. Feldberg, *Jour. Physiol.,* 87: 394, 1936.

Recent studies on curarized end plates present the electragonists another hard problem to solve. In experiments performed by Luco and Rosenblueth[24] two symmetrical muscles (*e.g.*, the soleus of each side) were arranged to record simultaneously. After a paralyzing dose of curare had been injected the motor nerve of one of the muscles was stimulated continuously at the rate of 60 shocks per sec. When the control muscle was periodically tested through its nerve, and by its response proved that complete recovery from curarization had occurred, the continuously stimulated muscle was usually not contracting at all. If given a brief rest and then again subjected to the stimulation, it contracted momentarily and thereupon promptly became inert; *i.e.*, it manifested extreme fatigue. Clearly, this was not a fatigue of the muscle, for the curare throughout the whole period of stimulation had protected the muscle from being forced into activity. And it was not a fatigue of the nerve, for that remarkable structure can carry impulses at the rate of 60 per sec. during an indefinitely long time. Furthermore, records of the spike potentials proved that the nerve impulses might be full-sized when they were having no effect. The fatigue occurred at the point of transmission. Even if curare should somehow block the nerve action current, the electragonists would have trouble solving this riddle, because the curare block disappeared while the continuous stimulation of the nerve was evoking no muscular contraction. In other words, at a time when both the electrical excitability of the muscle and the spike potentials of the nerve were quite normal, the nerve impulses failed to stimulate. Before this situation the electrical theory breaks down. The chemical theory, on the other hand, has no trouble in explaining it. Prolonged rapid stimulation results, after an initial large outburst of acetylcholine, in a gradually reduced discharge until the concentration falls below the effective range.[25] In these circumstances, according to the chemical theory, a muscular response would not occur. And it does not.

Evidence from experiments with eserine. In order to introduce another important series of facts supporting the theory of chemical transmission at muscular and ganglionic synapses, I wish to present evidence regarding the action of eserine. (That term will be used as equivalent to physostigmine and prostigmin). It will be recalled that eserine is able to protect acetylcholine against destruction by a cholinesterase. It will be recalled, also, that if acetylcholine is present in excess it is not a stimulating agent but a depressant.

As Rosenblueth and Morison[26] demonstrated, eserine has quite opposite effects on two symmetrical muscles, one recording responses to slowly repeated nerve impulses, the other responding to similar but rapid stimulation. A small intravenous injection of eserine causes the less frequently stimulated muscle to contract more strongly, and simultaneously it causes the corresponding, more frequently stimulated muscle to contract less strongly. This very striking difference of effect is not due to altered action currents, for the intensity of the nerve spike potential is not affected by eserine. Eccles (*Ergebn. d. Physiol.*, 38: 339, 1936) has suggested that eserine exerts its influence by increasing the sensitiveness of the responding structures. Obviously that suggestion does not apply to the depressant effect on the rapidly stimulated muscle. That effect has its explanation in the change produced in the functioning of such a muscle when acetylcholine is injected so that it is present in excess; the contractions are depressed as they are depressed by eserine. The action of eserine, therefore, can reasonably be accounted for, in these circumstances, by its shielding of the acetylcholine as it is set free by the nerve impulses, against attack by cholinesterase. Since the impulses are quickly recurring, the time for destruction is abbreviated, and with protective eserine at hand, the acetylcholine accumulates until it reaches a concentration which paralyzes some of the end plates. Thus would the chemagonists clear up the phenomenon.

But how account for the opposite effect in the slowly stimulated muscle? That, again, finds an explanation in the shielding of acetylcholine by eserine. Under normal conditions a single volley of nerve impulses evokes a single response in a muscle, as registered by the spike potential of the muscle fibers. If eserine is administered, however, a single volley from the nerve induces a repetitive response and hence a greater muscular shortening. As Brown, Dale and Feldberg[27] have pointed out, this sort of response to single volleys after eserine may reasonably be attributed to the persistence of the acetylcholine released by the motor impulse at the neuromuscular junctions.

The only difficulty which the chemagonists meet in clarifying the remarkably opposed effects of eserine on slowly and on rapidly stimulated muscles is that of understanding why these effects are prolonged. It is known that potassium ions are liberated simultaneously with acetylcholine, and they, as persistent agents, may play a rôle not yet evident. Whatever may be the final chapter of this story it is clear now that the results of injecting eserine are such as to

[24] J. V. Luco and A. Rosenblueth, *Am. Jour. Physiol.*, 126: 58, 1939.

[25] G. L. Brown and W. Feldberg, *Jour. Physiol.*, 88: 265, 1936.

[26] A. Rosenblueth and R. S. Morison, *Am. Jour. Physiol.*, 119: 236, 1937.

[27] *Loc. cit.*

place still unsurmounted obstacles in the path of the electragonists, while presenting the chemagonists additional support for their views.

Evidence for the chemical theory from experiments with different rates and periods of stimulation. Thus far the discussion has been concerned mainly with transmission at neuromuscular synapses. Many years ago Elliott[28] called attention to striking resemblances between characteristics of motor end plates and of cell bodies in sympathetic ganglia. For example, nicotine in small doses stimulates at both places, and in larger doses it paralyzes both; curare blocks transmission to skeletal muscle, and in greater concentration it has a similar influence in the ganglion; and, whereas the postganglionic axons can not replace motor fibers, the preganglionic axons, effective on the ganglionic cells, can be cross-sutured and will grow out and make good functional union with striated muscle cells. To these resemblances we can now add the following: acetylcholine in small doses excites at both synapses; and when present in excess acetylcholine, like nicotine, has a depressant or paralyzing influence. We have learned recently that there are still other remarkable similarities between these two transmission points. It will be convenient, therefore, to consider them together.

As shown by Rosenblueth and Morison,[29] if a muscle is stimulated through its nerve at a rapid rate—*e.g.*, 240 shocks per sec.—the muscle first shortens in a sharp strong contraction, then changes promptly to a low contraction or fails to contract at all, and thereupon it shortens in a strong contraction again. Thus with recurring impulses of a constant high frequency the muscular response manifests three distinct stages—a sequence of plus, minus, plus. Now if the stimulation is continued the muscle enters a well-known fourth stage, that of lessened efficiency or fatigue. If the stimulation is at a slow rate from the start, stages 2 and 3 drop out; then stage 1 merges directly with stage 4. Curiously enough, as revealed in the experiments of Rosenblueth and Luco,[30] continuance of the stimulation at a slow fatiguing rate (60 per sec.) causes the muscle to enter gradually a fifth stage in which its performance progressively improves; the contractions increase in strength—displaying a tension as great as 60 per cent. of the highest tension developed at the beginning—and this betterment may last for several hours.

By use of the nictitating membrane of the cat as an indicator of the discharge from the superior cervical sympathetic ganglion the effect of stimulation of preganglionic fibers on the ganglion cells can be examined. In experiments which Rosenblueth and I[31] performed we were able to duplicate, with rapidly repeated stimuli, the first four stages—plus, minus, plus, minus—seen in skeletal muscle. The only difference was a preliminary dose of eserine in experiments on the ganglion. And a few months ago Lanari[32] and Rosenblueth succeeded in recording in the ganglion performance the fifth stage, that of partial recovery of the original efficiency, if only the fatiguing stimulation is patiently continued.

Here is a challenging sequence of events. Why do muscle cells and ganglion cells, when continuously stimulated, respond well at first, then poorly; well again, then poorly again; and finally well for a long period? As proved by Rosenblueth and Luco,[33] who took records of the nerve action potentials periodically during four or five hours of stimulation, there may be a slow diminution in the height of the spikes or there may be no diminution, but in no case is there any correlation between the electrical phenomena of the nerve and the various stages of muscular activity. These conditions at motor end plates and at ganglionic synapses—and with normal blood supply, let it be noted—disclose a puzzle which the philosophy of the electragonists has not dreamed of. What clue to its solution can the chemagonists present?

As previously remarked, when a nerve is continuously stimulated there is at first a flush discharge of acetylcholine at the nerve endings, and then the discharge gradually diminishes. Now if the stimuli are repeated at very high frequency time will be lacking for destruction of the relatively abundant acetylcholine by cholinesterase; thereby the initial great outburst becomes accentuated and the acetylcholine accumulates until it is present in a paralyzing concentration. Thus, the primary contraction of the muscle (stage 1) is quickly followed by less contraction or an actual failure to contract (stage 2). In the ganglion a small dose of eserine, protective against the destructive cholinesterase, allows the acetylcholine to act in the same manner, so that it has the same paralyzing effect. As the primary outburst quickly subsides, the depressant concentration of acetylcholine ebbs away, to be replaced by an optimal lower concentration which is highly stimulative; hence the rise of the muscular tension into stage 3. That this is the correct explanation is proved by the injection of a small amount of acetylcholine during stage 2, whereupon the depressed condition is prolonged; or during stages 1 or 3, whereupon, not stimulation, but, instead, depression is induced. The fourth stage, the stage of fatigue, can be explained by so great a reduction in the output of

[28] T. R. Elliott, *Jour. Physiol.*, 35: 367, 1907.
[29] *Loc. cit.*
[30] *Loc. cit.*
[31] See footnote 10.
[32] A. Lanari and A. Rosenblueth, *Am. Jour. Physiol.*, 127: 347, 1939.
[33] *Loc. cit.*

acetylcholine that it fails to stimulate some of the cells; hence the lessened degree of response—*i.e.*, the appearance of "fatigue." That this interpretation is correct is proved by injecting at this stage the same dose of acetylcholine as that which caused depression in stages 1 and 3 and finding that it now causes an improvement of the response. This, of course, is strictly in accord with the theory of chemical transmission.

The astonishing recovery of function in the fifth stage has been investigated by Rosenblueth, Lissák and Lanari.[34] They have assayed the acetylcholine content of nerves, quickly frozen *in situ* by carbon-dioxide snow, or instantly excised and dropped into liquid air (in order to prevent any change), after continuous, rapid stimulation for various lengths of time. They found a progressive decrease in the content during the first ten minutes—a decrease which turned to an increase when there was a brief period of rest. In the fifth stage the concentration of acetylcholine had gradually increased over that in the fourth stage. If, now, it is assumed that depolarization of the surface membrane of the nerve fibers is attended by a greater permeability for the acetylcholine contained within them, the output per impulse would vary directly with the concentration. In the fifth stage, when the fibers have more acetylcholine, they give forth more, and the result is a larger response. This is the view presented by Rosenblueth, Lissák and Lanari. It is substantially based on the agreement between the evidence of acetylcholine liberation from nerves at the different stages of stimulation and the evidence of concentration of acetylcholine in nerve fibers.

I have described a group of events in the transmission of nerve impulses from neurone to neurone and from neurone to muscle cell. I can find no adequate explanation for these perplexing events in terms of the electrical theory. On the contrary, I can see that supporters of the theory of chemical transmission need only use demonstrated facts in order to give a reasonable answer to the whole intricate riddle.

Evidence for the chemical theory from degenerating nerves and muscles. The main assumption in the foregoing account of the five stages of transmission at peripheral synapses was that the output of acetylcholine per nerve impulse would vary with its concentration in the nerve fiber. The recent research by Rosenblueth, Lissák and Lanari, *loc. cit.*,[34] already noted, has furnished evidence that when a nerve has been cut and is undergoing degeneration there is a gradual reduction of the acetylcholine content after the first twenty-four hours. A highly significant fact is that during these first twenty-four hours no functional change is observable. As the concentration of acetylcholine diminishes in the second twenty-four hours, however, the possibility of evoking stage 5 is regularly lost, and stages 2 and 3 may largely disappear or be wholly absent. *At the same time the nerve spike potentials are of normal intensity.* The results are quite in accord with the assumption mentioned above, for, as will be recalled, tests revealed that stages 2 and 3 were due to an excess of acetylcholine and stage 5 to an improved production of it. In the degenerative process, with the acetylcholine of the nerve gradually becoming less, these periods of excessive production fade out and as they do so the attendant variations of activity likewise fade out. And, as might have been anticipated, the fourth stage, that of fatigue, becomes unduly emphasized—the transmission from degenerating nerves "fatigues" much sooner than normally. Now the further important fact emerges that while nerves in the late steps of degeneration can still conduct, as revealed by a definite, though reduced, spike potential, they may not stimulate across the synapse. All these observations harmonize perfectly with the chemical theory of transmission and find no illumination whatever in the electrical theory.

Perhaps no further testimony is needed to justify the claims of the chemagonists. There is one other observation, however, which is demonstrative and well worth presenting. As is now generally recognized, autonomic nerves do not end on striated muscle. They do end on blood vessels—vessels intimately distributed throughout striated muscle fibers. Among the autonomic nerves which give off acetylcholine is the lingual, supplied to the vessels of the tongue. In 1937 Rosenblueth and Luco recorded the action potentials of the tongue muscles which had been deprived of motor nerves for about a week. Stimulation of the lingual nerve induced the appearance of typical action currents in the completely denervated muscle fibers. Here is a stimulation of muscular units clearly not due to electrical transmission, for the nerve stimulated was not distributed to the muscle which responded, and, furthermore, the latent period (60 msec.) was too long for that occurrence. It was a clear instance of a contractile response to acetylcholine, liberated by nerve impulses, to be sure, but not nerve impulses delivered to the contracting fibers. Electrical transmission was definitely excluded. Until the electragonists can display an instance of electrical transmission without acetylcholine at neuromuscular and neuroneuronal synapses their argument can not be on the same footing as that of the chemagonists.

Meanwhile they have arduous tasks in explaining away the evidence which has repeatedly proved the adequacy of the chemical theory of nervous transmission.

[34] A. Rosenblueth, K. Lissák and A. Lanari, *Am. Jour. Physiol.* In press.