

is capable of doing so. We have also noticed that with the *Torpedo* organ we do not obtain true spike responses to acetylcholine. Perhaps we should arrive at a better understanding of its behaviour if we admit that this electric plate is mainly responsive to a co-operation of both actions. This is an idea that has not yet been submitted to direct experiment, but the data just mentioned are indirect indications of its value, nerve actions being taken as synonymous with acetylcholine action.

Finally, it is suggested that such co-operation, if it exists, may be only an example of these variously combined electrochemical operations that seem to characterize all synaptic transmissions and contribute to the diversity of their behaviour. What appear to-day as oddities in electric organs, when one compares them to muscles, may receive future explanation in terms of secondary factors, but these oddities may well be nothing but peculiarities that unfamiliar objects owe to their specific chemical, electrical and structural properties, for there is no reason why they should exactly conform to models suggested by more familiar junctions.

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## ASPECTS OF EXCITATION AND INHIBITION IN THE RETINA

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The work to be reported was begun twenty years ago, and my own contributions are all of a relatively early date inasmuch as they concern the retinal electrical response to illumination, the so-called electroretinogram. They were, in fact, made at the time when the retina was still mainly studied by inference from psychophysical experiments. Adrian & Matthews (1927*a, b*, 1928) had reported their pioneer work on the impulse activity in the eel's optic nerve, and this, of course, was the great landmark from which to start the journey into the unknown.

It seemed to me that in a structure such as the retina there must be processes of inhibition at work. The question was merely how to proceed in order to demonstrate it.

Since the attempts to analyze the electroretinogram provided the first key to the problem and is likely to provide the last key also, let us consider excitation and inhibition from this aspect first. It should be well known to-day that it proved to be possible by several means to establish that light induces in the retina two sequences of slow potential, *P*II and *P*III, which are of opposite electrical sign. There is also a still slower component *P*I which need not concern us. The methods used and the results obtained have been reviewed elsewhere (Granit 1947). With respect to the corneal lead we call *P*II positive and *P*III negative, but, since the vertebrate retina is inverted, these conventions, used by all, are inadequate.

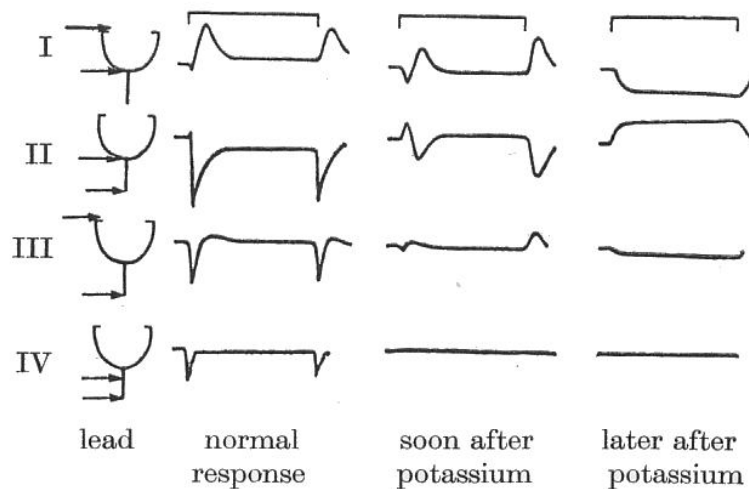


FIGURE 14. The effect of potassium chloride on the frog electroretinogram in different leads. Upper electrode positive plotted upwards.

*P*III appears to be faster than *P*II. Hence the electroretinogram generally begins with a negative dip of *P*III, then turns positive (*P*II), and runs its course as a balanced state varying with type of animal, state of adaptation, etc. Thus cone eyes tend to have large negative components, *P*III, and the state of balance may therefore become adjusted below zero level. The electroretinograms have a negative value. In rod eyes the electroretinograms are largely positive (*P*II). At cessation of illumination the negative component *P*III swings back and we succeeded in demonstrating (Granit & Therman 1937) that the positive off-effect in the frog eye involves fresh activation of *P*II and cannot be explained merely on the principle of algebraical summation of components disappearing at different rates. The positive *P*II was shown in several ways to correlate with excitation. My first task is to consider the evidence relating *P*III to inhibition.

To begin with, why is it of opposite sign relative to *P*II? Is this because of a different orientation of the structures concerned or because the membrane change itself is of an opposite character? Figure 14 summarizes the results of an experiment with a well-known depolarizing agent such as potassium chloride. It was presented at a meeting of the Physiological Society in Cambridge (Granit &

Therman 1938). Four arrangements of leads are indicated on the left, and the period of illumination is shown above each vertical row. I is the standard lead of electroretinography, producing the normal response of the frog's eye, in which 'on' and 'off' appear as positive deflexions and the initial negative dip is indicated. IV is the optic nerve lead, applied some distance from the bulb so as to exclude the electrotonic potential. In the frog eye the main discharge occurs at 'on' and 'off' with relatively little in between. It is recorded at low amplification with the d.c. amplifier used for all leads and is downwards because in all leads 'upwards' means that the upper electrode has turned positive relative to the lower one. II is an interesting lead inasmuch as it adds the large electrotonic component, absent in normal electroretinograms, to the total response.

We proceed to add a drop of 0.5% potassium chloride to the vitreous body of the opened bulb. The second vertical row shows that this quickly depolarizes the outermost layer of nerve cells forming the optic nerve. The nerve is silenced, the electrotonic component disappears and leads I and II record identical but inverted electroretinograms in which the positive *P*II already is somewhat depressed and delayed and the initial negative dip consequently increased. Somewhat later, in the third vertical row, the positive component *P*II has disappeared and light merely elicits the negative *P*III which in the two leads I and II is in a different direction. This state of affairs lasts for a considerable time.

The interpretation of this experiment is that *P*II, as we have many other reasons to believe, is a typical excitatory depolarization which cannot take place if the structure concerned already has become depolarized. *P*III, however, can be obtained in a depolarized retina and hence cannot be a depolarization. It is, on the contrary, a process of repolarization with light. *P*III can be, as it were, laid bare by many other methods than the one illustrated, but this method appears to be particularly significant.

Another type of experiment (Granit & Helme 1939) provided further evidence for the view that *P*III is of an opposite sign relative to *P*II because it represents an opposite process and not a similar process in a structure of opposite orientation. The frog retina was placed between two non-polarizable electrodes, arranged as in lead I above, and the electroretinogram again elicited during polarization of the eye with weak currents. Normal eyes were used as well as eyes from which *P*II had been removed and which therefore responded with *P*III. Both components of the electroretinogram were found to respond similarly to the polarizing current. Inside cathode augmented both *P*II and *P*III and inside anode depressed both *P*II and *P*III. If the two component potentials had been generated in similar structures of opposite orientation the one should have been favoured by the current running in one direction and the other by the current running in the opposite direction.

Having concluded from these experiments that *P*II and *P*III are truly opposite potential changes we proceed to review the evidence for the association of *P*III with inhibition. At the time when I was working on these problems, Dr L. A. Riddell came over from England and together we found a physiological situation in which there was selective activation of *P*III (Granit & Riddell 1934). The simple

expedient used was to re-illuminate the eye during the off-effect. Figure 15 shows the large negative dips of *P*III which are obtained by these means.

During the off-effect of *P*II in the electroretinogram there is a brisk off-discharge in the optic nerve, as demonstrated by Adrian & Matthews (1927*a*). Here then was a situation in which without any interfering agent one could find out what happened in the optic nerve during activation of *P*III. At a meeting of the Physiological Society\* in 1934 Therman and I presented the result shown in figure 16. Inhibition of the off-discharge was clearly the first effect of a flash of light timed so as to fall on top of the off-effect and elicit a deep trough of *P*III.

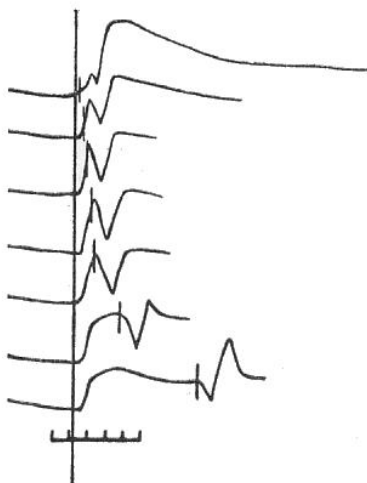


FIGURE 15. Effect of 40 ms flash, marked by vertical short lines, on off-effect. Vertical line marks end of illumination. Note the large negative dips that tend to re-establish level of potential during illumination. Time  $\frac{1}{10}$  s.

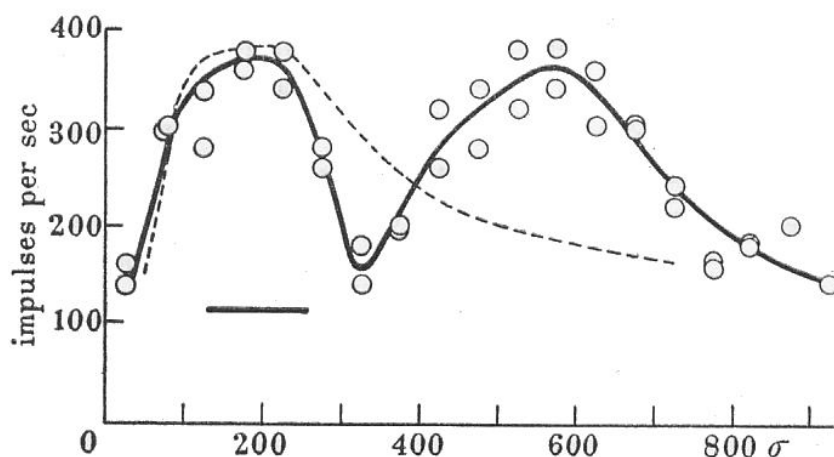


FIGURE 16. Effect of a flash (dark line, inset), superimposed on the off-discharge, on the frequency of impulses in the frog optic nerve. Dotted line: normal off-discharge. Continuous line: inhibition of discharge by the flash.

In a preliminary report of 1935, Hartline confirmed this result as far as the optic nerve was concerned. By obtaining single-fibre discharges he made the important discovery that this inhibition occurs in elements that have off-discharges. He did not work with the electroretinogram. Riddell and I had found that in frog eyes a large *P*III could be obtained by light adaptation and that it diminished in dark adaptation. Since the off-effect behaved similarly, it confirmed us in the opinion that the off-effect very largely depends upon the existence of a large and active component *P*III. Hartline's results clearly pointed in the same direction.

In alcohol Bernhard & Skoglund (1941) found a substance which selectively diminishes *P*III and, indeed, the alcohol-treated eye behaved very much like a dark-adapted eye despite its state of light-adaptation. Figure 17 shows the progressive effect of alcohol on the initial negative dip (above) and the test with the flash on the off-effect (below). The eye, after alcohol, lost its capacity for fast flicker, which requires *P*III and the concomitant inhibition, for cutting short the effect of the previous flash (Granit & Riddell 1934). Figure 18 illustrates in *a* the normal off-discharge picked up by an inside micro-electrode, in *b* the test for inhibition with re-illumination and in *c* the diminished and drawn-out off-discharge after alcohol and the likewise diminished inhibition.

In 1933 (Granit 1933) I had already developed the notion that the off-effect involves a release from inhibition when PIII swings back towards zero. To this our later work (Granit & Therman 1937) had added the fact that the frog retina

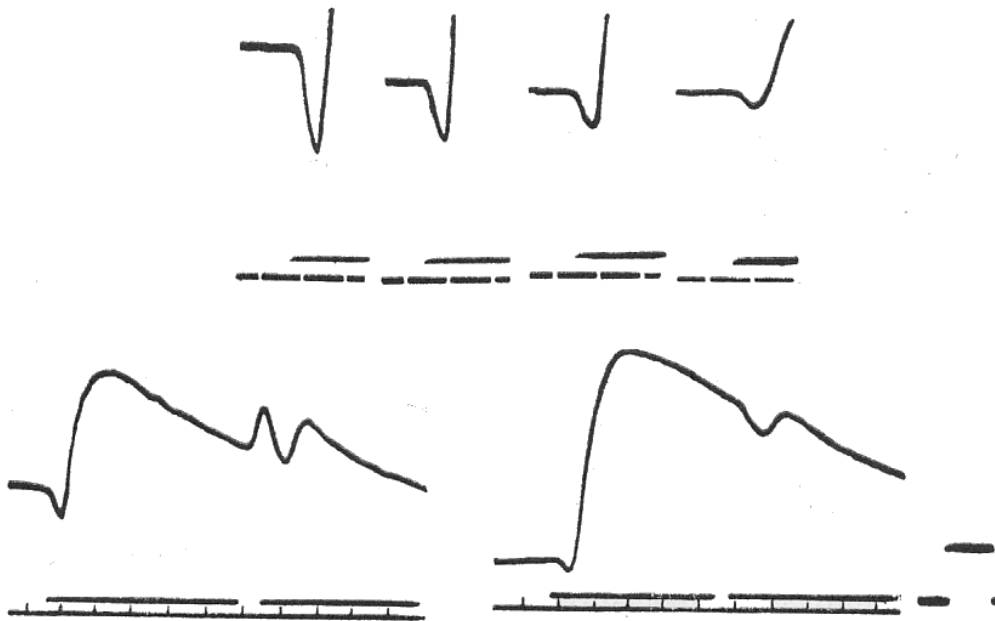


FIGURE 17. Effect of alcohol on the negative dip. *Upper*: Progressive diminution of initial negative dip (*a*-wave). *Lower*: Left, flash on normal off-effect causes large negative dip. Right, hardly any off-effect and small negative dip after alcohol. Frog eye. *Below*: stimulus signal and time in  $\frac{1}{5}$  s.

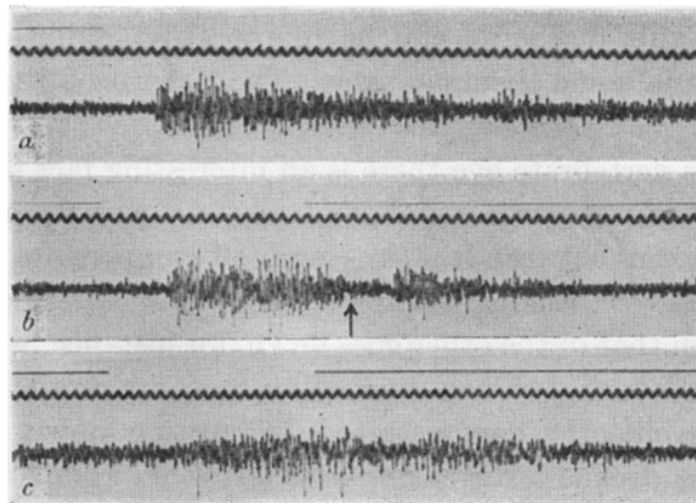


FIGURE 18. Effect of alcohol on the inhibition of the off-discharge as recorded by micro-electrode. *a*, normal off-discharge; *b*, normal initial inhibitory effect of re-illumination; *c*, decreased off-discharge and inhibition after alcohol. *Above*: light signal and time in 50 c/s.

at 'off' again swings positive above the baseline. From this point of view let us consider the important results of Parry (1947) obtained on the ocellus of a locust. This organ responds to illumination by increased polarization that can be recorded from the ocellar nerve as a positive electrotonic potential. At cessation of illumination this change is reversed, the ocellus becomes depolarized with an overswing, and a negative electrotonic component can be recorded from the ocellar

nerve. Neither change was found to be accompanied by any impulse activity in the nerve, but the polarization, due to light, and transmitted electrotonically to the next station in the ganglion cell, discharged a burst of impulses from the latter down the circumoesophageal commissural path when the positive potential reversed to negative. Here then is a clear case of polarization of a cell by light instead of depolarization, as in *Limulus* (Hartline & Graham 1932). From what is known about nerve excitation this would be expected to silence an active ganglion if it could reach it, whilst, similarly, the ensuing depolarization at 'off' would be expected to activate the ganglion. The distance between ocellus and ganglion, bridged by the exceptionally large ( $25 \mu$ ) ocellar nerve, is only 1 mm. This is a mechanism of the kind that was postulated for the retina on the evidence reviewed.

Before returning to other aspects of inhibition in the vertebrate retina something should be said about Hartline's recent lateral inhibition in the eye of *Limulus* (1949). This eye has proved to be far more complex than it originally was held to be. Hartline's single-fibre records are from the large fibre running out from the so-called excentric cell of the stalk of the ommatidium. But the ommatidium also contains a number of so-called retinula cells which appear as a large number of small fibres in the optic nerve. From these he has so far failed to obtain any impulse activity. We do not know to what extent these small fibres may spread laterally. Cajal emphasized the difficulty of staining the small insect fibres. There is, however, a very definite lateral inhibition in the eye of *Limulus*. If one ommatidium is kept actively discharging, say, in response to localized illumination or spontaneously, this discharge can be temporarily inhibited by illuminating another ommatidium some distance away. The nature of the effect has so far defied analysis.

Returning to the vertebrate eye there is an interesting fact that we have noted from looking over a very large number of records over several years, namely, a definite antagonism between the on- and off-components of retinal on/off-elements picked up by the microelectrode technique. The frequency of the one tends to go up when that of the other goes down, for instance, when light intensity is varied. This suggests that the on- and off-systems are mutually inhibitory. The effect can be conveniently demonstrated by cutting down time of exposure (Granit 1951). The discharges are long-lasting events compared with the flash and so the on- and the off-discharge will sooner or later collide. In this situation they do not add their effects upon the large ganglion cell which, according to Rushton (1950), generates the spike, but the one that has a shorter latency or otherwise is stronger impresses its frequency upon the cell and the effect of the other one is excluded. The same mutual exclusion of on- and off-components may also be shown by making stimuli of different colour clash, provided that the colour effects have different off/on-ratios (Granit 1949). The inhibition in this case cannot be localized at the ganglion cell itself because individually the on- and off-components can both excite it. But if, as we have reason to assume, the on- and off-components activate or are activated by different and opposite changes of potential their antagonism seems reasonable.

The on- and off-components, in the light of Hartline's microdissection or our microelectrode records, occur in a relatively pure state as on- and as off-elements responding to onset or cessation of light. The great majority of cells, however, are mixed on/off-elements and possibly the two pure types are pure merely because we work with dark-adapted eyes and limited stimulus strengths. Nevertheless, they are relatively pure compared with the on/off-elements. Gernandt and I (1947) have together and independently applied a routine test of polarization to these elements and by this test also the on/off-antagonism came to light. Polarizing electrodes were placed outside the bulb into the temporal and nasal corners of the eye and the microelectrode placed just inside the one polarizing electrode so as to test the effect of the current at that point. The pure on-elements were found to discharge at the cathode whilst the pure off-elements were excited at the anode.

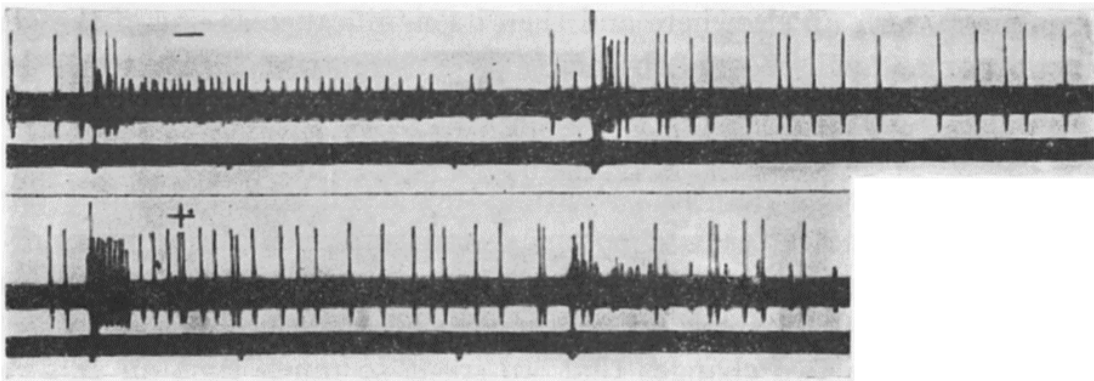


FIGURE 19. Large and small spike isolated in cat's eye by same micro-electrode. Eye is stimulated by current of 0.5 mA run between external nasal and temporal polarizing electrodes. Note small spike excited by nearby cathode and inhibited by anode, large spike vice versa.

Both behaved in an opposite fashion to the opposite pole. From the point of view of excitation the pure on-elements were cathodal and the pure off-elements anodal. The on/off-elements were of either type, apparently depending upon which component was the stronger. The record of figure 19 shows two elements of the on/off-type located by the same electrode, the small one cathodal, the large one anodal.

This may seem difficult to remember, but the result can be easily simplified in the statement that light and the cathode in this particular experiment always act in the same manner. The on-element that is excited by light is excited by the cathode; the off-element that is inhibited by light is likewise inhibited by the cathode which thus always acts as the natural stimulus. Returning now to our earlier experiments of 1939 on polarization in the frog's eye (Granit & Helme 1939), already mentioned, we recall that the cathode also had the same effect on *P*II and *P*III in the frog's eye. The simplest explanation is undoubtedly to state that the cathode, when used to excite the on-system, separated in an on-element, excites it the way light does by means of the mechanism of *P*II; whilst, when it is used to excite the off-system, separated as an off-element, it does so by means of the mechanism of *P*III, which represents the inhibitory system of opposite polarity.

In both cases light and the cathode must stimulate a structure capable of generating in itself, or downstream, the component potential concerned.

I am afraid that the retinal work, inasmuch as it has been developed in our laboratory, has been developed in a very unorthodox manner although the basic ideas have changed very little since 1932. This and the specific aspects of vision, coupling the problems to state of adaptation, flicker-fusion, rods and cones, etc., may have served to create the notion that the retina is a highly specialized organ unlikely to be of any general interest to those concerned with nervous problems. Feeling that this might be felt I inserted in my summary of 1947 (p. 40) the following sentences: 'Nowadays it is becoming clear that the generation of excitation and inhibition follows a limited number of patterns one of which may predominate in one organ and another in some other nervous structure, whether it be a sense organ, a peripheral nerve or some ganglion. The retinal pattern may, at any moment, turn up elsewhere and then its significance as one of the general nervous phenomena will become obvious.'

It seems as if the fascinating results reported by Professor Eccles at this meeting are in good agreement with the retinal findings inasmuch as they demonstrate the focal synaptic potential and its inhibitory counterpart of opposite polarity. The retina is a complex structure with three layers of cells and several interneurons. No doubt, as I have emphasized elsewhere (Granit 1947), several potential changes must occur in this structure, but it presents in a simplified form the two changes that we spent so much time on and named *PII* and *PIII*. To be as large and regularly occurring as they are, they must be favoured by an optimum orientation. They seemed at the time to suggest a natural starting point. For further work I think focal recording with fine micro-pipettes would be found very useful in this structure also, and in the last issue of the *Journal of Neurophysiology* (January 1952, vol. 15) I saw a beginning made along these lines by two Japanese authors, Tomita & Funaiishi, who, among other things, also came to the conclusion that *PII* was to be identified with Eccles's synaptic potential.

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CENTRAL EXCITATION AND INHIBITION FROM THE VIEW POINT  
OF CHEMICAL TRANSMISSION

BY W. FELDBERG, F.R.S.

Any discussion on the problem of how to explain central excitation and inhibition by chemical transmission must, I think, at the present stage of our knowledge be based mainly on transmission by acetylcholine at neuromuscular junctions and synapses in autonomic ganglia, in spite of the fact that there are characteristic differences between transmission at these sites of the peripheral nervous system and at central synapses. During the last years many of these differences have been explained by internuncial neurone activity. For instance, two very ingenious suggestions have been made to explain long-lasting facilitation and after-discharge: the theory of the delayed pathways by Forbes and that of the self re-exciting circuits by Lorente de Nó. Attempts have also been made to explain central inhibition by internuncial neurone activity. For instance, Gasser in his Harvey lecture in 1937 gives a diagram to show how reciprocal innervation could be explained on the assumption that the flexor and extensor pathways share an interneurone. As we have heard, Eccles's brilliant recent investigations with intracellular recordings no longer require such a scheme.

At the neuromuscular junction and at the autonomic ganglia the released acetylcholine excites the effector structures, the motor end-plates or the ganglion cells by depolarization, and this depolarization then excites the muscle or the nerve fibre. There is no difficulty in assuming a similar depolarizing action of acetylcholine at the central synapse. But there may be differences in detail in the mechanism of the release and synthesis of acetylcholine in the central cholinergic neurones as compared to the peripheral ones. They may be related to differences in the acetylcholine metabolism in brain.

From the relatively low acetylcholine content of the tissue of the central nervous system, combined with its relatively high choline acetylase content and the ability of brain tissue slices to release and synthesize acetylcholine on incubation, we may perhaps conclude that central cholinergic neurones—and this may apply particularly to the internuncials—are in a more labile state with regard to their acetylcholine than the peripheral neurones. There may be greater leakage and resynthesis of acetylcholine at rest than in peripheral neurones. To make clear my meaning I shall use a metaphor of a sluice or lock. Its opening compares to the arrival of a nerve impulse. But even at rest a little leakage takes place. In peripheral nerves this is of no importance unless we were to allow the acetylcholine to accumulate. That occurs after eserine and leads to fibrillation. With special methods we can detect this 'leakage' even without bringing about an