ACTION OF POTASSIUM AND NARCOTICS ON RECTIFICATION IN NERVE AND MUSCLE*

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INTRODUCTION

The characteristics of the cell membrane seem to be of primary importance in the case of both conduction and contraction. Among the membrane qualities recently discovered is electrical rectification; *viz.*, the ability of the membrane to permit electrical current to pass more easily outward than inward (Cole and Curtis, 1941; Guttman and Cole, 1941). The present paper constitutes an attempt to study the mechanism involved in tissue rectification by observing the effect of variation of the ionic medium bathing the cells.

It will be shown that the potassium ion decreases rectification in frog nerve and muscle and that this effect is reversible. It will also be shown that various narcotics, *e.g.* chloroform, isoamyl carbamate, veratrine sulfate, also have the ability to decrease the rectifying property of frog nerve and muscle and that lack of calcium, excess calcium, or barium, have no such effect.

While it has been known since 1941 that electrical rectification is exhibited by the single nerve fiber of the squid, it is of interest that this property can also be demonstrated in whole nerve and in whole muscle (*cf.* Katz, 1942), and moreover in the classical preparations long used in physiological laboratories; *i.e.*, frog sciatic nerve and frog sartorius muscle. A few experiments carried out in Woods Hole in the summer of 1941 indicate that rectification occurs also in the single nerve fiber of *Ommastrephes illecibrossus*, the northern squid. Whether the rectifying property is a general property present in the plasma membrane of all types of cells, or is confined to nerve and muscle cells, where a propagated type of activity occurs, is something which should be worth investigation.

Preliminary Experiments

Preliminary experiments were carried out in Woods Hole in the summer of 1940 and 1941. There the material used was the giant nerve fiber of the hindmost stellar nerve of the squid, *Loligo pealii*, and also of the northern squid, *Ommastrephes illecibrossus.*

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Immediately after dissection the axon was threaded through a glass U tube. Then the U tube was suspended by means of a glass rod cemented to it in such a way that one end of the fiber dipped into sea water, where it remained in good condition for many hours and the other end into a 0.48 M KCl solution twenty times as concentrated with respect to the K ion as sea water or else into 0.52 M KCl approximately isosmotic with sea water, which injured that end. Experimental solutions were then substituted for the sea water at the uninjured end. In previous experiments (Guttman and Cole, 1941) the interelectrode region (which was about 1 cm. long) lay in oil, but in these experiments it was found that many experimental difficulties, *e.g.* creeping of the electrode levels, were alleviated if air was substituted for the oil. The measuring circuit was much the same as that described in a previous paper (Fig. 1, Guttman and Cole, 1941) except that an amplifier and cathode ray oscillograph were substituted for the galvanometer previously used as a detector in the D.C. Wheatstone bridge circuit.

In these preliminary experiments only relative values of rectification were obtained. Degree of deflection, *i.e.* offbalance of the stationary spot of the oscillograph was plotted against the amount of the voltage imposed upon the fiber for currents flowing first in one direction and then in the other (anodal and cathodal currents).

Material and Methods

The sciatic nerve and the sartorius muscle of *Rana pipiens* were used in these experiments. (All frogs used were winter frogs.) In the case of the sciatic nerve, the sheath was either partially removed or else slit with Swiss watchmaker forceps while in Ringer's solution under a binocular microscope. This was done to insure easy penetration of the chemical agents used.

The amphibian Ringer's solution used was buffered to pH 7.4 with sodium bicarbonate. Except where otherwise noted all solutions used were isosmotic with Ringer's solution. However, it was found that slight variations in osmotic pressure had no appreciable effect upon the amount of rectification observed.

After dissection the tissue was mounted in a chamber similar to that used in a previous paper (Guttman, 1940). It consisted (Fig. 1) of a box containing paraffin, in which were imbedded at right angles two U shaped glass tubes, BB' and CC' and a blind glass tube, A. The distance (outside measurement) between A and C was about 4 cm.

Tube A was filled with Ringer's solution. Tube BB' was filled either with Ringer's or some test solution, whose effect upon the tissue was being investigated. Tube CC' was usually filled with isosmotic KCl (0.116 M).

Two glass supports (S), imbedded in the paraffin, suspended the nerve or muscle in such a way that one end dipped into the Ringer's solution at A, the middle dipped into Ringer's solution or test solution at B, and the other end dipped into the isosmotic KCl solution at C, which served to injure that end.

A moist chamber ($M.C.$), whose edge was smeared with vaseline, fitted into a circular groove in the paraffin and enclosed the tissue, portions A, B, and C of the tubes,

and the glass supports (S). The glass supports were coated with vaseline and the surface of the paraffin was kept dry to avoid creeping. One calomel half cell made contact with the tissue at B' and another at C'.

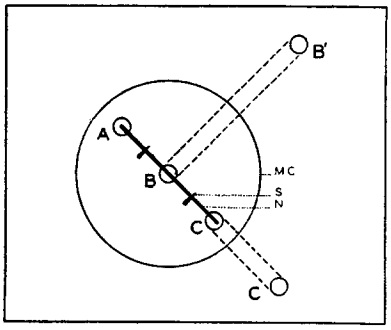

FIG. 1. Mounting chamber for measuring electrical rectification and resting potentials of frog nerve or muscle, top view. Dotted line indicates portion of glass tubing below the surface of solid paraffin. A, blind glass tube containing Ringer's solution; BB', glass U tube containing Ringer's or test solution; CC', glass U tube containing isosmotic KCl; S, glass support; $M.C.$, moist chamber; N, nerve or muscle.

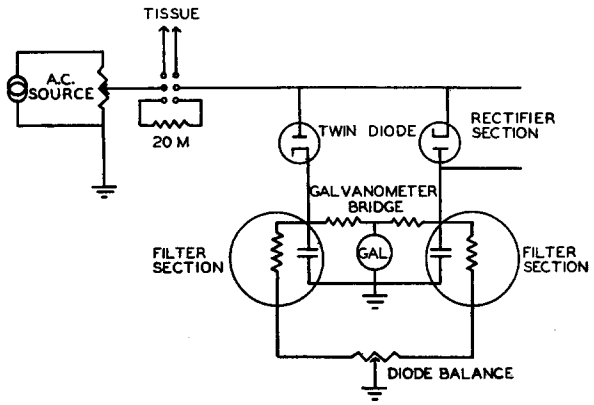

FIG. 2. Measuring circuit. Explanation in text.

Apparatus

The A. C. tissue rectification measuring circuit as used in the main body of experiments is shown in Fig. 2. The circuit impresses a sinusoidal voltage (of 1 volt, root mean square) across the tissue and the measuring circuit in series. When the tissue exhibits rectifying properties the voltage drop across the tissue differs for the positive and negative half cycles of the applied E. M. F., due to a greater resistance of the tissue to current flow in one direction. The measuring circuit consists of a twin diode, each

section being used to rectify one polarity of the applied wave. A filter circuit employed with each diode section develops a D. C. voltage nearly equal to the peak value of the applied E. M. F. Since the positive and negative half cycles of the wave are of unequal amplitude due to partial rectification in the tissue, the D. C. output voltages of the diode sections will also be unequal. The difference between these two voltages causes the galvanometer to deflect in proportion to the degree of rectification.

The instrument is calibrated by introducing a small D. C. voltage at the tissue test terminals. This shifts the axis of the A. C. wave, simulating partial rectification. A calibration plot is then made of galvanometer deflection *vs.* percentage rectification. Since the peak voltage of the 1 volt root mean square applied sine wave is $\frac{1}{0.707}$ or 1.414 volts we can get a measure of the rectification by comparing the D. C. component of the rectified wave with this figure. Thus

$$\text{Per cent rectification} = \frac{V}{1.414} \times 100 \text{ per cent}$$

where V equals the D. C. component in volts or "shift" in the A. C. axis of the sine wave. This method is sufficiently accurate for the small percentages of rectification encountered in this work. For high percentages a wave form factor would have to be included in the formula.

Although this measuring circuit was quite satisfactory and was very simple, an improvement in the sensitivity can be obtained by replacing the galvanometer with a vacuum tube voltmeter. This is contemplated in future experiments.

Frequency was varied from 30 cycles to 10,000 cycles without perceptible differences in results, by means of a General Radio 713 B beat frequency oscillator. Most of the data were taken at 60 cycles.

Resting potentials were measured at the calomel half cells by means of a potentiometer and galvanometer.

The rectification varied between 1 and 3 per cent in the experiments with frog material. The experimental error was estimated to be below 10 per cent. Rapidity of making measurements before the tissue had a chance to change resistance or the apparatus to become unbalanced was a means of obtaining significant measurements of small amounts of rectification. On reversing the current, similar rectification was always obtained.

RESULTS

Eighteen preliminary and twenty-seven subsequent experiments were done in all. All figures represent typical results.

Effect of Potassium upon Tissue Rectification.—In all cases potassium decreases tissue rectification, the degree of the effect depending upon the concentration. This effect is either partially (Fig. 3 A) or completely (Fig. 3 B) reversible, depending upon the concentration used and the length of time the solution is permitted to act.

In the experiments represented by Fig. 3, the reference end of the tissue was placed in isosmotic KCl (0.116 M) and the experimental end treated alter-

nately with Ringer and KCl solution, to show the effect of KCl upon the tissue. In another type of experiment, a slightly different technique was used; *viz.*, the reference end of the tissue remained in Ringer's solution and the experimental portion was referred to this untreated end. In such a case, when both portions were in Ringer's solution no rectification was observed, but when the experimental end was treated with potassium, rectification appeared.

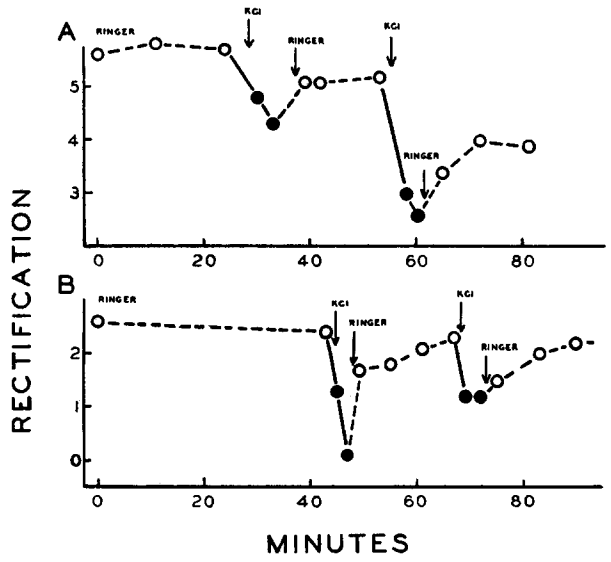

FIG. 3. Effect of potassium upon rectification in A, sartorius muscle and B, sciatic nerve of *Rana pipiens*. Rectification in arbitrary units directly proportional to D. C. component of the current passing through the tissue *vs.* time in minutes. "Ringer" indicates that one electrode region was immersed in Ringer's solution and the other in isosmotic KCl (0.116 M). "KCl" indicates that both electrode regions were immersed in isosmotic KCl. A decrease in resting potential accompanied every decrease in rectification. Both rectification and resting potential changes are reversible, wholly or in part.

Narcotics Decrease Rectification.—Narcotics are another group of substances which are known to affect the membrane profoundly. As might have been expected, narcotics, like potassium, reversibly decrease rectification. This was shown a few years ago in the case of cocaine and the single nerve fiber of the squid (Guttman and Cole, 1941). It can also be demonstrated for frog sciatic and sartorius using chloroform, veratrine sulfate and isoamyl carbamate (Fig. 4).

Excess Calcium and Barium, and Lack of Calcium Do not Affect Rectification.—Because of the very suggestive effect of low calcium in initiating spontaneous

chemical firing in nerve (Brink and Bronk, 1937) and in causing striking oscillations of potential (Arvanitaki, 1939), the effect of this agent upon tissue rectification was investigated. The procedure used was to soak the tissue first in 0.116 M NaCl and then in 0.116 M NaSCN (Sjöstrand, Brink, and Bronk, 1938). No effect upon rectification was observed, however, with low calcium. Neither do excess calcium (0.083 M) nor barium chloride (0.083 M)

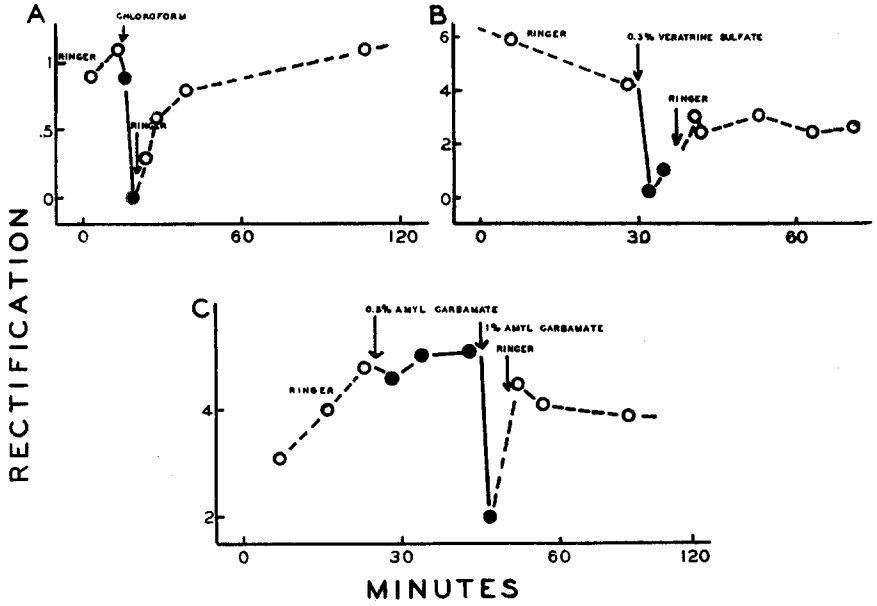

FIG. 4. Effect of narcotics upon rectification in sciatic nerve (A) and sartorius muscle (B and C) of *Rana pipiens*. Rectification in arbitrary units directly proportional to D. C. component of the current passing through the tissue *vs.* time in minutes. A decrease in resting potential accompanied every decrease in rectification. Both rectification and resting potential changes are partially or completely reversible. (Chloroform was applied as a saturated solution in Ringer.)

affect tissue rectification. In a previous paper, it has been shown that even where the alkaline earth ions have in themselves no effect they are capable of neutralizing the depressing effect of potassium upon resting potentials (Guttman, 1940). No similar neutralizing effect of alkali earths upon the depression of rectification by potassium was observed.

Only one experiment was done to investigate the effect of acetylcholine upon tissue rectification. After soaking frog sartorius for ½ hour in freshly made up 1–5000 eserine sulfate in Ringer's solution (at pH 7.2), 100 gamma per cc. acetylcholine had no effect upon rectification. Of course, no conclu-

sions can be drawn from an isolated trial of this kind, but the result is mentioned inasmuch as the subject is of interest to many.

DISCUSSION

Rectification Effect Located in Membrane.—The fact that the potassium ion decreases rectification in the tissue may be of assistance in determining where in the cell rectification occurs. One would not expect the potassium ion to be able to affect the rectification of (1) the Ringer's solution, (2) the connective tissue, or (3) the protoplasm of the nerve or muscle cell interiors, so as to alter the degree of rectification exhibited by them, supposing for the moment that these media were capable of rectification. We do know, on the other hand, that potassium profoundly influences the membrane structure of nerve and muscle cells. Since the data here presented demonstrate that potassium markedly affects the degree of rectification exhibited by nerve and muscle cells, there is some indication that the seat of rectification is in the cell membrane. (Evidence for the assumption that the cell interiors and the external media act merely as electrolytes may be obtained from the transverse resting impedance data of Cole.) These data may thus be considered as experimental verification of Cole's suggestion that selective ion permeability of a membrane may be expected to give rise to rectification (Cole, 1941) since they show that when the membrane is externally in contact with ions to which it is more permeable or has its permeability increased by narcotics rectification falls off.

Mechanism of Rectification.—The experiments with potassium offer experimental verification of Cole's suggestion (1941) that the mechanism of rectification may probably be explained on the basis of ionic conduction rather than electronic conduction in the cell membrane. They are of especial interest in connection with his more specific supposition that rectification may possibly be explained on the basis of conduction by potassium ions alone. Since the external concentration of potassium is low an inwardly flowing current would decrease the potassium ions in the membrane and decrease its conductivity. An outwardly flowing current would have the opposite effect since the internal concentration of potassium ions is relatively high. Thus rectification in the membrane, *viz.* a change in resistance with change in direction of current flow, may possibly be explained in terms of a change in the concentration of potassium ions in the membrane.

In the potassium experiments described in this paper, rectification was decreased or disappeared when the concentration of potassium ions in the outer medium was increased and the gradient of potassium ions in the membrane thus lessened. This work constitutes experimental verification of a possible explanation for rectification in living tissues.

Such an explanation for rectification is similar to that long ago suggested for resting potentials. Höber (1905) has shown for frog muscle, Osterhout (1931)

for *Nitella*, and Cowan (1934) for crab nerve that bathing cell exteriors with KCl solutions and thus lessening the potassium gradient normally present in the membrane will decrease the potential, and Blinks (1930) showed that increasing external KCl increased the conductivity in *Nitella*. In almost every experiment reported in this paper, resting potentials were observed at the same time as rectification. Invariably, whenever the rectification of the tissue was decreased, the resting potential also declined. This was true not only when the cells were treated with potassium but also when narcotics were used. (That narcotics decrease resting potentials has long been known (Höber, Andersch, Höber, and Nebel, 1939; Guttman, 1940).)

That it was possible to lessen rectification (and resting potentials) by narcotics as well as by increasing the external potassium, may indicate that narcotics increase permeability to all ions.

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SUMMARY

Electrical rectification was demonstrated in whole sartorius muscle and sciatic nerve of *Rana pipiens* and also in the single giant nerve fiber of the northern squid, *Ommastrephes illecibrossus*. It is probably a property of the plasma membrane.

Rectification decreases reversibly under the influence of increased concentrations of the potassium ion and with chloroform, veratrine sulfate and isoamyl carbamate. No effect was found with lack of calcium, excess calcium, or barium chloride.

Decrease in rectification is invariably accompanied by simultaneous decrease in resting potential.

A proposed explanation of the mechanism of rectification is discussed. Rectification in a living membrane, *viz.* a change in resistance with change in direction of current flow, may possibly be explained in terms of a change in the concentration of potassium ions in the membrane.

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