PACEMAKERS IN NITELLA

II. Arrhythmia and Block

By W. J. V. OSTERHOUT and S. E. HILL

(*From the Laboratories of The Rockefeller Institute for Medical Research*)

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Irregular rhythm (arrhythmia) and failure to transmit impulses (block) seem to be essentially similar in the vertebrate heart and in *Nitella*.

In *Nitella* arrhythmia and block (partial or complete[1]) have been produced by the following treatment. Cells (freed from neighboring cells) are kept for 6 weeks or more in a nutrient solution (Solution A[2]). Exposure for 2 or 3 hours[3] to 0.01 M NaCl, 0.01 M NaSCN, or 0.01 M guanidine chloride[4] then reduces the time required for the action current to about 1 second (the normal time is from 15 to 30 seconds).

The leads[5] are shown in Fig. 1. At the common contact *F* we place 0.01 M KCl which prevents changes[6] at that spot, and thus renders the action currents monophasic: it reduces the P.D. approximately to zero. (The location of the zero in the figures in this paper is made on the assumption that the P.D. at *F* is zero, hence it is only an approximation.)

Owing to the presence of 0.01 M KCl, *F* usually acts as a pacemaker:[2] in the present case the rhythm is about 1 a second. Apparently certain parts of the cell are unable to follow this rapid pace and hence fall

[1] Complete block is sometimes found in untreated cells.

[2] *Cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 87.

[3] *Cf.* Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22,** 91.

[4] Unpublished.

[5] The experiments were performed on *Nitella flexilis*, Ag., using the technique described in former papers (*cf.* Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937–38, **21,** 541).

[6] Any change at *F* can be detected by simultaneous changes in the records of *C*, *D*, and *E*.

into irregular rhythms and fail to register all the impulses (partial block). If this does not occur and if all parts of the cell follow the rhythm perfectly, we obtain such records as are shown in Fig. 2. Here the action curves[7] are practically the same at each of the recorded spots.

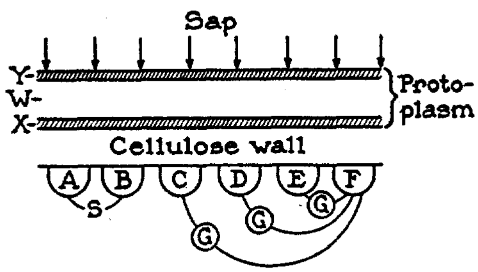

FIG. 1. Diagram to show the arrangement of leads and the supposed structure of the protoplasm which is assumed to consist of an aqueous layer *W*, an outer non-aqueous layer *X*, and an inner non-aqueous layer *Y*.

The arrows show the outwardly directed (positive) P. D. whose seat is supposed to be chiefly at *Y* when the cell is in pond water: hence the P. D. at *X* is regarded as negligible and is not shown. But under some conditions the P. D. at *X* may become important.

Each lead is connected to a separate amplifier and to one string of the 3-string Einthoven galvanometer.

Figs. 3 and 4 show certain differences in the forms of the action curves at different spots. The sudden appearance of a more striking difference is seen in Fig. 5 which records action currents at *C* (top) and *D* (bottom). At first each impulse passes from *D* to *C* but later, *C* appears to be unable to follow the pace and hence it records every second impulse.[8] This recalls a situation often found in auricular flutter of the heart[9,10] when the auricular rate becomes so rapid that

[7] Regarding the form of these curves see Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22,** 91.

[8] The chance of finding such cases, and of observing blocks, naturally increases as the distance between the leads increases.

[9] White, P. D., Heart disease, New York, The Macmillan Co., 2nd edition, 1937.

[10] Bethe, A., *Arch. ges. Physiol.*, 1937, **239,** 41; *Z. vergleich. Physiol.*, 1937, **24,** 613. Wenckebach, K. F., and Winterberg, H., Die unregelmässige Herztätigkeit, Leipsic, W. Engelmann, 2nd edition, 1927.

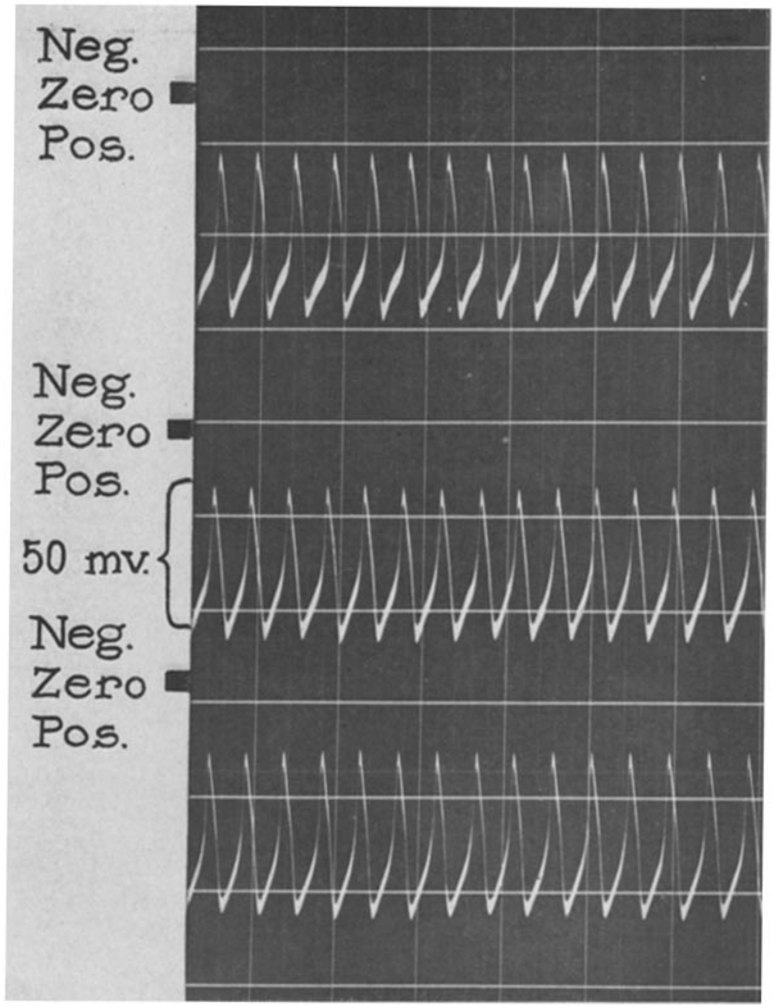

FIG. 2. Shows nearly identical responses at *C*, *D*, and *E* (*cf.* Fig. 1): the pacemaker is at the right (probably at *F* which is in contact with 0.01 M KCl) since *E*, lowest string, moves first. The other spots are in contact with 0.01 M NaCl.

The cell was freed from neighboring cells and kept 40 days at 15 ± 1°C. in Solution A: it was then placed for 3 hours in 0.01 M NaCl at 21 ± 2°C. before the record was made.

The leads are 6 mm. apart.

Time marks 5 seconds apart.

the ventricle can record only every other impulse coming from the auricle.

Such ratios as 3:1 and some others found in the heart appear in *Nitella* (*cf.* Figs. 6–8).

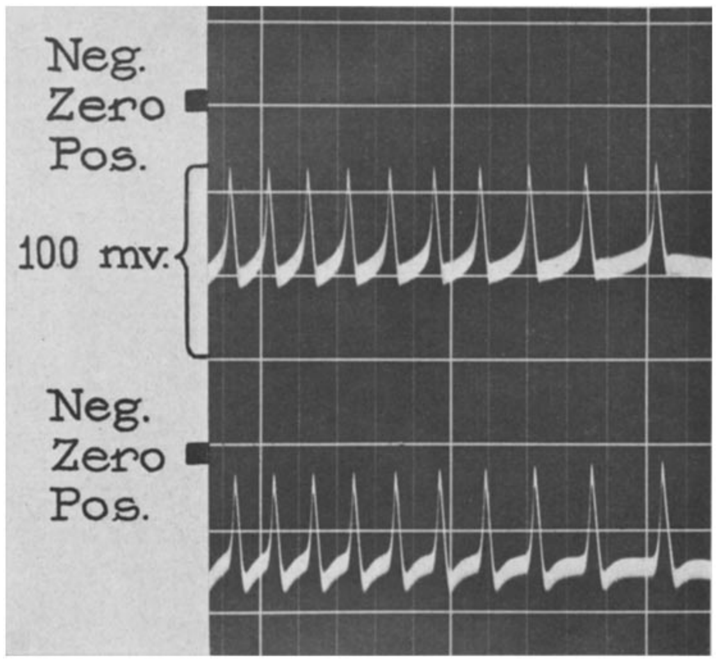

FIG. 3. Shows action curves at C (top) and D (bottom; *cf.* Fig. 1) which are similar but not identical. The pacemaker is at the left of C (since the top string, C, moves first).

F is in contact with 0.01 M KCl; the other spots are in contact with 0.01 M NaCl. The leads are 28 mm. apart.

The cell was freed from neighboring cells and kept 40 days in Solution A at 15 ± 1°C.; it was placed for 2.5 hours in 0.01 M NaCl at 22°C. before the record was made.

Heavy time marks 5 seconds apart.

We also find in *Nitella* cases where one or both spots are irregular (*cf.* Figs. 7 and 8).

In Fig. 9 we see that 26 action currents are recorded at C (top)

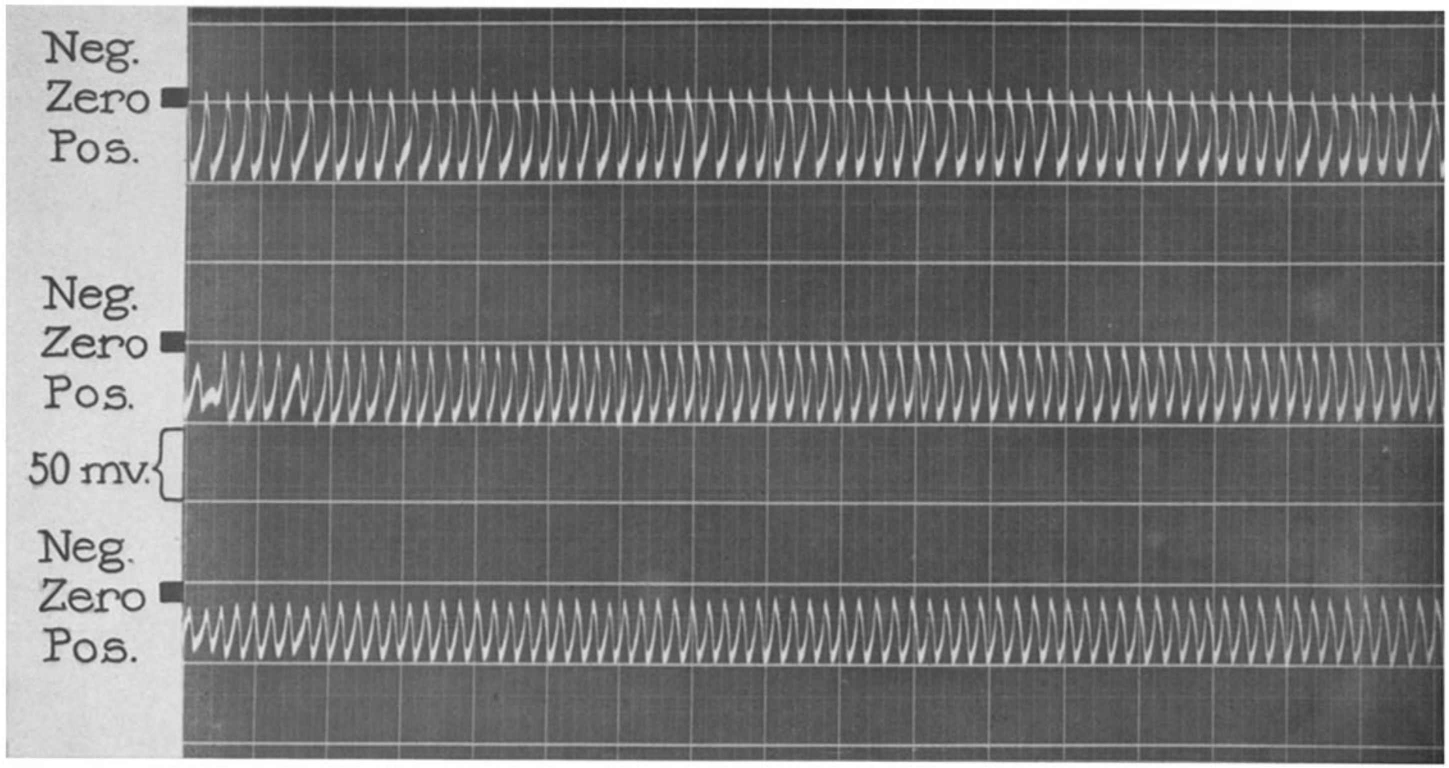

FIG. 4. Shows action curves at C (top, *cf.* Fig. 1), D (middle), and E (bottom) which are similar in some cases but in others differ considerably. Since the bottom string, E, moves first the pacemaker is at the right of E, probably at F, which is in contact with 0.01 M KCl; the other spots are in contact with Solution A. The leads are 10 mm. apart.

The cell was freed from neighboring cells and placed for 50 days in Solution A at 15 ± 1°C.; it was then placed in 0.01 M guanidine chloride for 3 hours at 25 ± 2°C. before the record was made.

Heavy time marks 5 seconds apart.

while 33 are being recorded at *D* (bottom).[11] Similar situations are also found in the heart.

Fig. 10 shows groups[12] of action currents separated by intervals of rest. Each action current at *C* (top) passes over to *D* (bottom) but the forms of the curves are not identical in all cases.

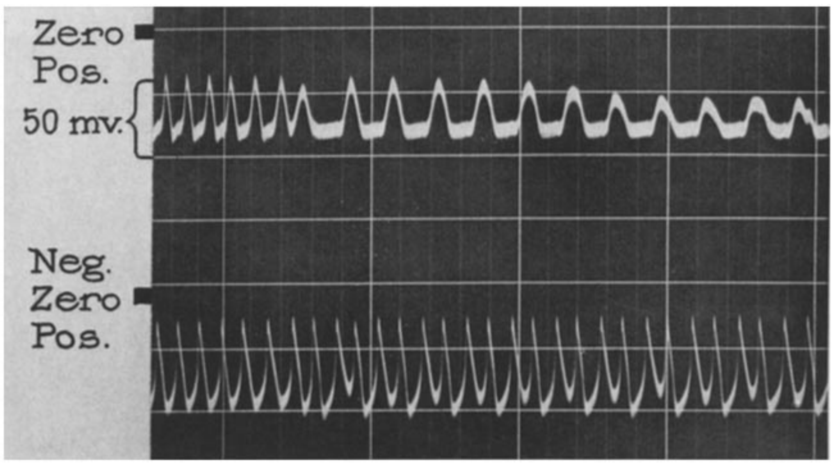

FIG. 5. Shows the sudden appearance of a 2:1 ratio. The first 7 action currents at *D* (bottom, *cf.* Fig. 1) are followed by action currents at *C* (top) but after this every other impulse registers at *C* and most of the time there is incomplete recovery of every other action current at *D*, *i.e.* the stimulus from the pacemaker arrives before recovery is finished and in these cases the action current does not register at *C*.

Since *D* (bottom) moves first the pacemaker is at the right of *D*, probably at *F* which is in contact with 0.01 M KCl (the other spots are in contact with 0.01 M NaCl). The leads are 28 mm. apart.

The cell was freed from neighboring cells and kept for 40 days in Solution A at 15 ± 1°C. It was then placed in 0.01 M NaCl for 3 hours at 25 ± 2°C.

Heavy time marks 5 seconds apart.

In Fig. 11 we see groups of action currents but the rhythms of *C* (top) and *D* (bottom) appear to be independent, indicating a com-

[11] Whether *C* and *D* are acting independently is not evident.

[12] Such groups are not uncommon in *Nitella*. *Cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18,** 499. Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22,** 91.

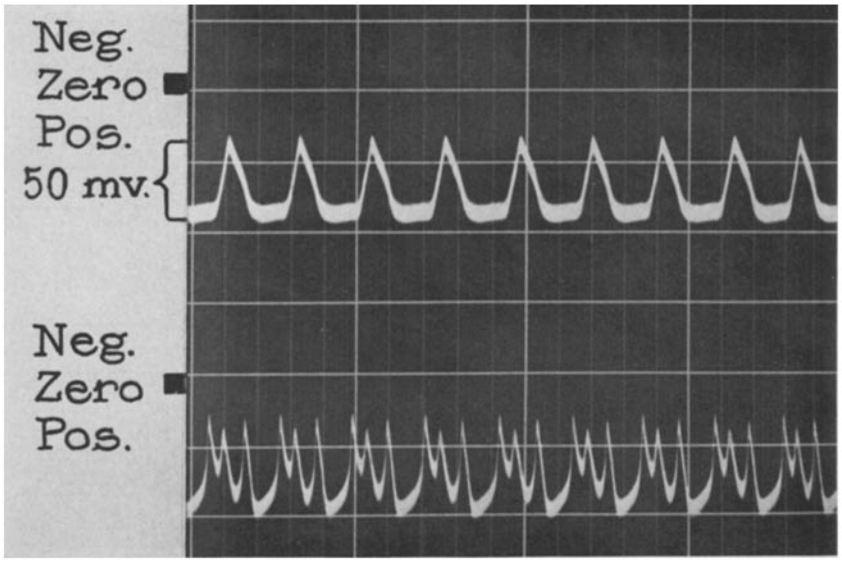

FIG. 6. Shows a 3:1 ratio. Every third impulse passes over from *D* (bottom, *cf.* Fig. 1) to *C* (top). Since the bottom string, *D*, moves first the pacemaker is at the right of *D* and is probably at *F*, which is in contact with 0.01 M KCl; the other spots are in contact with 0.01 M NaCl. The leads are 28 mm. apart.

The cell was freed from neighboring cells and kept for 40 days in Solution A at 15 ± 1°C. It was then placed for 3 hours in 0.01 M NaCl at 25 ± 2°C. before the record was made.

Heavy time marks 5 seconds apart.

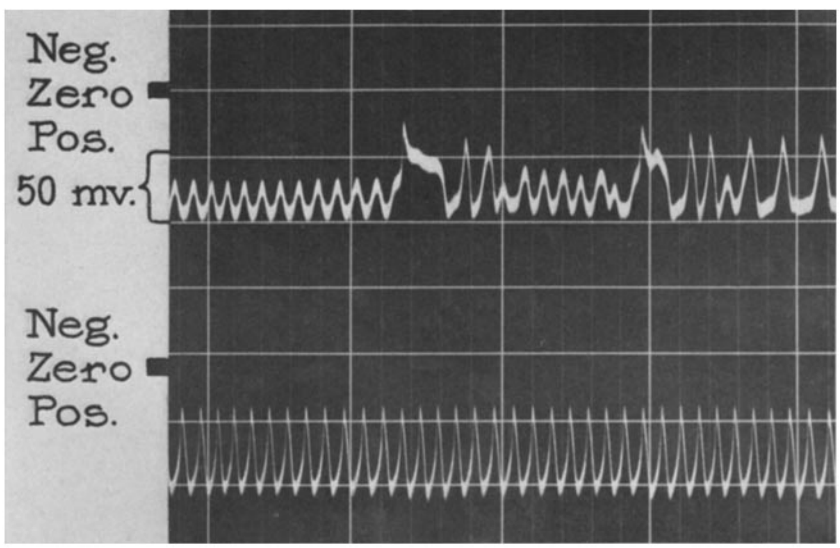

FIG. 7. Here *D* (bottom, *cf.* Fig. 1) is regular and *C* (top) is partly irregular. Since *D* moves first the pacemaker is at the right of *D*, probably at *F*, which is in contact with 0.01 M KCl: *C* and *D* are in contact with 0.01 M NaCl. The leads are 28 mm. apart.

The cell was freed from neighboring cells and kept for 40 days in Solution A at 15 ± 1°C. It was then placed for 3 hours in 0.01 M NaCl at 25 ± 2°C. before making the record.

Heavy time marks 5 seconds apart.

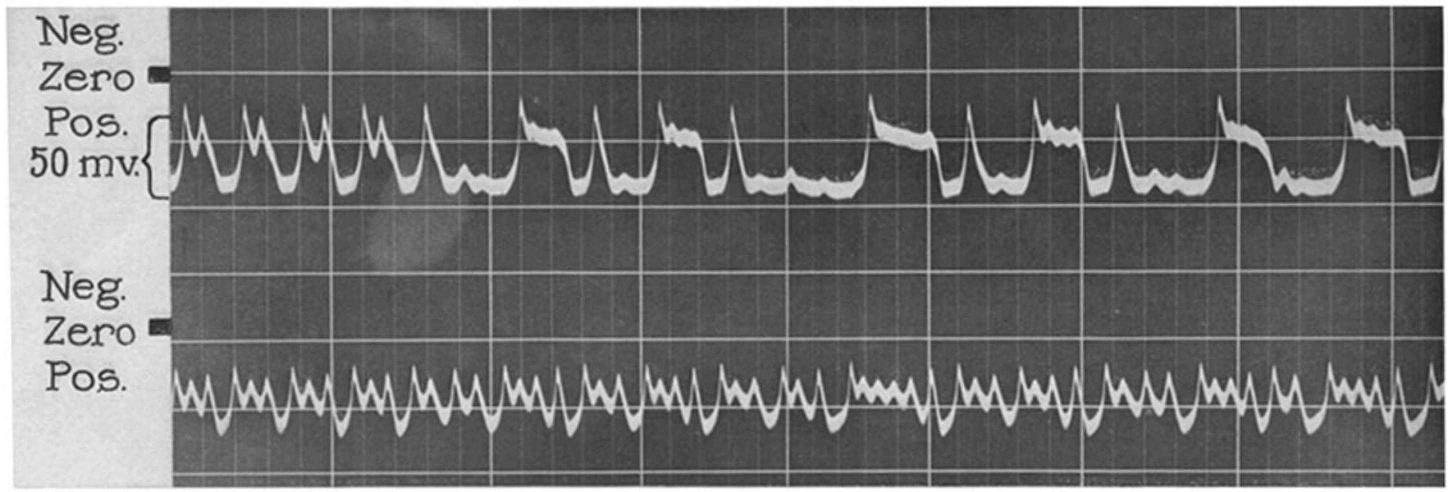

FIG. 8. Both C (top, *cf.* Fig. 1) and D (bottom) are irregular. The first part of the record appears to show that D moves first so that the pacemaker is at the right of D, probably at F which is in contact with 0.01 M KCl: C and D are in contact with 0.01 M NaCl. The leads are 28 mm. apart.

The cell was freed from neighboring cells and kept for 41 days in Solution A at 15 ± 1°C. It was then placed in 0.01 M NaCl for 2.5 hours at 25 ± 2°C. before the record was made.

Heavy time marks 5 seconds apart.

plete block between them. A striking instance of complete block is seen in Fig. 12. The form of action curve seen at *C* (top) is common in cells treated[3] with NaCl but is seldom present in the same cell and at the same time as the quick action currents seen at *D* (bottom).

Groups of action currents and complete block are also found in

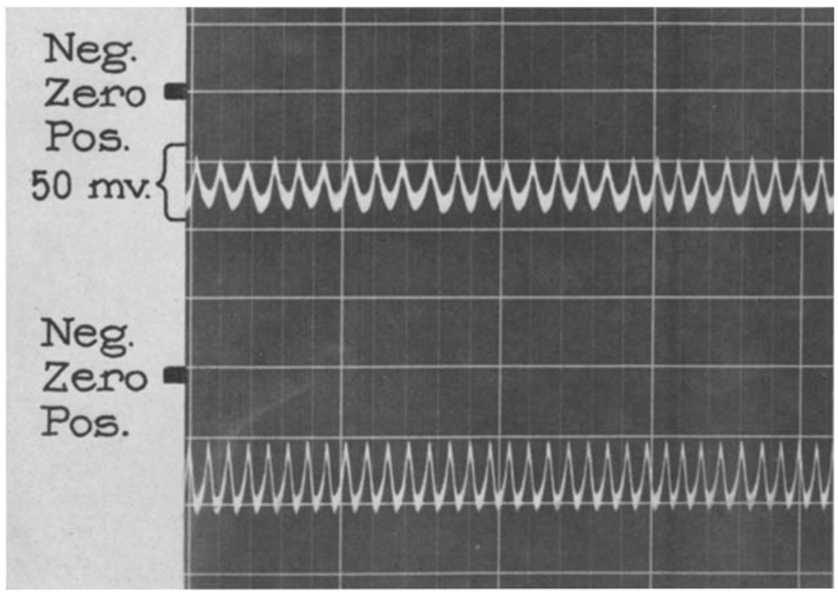

FIG. 9. Here 33 action currents are recorded at *D* (bottom, *cf.* Fig. 1) while 26 are being recorded at *C* (top). As *F* was in contact with 0.01 M KCl it is probably the pacemaker for *D* (*C* and *D* are in contact with 0.01 M NaCl). The leads are 28 mm. apart.

The cell was freed from neighboring cells and kept for 40 days in Solution A. It was then placed for 2.5 hours in 0.1 M NaCl at 25 ± 2°C. before the record was made.

Heavy time marks 5 seconds apart.

the heart.[9,10] A great variety of other irregularities found in the heart may be duplicated in *Nitella*. One of these, electrical alternans,[13] is shown in Fig. 13. Another shows groups in each of which the amplitude of the action curve first increases and then diminishes.[14] Still another is an analogue of auricular extra systole (Fig. 14).

[13] *Cf.* Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22,** 91, Fig. 13.

[14] *Cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18,** 509.

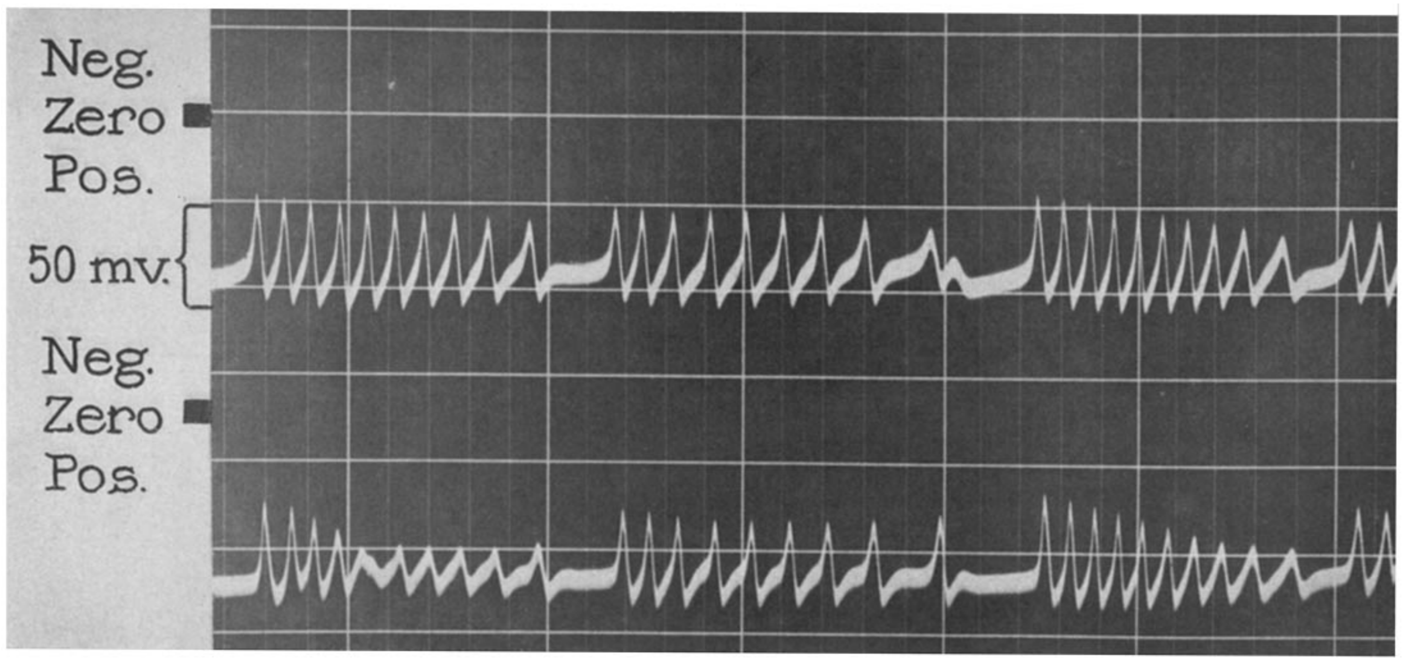

FIG. 10. Shows groups of action currents with intervals of rest. Since *C* (top, *cf.* Fig. 1) moves before *D* (bottom) the pacemaker is at the left of *C* (*C* and *D* are in contact with 0.01 M NaCl and *F* with 0.01 M KCl). The leads are 28 mm. apart.

The cell was freed from neighboring cells and kept for 41 days in Solution A at 15 ± 1°C. It was then placed in 0.01 M NaCl at 25 ± 2°C. for 2.5 hours before the record was made.

Heavy time marks 5 seconds apart.

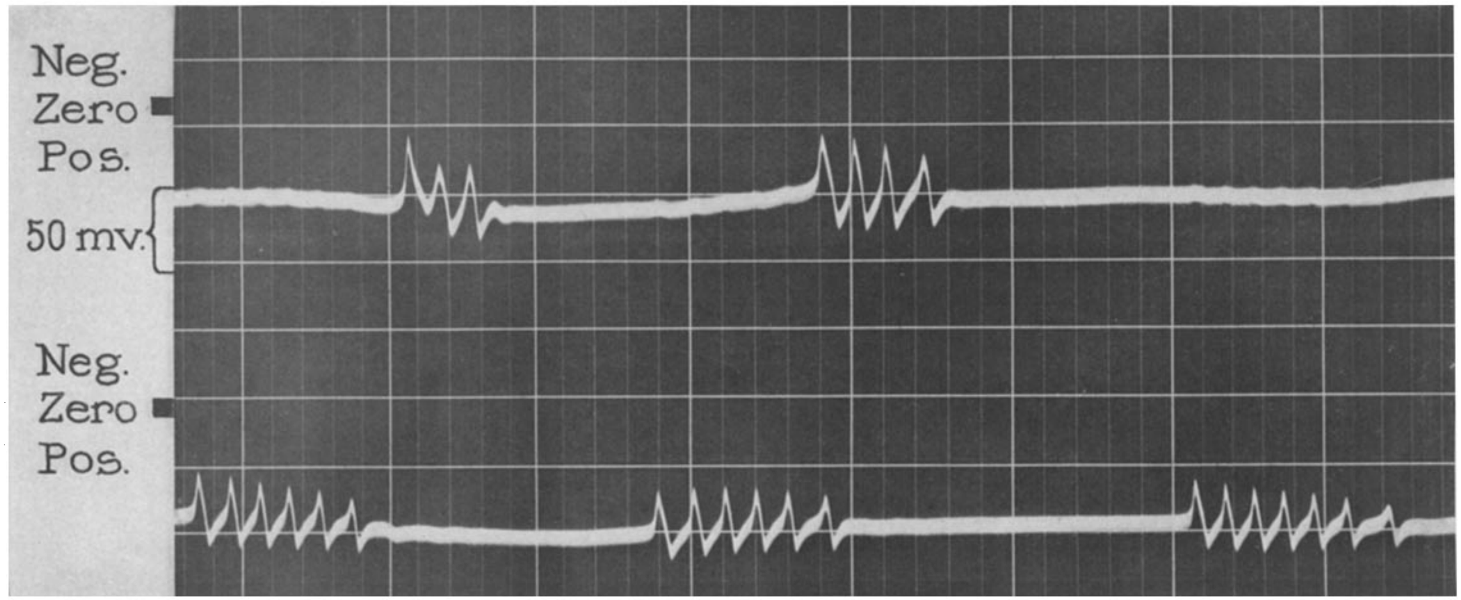

FIG. 11. Shows complete block and groups of action currents. *C* (top, *cf.* Fig. 1) appears to be independent of *D* (bottom): *C* and *D* are in contact with 0.01 M NaCl and *F* with 0.01 M KCl. The leads are 12 mm. apart.

The cell was freed from neighboring cells and kept for 42 days in Solution A at 15 ± 1°C. It was then placed for 3 hours in 0.01 M NaCl at 22 ± 2°C. before the record was made.

Heavy time marks 5 seconds apart.

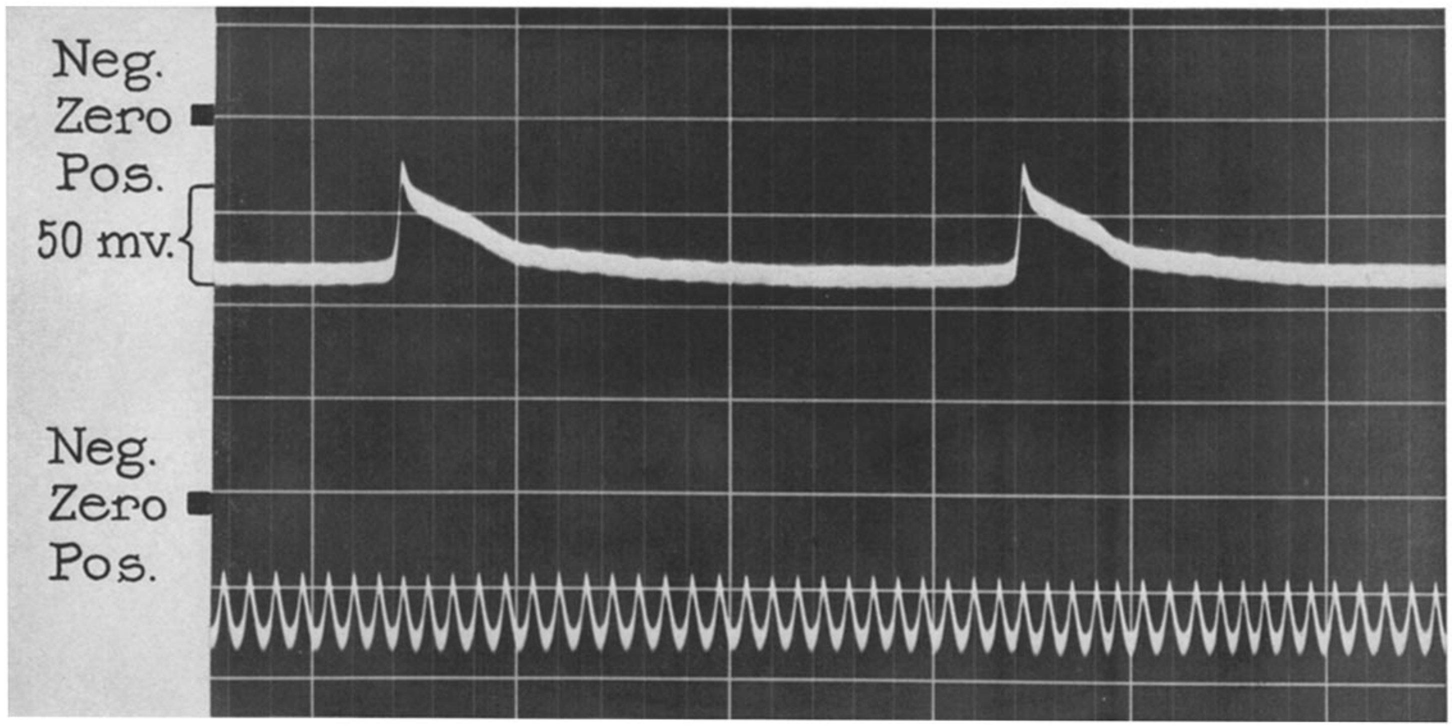

FIG. 12. Shows complete block. *C* (top, *cf*. Fig. 1) and *D* (bottom) appear to be independent. The form of the action curve at *C* is one commonly found in cells treated with NaCl. *C* and *D* are in contact with 0.01 M NaCl, *F* with 0.01 M KCl. The leads are 28 mm. apart.

The cell was freed from neighboring cells and kept for 40 days in Solution A at 15 ± 1°C. It was then placed in 0.01 M NaCl for 3 hours at 25 ± 2°C. before the record was made.

⌊ Heavy time marks 5 seconds apart.

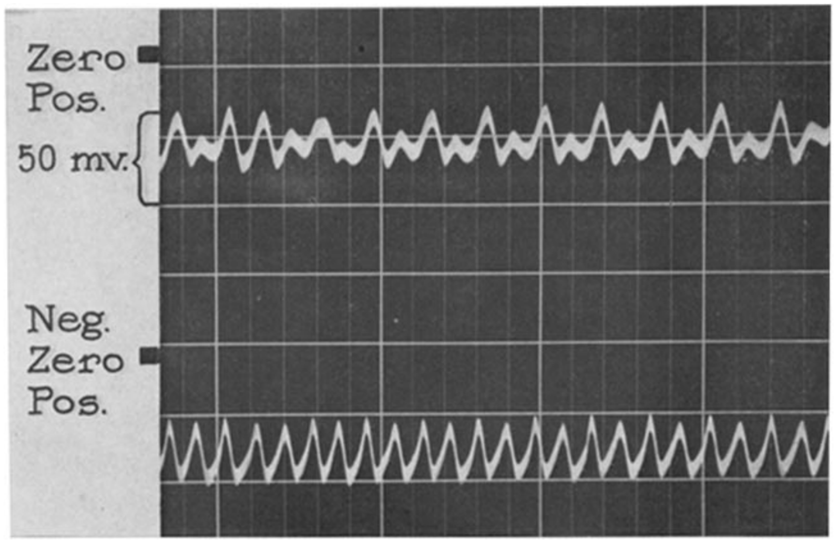

FIG. 13. Shows "electrical alternans." Each action current at *D* (bottom, *cf.* Fig. 1) is followed by one at *C* (top) where every other action curve is of smaller magnitude. As *D* moves first the pacemaker is at the right of *D*, probably at *F* which is in contact with 0.01 M KCl (*C* and *D* are in contact with 0.01 M NaCl). The leads are 18 mm. apart.

The cell was freed from neighboring cells and kept for 42 days in Solution A at 15 ± 1°C. It was then placed for 3 hours in 0.01 M NaCl at 25 ± 2°C. before the record was made.

Heavy time marks 5 seconds apart.

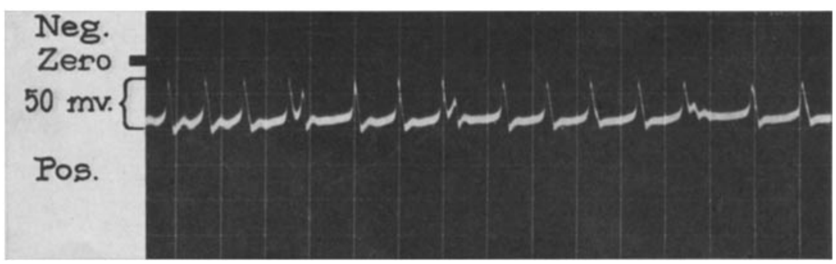

FIG. 14. Shows changes like those observed in extra systole of the auricle, *i.e.* an occasional premature impulse followed by an extra long pause (but not a pause of double length as in ventricular extra systole).

D (*cf.* Fig. 1), which is recorded, is in contact with 0.01 M NaCl: *F* is in contact with 0.01 M KCl and is apparently acting as pacemaker. Whether there is a competition of pacemakers is not clear.

The cell was freed from neighboring cells and kept for 30 days at 15 ± 1°C. in Solution A. It was then placed for 3 hours in 0.01 M NaCl at 22 ± 2°C. before the record was made.

Heavy time marks 5 seconds apart.

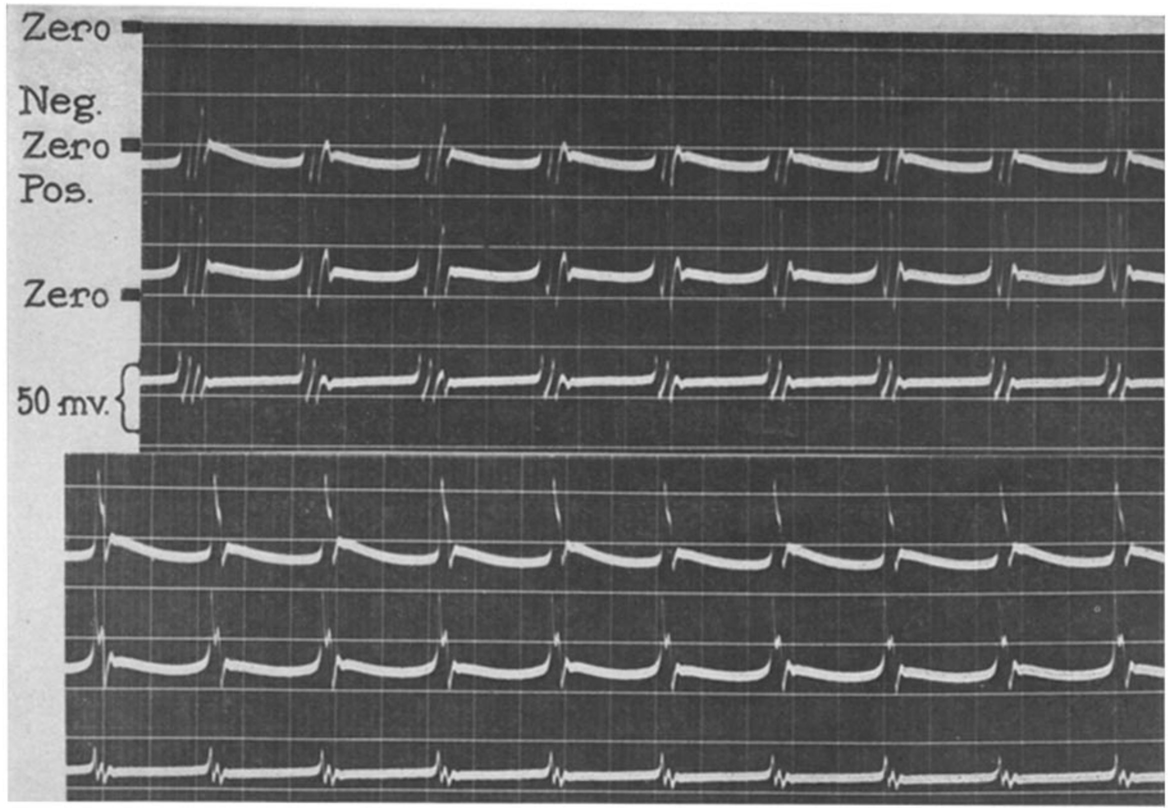

FIG. 15. Shows a progressive change in the ability of C (top, *cf.* Fig. 1) to follow in detail the impulses coming from E (bottom). The pacemaker is at the right of D, probably at F which is in contact with 0.01 M KCl (the other spots were in contact with 0.01 M NaCl). The leads are 28 mm. apart.

The cell was freed from neighboring cells and kept for 30 days in Solution A at 15 ± 1°C. It was then placed in 0.01 M NaCl for 5 hours at 24 ± 2°C. before the record was made.

Heavy time marks 5 seconds apart.

The continuous record is cut into sections for purposes of reproduction.

Competition between pacemakers and shifts from one pacemaker to another which occur in the heart are also found in *Nitella*: these will be described elsewhere.[15]

DISCUSSION

It may seem surprising that so many of the irregularities found in the heart have analogies in *Nitella*. Evidently, two regions of a *Nitella* cell, only a short distance apart, can react differently and this appears to be the fundamental requisite for duplicating many of the differences of rhythm between auricle and ventricle.

Since the irregularities of conduction in *Nitella* so closely resemble those found in disorders of the heart it may be asked whether the cells of *Nitella* used in these experiments were in any way pathological. Regarding this it may be said that they were normal in appearance and that they remained so after a much longer exposure to Solution A: also that they remained normal in appearance after an exposure of several weeks to 0.01 M NaCl.

It may be added that in many cases the heart is quite normal in appearance when it shows the irregularities here discussed.[16] It therefore seems that similar irregularities can be brought about in the heart and in *Nitella* by certain conditions which do not produce abnormal appearances. To what extent these conditions are similar must be decided by future investigation.

In conclusion we may say that when recovery in a stimulated region is so slow that the region cannot respond normally to the next stimulus there may be arrhythmia and partial block. Much therefore depends on the rate of recovery. This may undergo a progressive change[10] as seems to be the case in Fig. 15.[17] As already indicated (p. 115) the rate of recovery may be greatly increased by exposure to certain salt solutions. It may be added that recovery can be (rever-

[15] Regarding pacemakers see Auger, D., Comparaison entre la rythmicité des courants d'action cellulaires chez les végétaux et chez les animaux, Actualités sc. et indust. No. 314, Paris, Hermann et Cie., 1936.

[16] White, P. D., Heart disease, New York, The Macmillan Co., 2nd edition, 1937, pp. 610 *ff.*

[17] Sometimes the rate of recovery seems to slow down and then recover, *cf.* Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1935–36, **19**, 307.

sibly) suppressed, producing complete block. This appears to offer a promising field for further investigation.

The occurrence of closely similar phenomena in structures as different as the vertebrate heart and *Nitella* suggests that all tissues capable of conducting impulses are similar in certain fundamental properties and appears to justify the use of simple tissues to make clear the mechanism of more complex structures.

SUMMARY

Many forms of irregular rhythm and of partial block occurring in the vertebrate heart can be duplicated in *Nitella*.

In order to observe these phenomena the cells of *Nitella* are kept for 6 weeks or more in a nutrient solution. They are then exposed for 3 hours or less to 0.01 M NaCl, NaSCN, or guanidine chloride, which reduce the time required for the action current to about 1 second (the normal time is 15 to 30 seconds).

A pacemaker is established at one end of the cell by placing it in contact with 0.01 M KCl. This produces action currents at the rate of about 1 a second. Apparently some parts of the cell are unable to follow this rapid pace and hence fall into irregular rhythms (arrhythmia) and fail to register all the impulses (partial block).

We wish to express to A. E. Cohn and to A. G. Macleod our thanks for valuable criticisms.