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THE DEPOLARIZATION OF CRUSTACEAN NERVE BY STIMULATION OR OXYGEN WANT.

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In 1927, at the time of his sudden untimely death, Levin, working with crustacean nerve, was investigating a remarkable characteristic of the action current, which he termed the "retention of action current," and the extreme fatigability of the nerve in question (1). His chief results may be summarized as follows:

1. The galvanometer deflection (the "negative variation of the injury current") due to a continued tetanus through a single pair of stimulating electrodes increased with the time, reached a maximum in about 20 sec., and then gradually declined. The galvanometer leads were monophasic, non-polarizable electrodes were used, and the nerve was in moist air.

2. If stimulation were stopped within two minutes the galvanometer moved quickly back to a certain position and then returned only slowly towards its original place. The size of the rapid swing back decreased as the duration of the stimulus increased: no back swing at all was observed after two minutes of continuous tetanization. The time required for the galvanometer to return to its original position after the end of a stimulus depended upon the duration of that stimulus, being from 1 to 10 min. or more. Levin described this phase of slow return by the term "retention of action current."

3. The maximum displacement of the galvanometer from its earliest unstimulated position was the same in a succession of tetanic stimuli (the "ceiling effect"), whatever the position of the galvanometer at the beginning of any particular stimulus, provided that the nerve was not over-stimulated by too strong a faradic current, or unduly fatigued by too long a stimulus. In other words, the sum of (*a*) the "retention," and (*b*) the action current produced by a stimulus, was constant.

4. Fatigue set in particularly quickly in the neighbourhood of the stimulating electrodes.

Levin suggested that the disappearance of his "retention" was due to some kind of restitution process following activity.

At the beginning of 1928 Prof. A. V. Hill suggested to me that I should further investigate these remarkable properties of crustacean nerve. The present work, therefore, was undertaken at the Marine Biological Laboratory at Plymouth and was continued through the year. A brief account of the earlier experiments was communicated to the Physiological Society (2).

Method. The method employed was nearly the same as Levin's, with a few minor alterations from time to time. A high resistance sensitive moving-coil galvanometer of long period (Campbell galvanometer by the Cambridge Instrument Company) was at first employed, as the moisture of the sea air had injured the moving magnet instrument employed by Levin. The disadvantage of the Campbell galvanometer for this type of experiment is its very long period (25 to 30 sec.), and at the end of March it was changed for a sensitive and rapid moving-magnet instrument constructed and erected by Mr A. C. Downing. This galvanometer with four high resistance coils (20,000 ohms in series) can be adjusted to as high a sensitivity as 1 mm. $= 5 \times 10^{-13}$ amp. on a scale two metres distant, with a deflection time of about 2 sec. when critically damped. Such a sensitivity, however, is not required except when a single impulse only is investigated. Usually a much lower sensitivity was employed, the galvanometer being critically damped with a deflection time of about 1 sec. The preparation of the nerve was modified from Levin's practice. The leg (usually of a spider crab, but sometimes the claw of a lobster or the leg or claw of an edible crab) was severed near the body, the two junctions between the third and fourth segments were cut by bone forceps, and the muscles attached to the fourth segment were cut away. The two portions thus made were then gently pulled apart, the nerve remaining attached to the fourth segment. The whole process of "pulling out" the nerve may take less than half a minute. The nerve so obtained was washed immediately in sea-water. Lastly, the silver stimulating electrodes were replaced by narrow strips of filter paper soaked with sea-water. These paper electrodes were connected to the copper leads from the secondary of a Harvard stimulating coil (about 50 make and 50 break shocks per second) through holes bored in the block of wax constituting the nerve chamber, the holes being filled with sea-water gelatin. Thus the spread of copper ions into the stimulating electrodes was prevented. The chief reason for this change is to avoid the damage caused by silver wire to the nerve which is much more liable to mechanical injury than a medullated nerve.

Repetition of some of Levin's experiments. Some of Levin's experi-

ments were repeated, employing the moving-coil galvanometer. The deflection during the period of "retention" was recorded every half minute. The deflection ("negative variation") produced by tetanic stimulation increases at first with continuance of the stimulus, passes through a maximum, and then declines gradually. When stimulation ceases before three minutes there is a rapid back swing, the amount of which depends upon the duration of the stimulus: it vanishes after about three minutes' continuous stimulation. The back swing is followed by a long period during which the "retention" gradually disappears and the galvanometer slowly returns to its original position. The complete disappearance of "retention" is achieved within 17 to 50 min., according to the period of stimulus given (10 to 37 sec.). In the present experiments the periods of "retention" are more exaggerated than those observed by Levin. They cannot of course be measured very accurately as the galvanometer returns asymptotically to its final position. The duration of the stimulus in a number of experiments, the maximum deflection, and the period of "retention" are given in Table I.

TABLE I. Periods of action current "retention" in air.

Date (1928)	29. i.	29. i.	29. i.	30. i.	30. i.	30. i.	15. ii.	16. ii.	13. iii.	13. iii.
Experiment	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)			(*a*)	(*b*)
Duration of stimulation (sec.)	17	18	20	32	35	26	10	37	35	31
Maximum deflection (mm.)	422	430	387	287	830	481	278	850	181	535
Period of retention (min.)	23	30	32	21	30	30	17	50	43	22

From Levin's work it is clear that the magnitude of the deflection, caused by a second tetanic stimulus given at an interval after a first, is determined by the amount of the first action current still "retained" at the moment. The second response, therefore, can be fully displayed only when the first "retention" has disappeared completely. Fig. 1 shows the time curve of the height of the second deflection. Two stimuli were given at an appropriate interval. In order to avoid unnecessary fatigue at the stimulating electrodes the stimulus was cut off as soon as the deflection had reached a maximum. At the desired interval after the first stimulus the potential divider which supplied current to balance the injury potential was adjusted so as to bring the galvanometer to zero, and the second stimulus was applied. The ratio of the two deflections thus obtained is given in Fig. 1. An ample interval (30 to 50 min.) was allowed between two successive pairs of observations. The percentage recovery of the second deflection, beginning at a low value immediately

after the first stimulus, attains 50 p.c. in 2 to 4 min., then increases steadily, and at about 15 min. may reach 80 p.c. or more. These experiments, and all subsequent ones unless otherwise stated, were made in air.

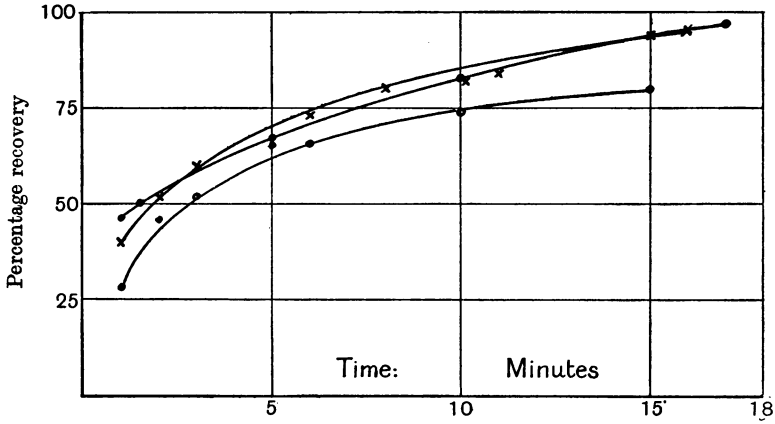

Fig. 1. Size of action current ("negative variation") in response to a second tetanus at various intervals after a first one: second response expressed as a percentage of first: three separate experiments: nerve in air.

The results show clearly that, in order to obtain the full value of a second deflection, the "retention" of the first action current must be completely eliminated, and that in the "retention" phenomenon we are concerned with some restitution process, as Levin suggested.

Injury potential and maximal "negative variation." During the earlier experiments a peculiar relation was noticed between the maximum deflection due to a tetanic stimulus given by a single pair of electrodes, and the injury potential existing at the moment. The two quantities seem to be connected in a simple manner. Altogether nineteen sets of observations were made during February, 1928, of which typical examples are shown in Fig. 2. The magnitude of the injury potential is a function of the time from the moment of preparation of the nerve. It increases often during a short period after the nerve is first prepared, then decreases rapidly for 30 min. or so, and then very slowly, at an approximately constant rate, until finally it disappears. The maximum deflection obtained on tetanizing follows the injury potential in an approximately linear manner, and tends also to disappear when the injury potential approaches zero. The periods of observation extended from 2 to 23 hours. Such a remarkable relation could hardly be expected unless we assume that the injury current and the action current are in some way related.

At present, however, in the nerves or other tissues of the various animals studied, there is little evidence suggesting any such relation. In the case

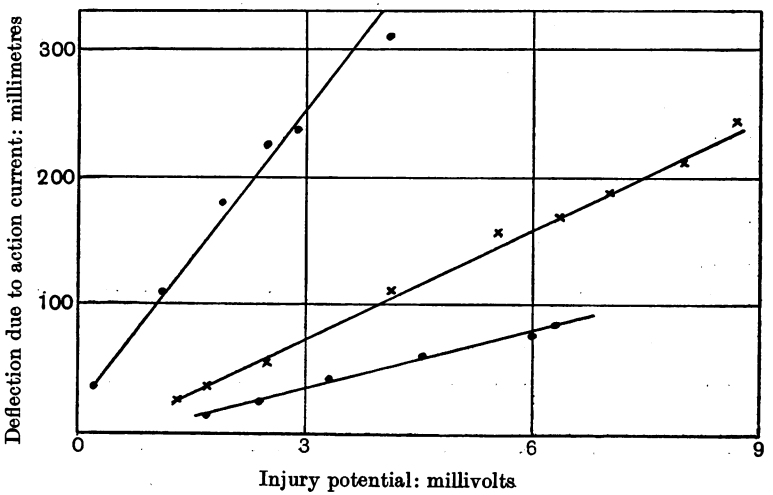

Fig. 2. Deflection due to action current, as a function of the magnitude of the injury potential existing at the moment: injury potential diminishing spontaneously with time: three separate experiments: nerve in air.

of frog's nerve, for example, it has frequently been observed that even when the injury potential has almost completely vanished the action current remains approximately unchanged.

The injury potential can be diminished not only by time but by stimulation. Take the case of two tetanic stimuli at an appropriate interval: denote the first maximal deflection by d_0 and the injury potential before stimulation by p_0. After a short interval (1 to 2 min.) from the first stimulus, adjust the potential divider to a value p_1 to bring the galvanometer deflection to zero. Apply a second stimulus, resulting in a maximum deflection d_1. The ratio $\frac{d_0 - d_1}{p_0 - p_1}$ gives us the change of action current (say in mm. deflection) associated with a change of one unit of injury potential (say 1 millivolt). This quantity is then compared with the deflection caused by one unit of potential applied externally, by means of the potential divider, through the galvanometer and nerve. Seven experiments of this nature were performed during February, 1928, with satisfactory results. One of these is described in Table II.

The agreement between the two values found is remarkable, the mean value of the ratio being 220 mm. per millivolt, while 221 mm. was obtained by the external application of the same difference of

TABLE II. Change of action current per unit change of injury potential produced by stimulation.

Int.	Time p.m.	Injury potential millivolts	Action current deflection mm.	$\frac{d_0 - d_1}{p_0 - p_1}$
	3 hr. 50 min.	9·51	795	221
1 min. 39 sec.		7·34	315	
	4 hr. 47 min.	8·55	725	222
1 min. 40 sec.		6·60	292	
	6 hr. 32 min.	6·98	618	213
1 min. 37 sec.		5·29	258	
	7 hr. 36 min.	6·23	567	225
1 min. 39 sec.		4·80	245	
	8 hr. 31 min.	5·72	537	222
1 min. 43 sec.		4·34	230	

One millivolt applied externally gave 221 mm. deflection. "Int." signifies the interval allowed between the cessation of the first stimulation and the beginning of the second stimulation in a given pair of observations.

potential. We may conclude, therefore, that a unit of potential difference is associated with the same deflection, whether it be a fall of injury potential produced by stimulation, or be applied externally. The relation found can scarcely be accidental, and it suggests that the injury potential, or whatever it implies, is in a sense, in crustacean nerve, the cause of the action current, just as an applied electromotive force is the cause of the resulting deflection.

The temporary depolarization of nerve. We have just shown that the ratio

$$\frac{\text{(decrement in the response to stimulation)}}{\text{(decrement in injury potential)}},$$

both decrements being the result of a previous stimulus, is equal to

$$\frac{\text{(deflection due to an applied E.M.F.)}}{\text{(E.M.F. applied)}}.$$

It might have been expected that each would be equal to

$$\frac{\text{(total response to stimulation)}}{\text{(total injury potential)}}.$$

This expectation is not verified. The ratio last named, with a single pair of stimulating electrodes, is usually much less than the others. The discrepancy might be accounted for if the strength of the stimuli were not sufficient to stimulate all the nerve fibres, or if local fatigue at the stimulating electrodes were so profound as to prevent further activity in the nerve trunk. The latter seems the more probable, but the former cannot be neglected, as was found in later experiments with the Downing galvanometer, when for other purposes a single impulse was recorded. If the second suggestion were correct local fatigue could be avoided by stimulating a fresh point on the nerve when signs of fatigue had set in

at the first electrodes. Accordingly a wax chamber with three pairs of stimulating electrodes was made. Three pairs were found to be sufficient for the present purpose. These electrodes were used in such a way that as soon as the deflection caused by stimulating at the first electrodes reached a maximum, the stimulus was switched over quickly to the second pair, and similarly later to the third pair. This type of experiment can be performed successfully only with a galvanometer of short period.

The object of the present experiments is as follows. Supposing that we can effectively avoid local fatigue at the stimulating electrodes, how closely can the ratio

$$\frac{\text{(total response to stimulation)}}{\text{(total injury potential)}}$$

be made to approximate to the ratio

$$\frac{\text{(decrement in response to stimulation)}}{\text{(decrement in injury potential)}}?$$

The question can really be expressed as follows: How far can we abolish the injury potential during, and by, stimulation if we avoid appreciable local fatigue? In the following experiments the injury potential was not balanced. The sensitivity of the galvanometer was suitably reduced and the deflection on the scale was a direct consequence of the injury potential. The electrical response to stimulation diminished the deflection. Our problem, therefore, was, how closely can the galvanometer be brought to zero during, and as the result of, stimulation?

Certain precautions necessary for this type of experiment must be described. Firstly, the injury must be complete, otherwise an apparent reversal of the injury potential may be obtained as the result of stimulation, as the following experiments show:

Injury potential (millivolts)	9·68	7·70	8·72
Deflection due to injury potential (mm.)	225	210	222
Residual deflection after stimulating (mm.)	−90	−80	−38

On some occasions a nerve trunk was found to have opaque patches (the normal nerve is quite transparent and has a very faint bluish tint) which block conduction. In one of these cases the two galvanometer leads were placed at intact points on either side of an opaque region. There was only a small potential difference (0·92 millivolt) between the two leads, which may be attributed to a difference of the nerve's resting activity at the two points. On stimulation, the maximum deflection (one pair of stimulation electrodes used) corresponded to 2·57 millivolts. This means that if the resting activity were equal on both sides of a block, we might record action current without injury potential. An incomplete injury may act as a partial block and leave some of the partially injured fibres inactive, though not exhibiting an injury potential. This may explain the apparent reversal, under such circumstances, of the injury potential as the result of stimulation.

Secondly, the strength of the stimulating current must be just, and only just, sufficient to give a maximal effect. Over-stimulating induces rapid local fatigue, or may inflict damage on the nerve around the stimulating electrodes, even though the stimuli used be no stronger than those employed for frog's nerve. The procedure adopted was to use at first a weak stimulus and then gradually to increase its strength in successive observations until finally a maximum degree of depolarization was obtained. An example will show the importance of not employing too strong a stimulus.

Time	Injury potential millivolts	Deflection due to injury potential mm.	Coil distance cm.	Residual deflection during stimulation mm.
11.57 a.m.	5·57	284	9	10
1.30 p.m.	5·23	260	8	33
3.30 „	5·18	275	7	90
4.25 „	5·06	319	5	160

The residual deflection is seen to *increase* with stronger stimulation instead of decreasing. Probably some of the fibres were put out of action by the strong current employed. The third, and the most important, factor for a successful experiment is the condition of the nerve itself. Uninjured points of this should be at, or approximately at, the same potential, and the excitability should be constant. These conditions are not always readily attained. To attain them requires care in the preparation of the nerve. It is often found that excitability varies considerably along the length of the nerve. Another important source of error lies in the onset of the type of inexcitability described below. In spite, however, of these difficulties, it is possible, on occasion, to obtain decisive results.

A series of preliminary experiments was made with the slow-moving Campbell galvanometer, the results of which are given in Table III.

TABLE III. Diminution of injury potential by stimulation: slow-moving galvanometer.

Date (1928)	6. iii.	7. iv.	8. iv.	9. iv.	10. iv.	11. iv.	13. iv.
Initial injury potential (millivolts)	4·23	6·88	2·59	4·04	2·54	3·21	5·29
Residual injury potential at end of stimulus (millivolts)	1·27	3·03	0·01	0·49	0·88	0·37	0·08

As mentioned above, this type of galvanometer is unsuitable for the present experiments. It can, nevertheless, be seen that the injury potential falls temporarily to a small fraction of its original value after a short tetanic stimulation at several pairs of electrodes. After this preliminary success the experiments were continued with the Downing galvanometer. Owing to its short deflection time (about 1 sec.) it was

extremely satisfactory. It was suitably shunted in order to keep the spot of light within a convenient range on the scale. The total time of stimulation was reduced to 5 to 10 sec. instead of the 20 to 30 sec. required with the Campbell galvanometer. The delay in attaining the maximum with the Downing instrument—5 to 10 sec.—is a genuine effect to be attributed to the properties of the nerve; the deflection time of the galvanometer itself is much less. The results of experiments with the Downing galvanometer are given in Table IV.

TABLE IV. Temporary total depolarization by stimulation: rapid galvanometer.

Date (1928):											
5. iv. (*a*)	5. iv. (*b*)	12. iv.	14. iv.	16. iv.	20. iv.	23. iv.	24. iv.	25. iv.	28. iv.	3. v.	4. v.
Injury potential (millivolts):											
2·61	3·90	6·73	1·14	12·80	2·08	10·87	5·57	7·99	5·04	10·12	6·99
Deflection due to injury potential (mm.):											
64	86	303	410	212	312	249	284	262	85	250	160
Residual deflection (mm.):											
1	20	0	0	0	4	0	10	0	0	5	0
Percentage abolition:											
99	77	100	100	100	98	100	96	100	100	98	100

The residual deflection given in the fourth row can be maintained only for a short period as fatigue at the stimulating electrodes causes a quick return of the spot of light towards its original position. It must be stated that the results given in Table IV are the best performances in a given set of observations. Several observations were generally made before the result shown was obtained (compare the second precaution discussed above). The injury potentials observed in these cases are higher than those of Table III. In seven cases out of twelve the deflection due to the injury potential was completely abolished, that is to say, the galvanometer had returned precisely to its zero at the moment when the "negative variation" had reached its maximum. In these experiments there was no over-shooting of the deflection beyond zero. It might be suggested that the absence of over-shoot was accidental and that the "negative variation" might more than abolish the injury current. The following example shows how, in successive observations over a considerable interval, the maximum response to stimulation may continue exactly to abolish the injury current deflection.

Time	Injury potential millivolts	Deflection due to injury potential mm.	Residual deflection mm.
2.55 p.m.	12·80	212	0
3.50 ,,	11·52	203	0
5.10 ,,	9·94	182	0

The first result of this experiment has already been recorded in Table IV (April 16). Two more observations were made later. The residual deflections remained nil. The last observation was made two and a quarter hours after the first one, when the injury potential had fallen by about 3 millivolts. It would be difficult, therefore, to attribute the equality of the two deflections to coincidence. In every other experiment shown in Table IV, in which the residual deflection was zero, at least one more observation was performed after the one recorded. The result was invariable, namely the residual potential was nil at the moment when the negative variation had attained its maximum.

If we assume that, in the injury potential, we are measuring the membrane potential across the boundary of the nerve trunk, the "uninjured" lead being connected to the outside charge, the "injured" lead to the inside charge, then the present result, namely that the injury potential may be just abolished by the negative variation, means no more and no less than a temporary total disappearance of the membrane potential; or, in other words, the temporary total depolarization of the nerve as the result of a tetanic stimulus of short duration.

The absolute depolarization of nerve. The amount of action current "retention" observed in the experiments just described was naturally small owing to the short duration of the stimulus. The question arises, since we know now that a nerve can be temporarily totally depolarized, whether it is possible to depolarize a nerve completely by prolonged stimulation. Putting the question in another way: Can we increase the "retention" until the "retention" is equal to the injury current? This result may best be realized if a sufficiently large number of pairs of stimulating electrodes be provided so as to avoid local fatigue, and if stimulation be applied long enough to exhaust the nerve trunk entirely. Accordingly in the experiments to be described five pairs of electrodes were employed, each pair being used for a period long enough to make up the total duration of stimulation to several minutes. The precautions and the procedure were as described for the case of temporary depolarization. The condition of the nerve plays an even more important rôle than it did there. The results of several experiments of this type are given in Table V. It must be stated that each result given is the highest value obtained in the set of observations referred to. At the end of a long stimulus there was only a small back swing (if any) of the galvanometer, so that in the present case we are dealing with an action current which is entirely "retained." In three cases the residual deflections were practically nil, and in several other cases quite small. We may

TABLE V. Permanent total depolarization produced by stimulation for several minutes at five pairs of electrodes.

Date (1928):										
23. ix.	24. ix.	27. x. (*a*)	27. x. (*b*)	27. x. (*c*)	29. x. (*a*)	29. x. (*b*)	1. xi.	19. xi. (*a*)	19. xi. (*b*)	20. xi.
Injury potential (millivolts):										
3·88	4·35	7·38	5·47	7·74	8·08	8·00	10·07	6·00	5·74	6·74
Deflection due to injury potential (mm.):										
194	235	198	167	283	312	360	487	175	199	193
Duration of stimulation:										
5′ 14″	3′ 0″	3′ 56″	3′ 4″	1′ 13″	2′ 25″	1′ 33″	1′ 52″	4′ 45″	7′ 40″	6′ 30″
Residual deflection (mm.):										
1	22	95	8	85	25	110	160	32	1	66
Percentage depolarization:										
99·5	91	52	95	70	92	70	67	82	99	66

conclude, therefore, that under certain favourable conditions the "retention" of the action current can be made equal to the injury current; or, in other words, an absolute total depolarization of the nerve can be obtained by long continued stimulation, if local fatigue be sufficiently avoided.

The oxidative nature of the injury potential. In view of the structure of, and the nature of the material composing, a nerve fibre, the injury potential can hardly be regarded as due to a surface charge produced by purely physico-chemical causes at a membrane. It is more reasonable to think of it as resulting from a dynamic equilibrium between a constant discharge and a recharge which is effected by the resting activity of the living fibre. The first attempt to identify the source of energy of the injury potential was made as follows. The nerve was placed inside a wax chamber through which passed a constant stream of nitrogen. The gas was purified from oxygen by the method of Kautsky and Thiele(4) as employed by Hill(5), commercial nitrogen from a cylinder being forced through a filter candle into an alkaline solution of sodium hydrosulphite. In nitrogen the injury potential falls gradually and tends to reach a minimum, as shown in Fig. 3. It returns towards, or to, its original value when the nitrogen is replaced by air. The results of thirteen such experiments are given in Table VI.

The second row gives the injury potential at the moment when the nitrogen was turned on; the third row the minimum potential in nitrogen; the fourth the highest potential attained after recovery in air. The percentage drop of injury potential, given in the fifth row, was estimated as follows. As already stated, the injury potential diminishes with time. After the initial rapid change the rate of fall is usually very uniform.

It has been assumed, therefore, that a linear relation obtains between injury potential and time. The probable value of the injury potential

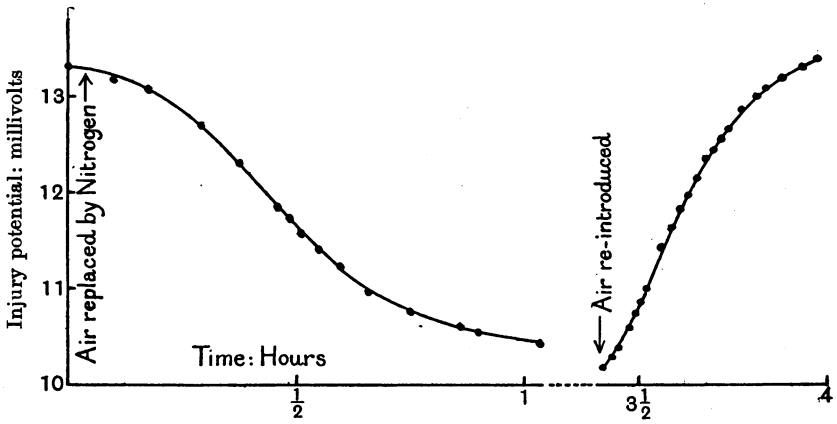

Fig. 3. Fall of injury potential in absence of oxygen: recovery in oxygen.

TABLE VI. Fall of injury potential in absence of oxygen.

Date (1928):	10. ii.	13. ii.	14. ii.	15. ii.	20. x.	24. x.	25. x.	26. x. (*a*)	26. x. (*b*)	27. x.	28. x.	29. x.	30. x.
Initial injury potential in air (millivolts):	8·36	14·02	13·40	12·39	13·75	18·58	15·01	27·33	17·47	14·74	16·01	22·00	12·61
Minimum injury potential in nitrogen (millivolts):	3·93	7·45	10·16	6·71	8·82	6·00	6·42	17·06	1·76	5·46	9·09	10·70	5·71
Injury potential after recovery in air (millivolts):	6·77	9·92	13·34	10·76	10·63	10·03	11·51	25·82	6·05	9·91	14·78	19·62	12·41
Percentage drop in injury potential:	49	25	24	40	22	62	51	35	78	55	40	47	54

is then interpolated, for the case of air, for the moment when the minimum potential is reached in nitrogen. From the interpolated value and the observed minimum in nitrogen the percentage drop is obtained. The first four experiments, carried out in February, indicate that with the wax chamber employed a considerable fraction, 24–49 p.c., of the injury potential may be abolished by asphyxia. It was considered, however, that the wax of the chamber might dissolve an appreciable amount of oxygen and give it out again, so contaminating the nitrogen. To avoid this risk further experiments were made in November, in which the wax chamber was replaced by a glass bottle with a thick rubber stopper through which three glass tubes were inserted. One of these was used for the gas exchange and the other two for the necessary electrical connections. The latter two tubes, 10 and 5 cm. long respectively, each

had a side hole made at the inner end and was filled with sea-water gelatin to within 2 or 3 cm. from the free end outside the stopper. The empty outer ends were filled with zinc sulphate solution to receive the galvanometer leads. The nerve was placed on the 10 cm. tube. The injured end was brought in contact with the gelatin inside the tube at the side hole. Connection with an uninjured point was made by a strip of filter paper soaked with sea-water, bridged over from the side hole of the second tube to the first. When the nerve had been mounted the bottle was evacuated with a water pump. It was then filled with hydrogen and evacuated again. This process was repeated several times. The results are shown in the last nine entries of Table VI. The percentage diminution of the injury potential is higher in general than in the experiments of February: the highest value was 78 p.c. The absolute drop of the potential is perhaps more conspicuous, being from 2·5 to 10 millivolts. The average drop is 6·8 millivolts.

The large fall of injury potential in the absence of oxygen, and its recovery when oxygen is readmitted, strongly indicate that oxidation provides the energy required to maintain the potential. The question arises, why is not the injury potential completely abolished by lack of oxygen? In this connection A. V. Hill has recently calculated (6) the depth to which oxygen can penetrate into a sheet of resting muscle exposed on one surface only to a given oxygen mixture. Assuming Krogh's coefficient of oxygen diffusion and a resting metabolism equal to that of frog's muscle, his calculation shows that in nitrogen containing 0·01 p.c. of oxygen the gas should be able to penetrate to a depth of 0·02 mm. Taking the case of a cylindrical tissue ((6), p. 60), and employing Gerard's value for the resting metabolism of nerve, it appears that 20 p.c. of the whole cross-section may be supplied with sufficient oxygen in a gas mixture containing only 0·025 p.c. of that gas. Without the most stringent precautions, therefore, to eliminate the last traces of oxygen, it is easy to understand that there might still be enough present to maintain in sufficient activity a considerable fraction of the whole substance of the nerve.

We have already identified the action potential as a temporary reduction of injury potential, and "retention" as a more permanent reduction, and it has been suggested that the gradual disappearance of the "retention" is due to some kind of recovery process. If, now, the injury potential were ultimately due to an oxidative process we might expect that the gradual disappearance of the "retention" would be much slower in the relative absence of oxygen. It is interesting to note

in this connection that excitability was often lost under anaerobic conditions although the injury potential still persisted. The loss of excitability by asphyxia is a well-known phenomenon in medullated nerve. In Fig. 4 in the upper two curves are shown two different types

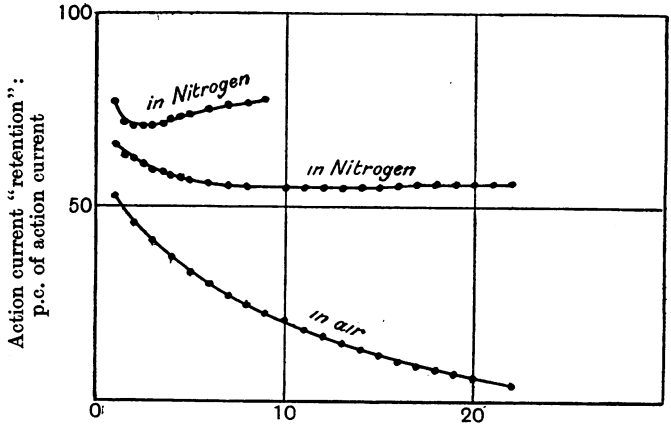

Fig. 4. Disappearance of action current "retention" in presence of oxygen: persistence of "retention" in absence of oxygen.

of time-course of the "retention" process after stimulation in nitrogen. The lower curve, taken in air, is given for the purpose of comparison. Nearly the same maximum deflection was attained in each of the three experiments, the duration of the stimulus being about 20 sec. The initial rapid drop represents merely the back swing on the cessation of the stimulus, the latter not having been long enough to abolish the action current completely. In the case of the experiments in nitrogen the deflection ceases within a few minutes to fall further, then either remains constant or even increases gradually again. In air the curve falls regularly and finally reaches its zero position. There is no doubt therefore that the gradual disappearance of the "retention" involves a restitution process accompanied by oxidation. The injury potential is maintained, and can be restored after stimulation, only in the presence of an adequate supply of oxygen.

In a recent paper Meyerhof describes experiments on the oxygen consumption of Maia's nerve (7). The resting oxygen consumption, and the extra oxygen used as the result of stimulation, are astonishingly high—10 and 20 times, respectively, those of the frog's sciatic nerve at the same temperature. The maximum rate of the extra oxygen consumption due to stimulation reached 40 p.c. of the resting value. Thence it declined slowly until the normal rate was reached somewhere between one-half and three-quarters of an hour,

after a tetanic stimulation lasting 2½ to 10 min. The last result is in good agreement with the conclusion reached above, that the disappearance of "retention" is due to a restitution process involving oxidation.

Discussion. The "negative variation" of the injury current resulting from a tetanic stimulus is produced by the summation of a large number of monophasic action current waves, following one another in rapid succession. Its diminution as the result of prolonged stimulation might conceivably be due to any one of three causes:

(*a*) A prolonged refractory period, allowing fewer impulses to pass per second;

(*b*) a recovery in the injured region, allowing the individual action current waves to become diphasic, so that each second phase would neutralize the first;

(*c*) diminished polarization in the uninjured region, as suggested above.

Of these, (*a*) would afford no explanation of the "retention" effect described by Levin. As shown already, the diminution in the response to a tetanus caused by previous stimulation is exactly equal to the diminution in the injury current also so caused. Thus possibility (*a*) may be dismissed. With regard to (*b*), it seems very unlikely that absence of oxygen would *improve* the condition of the injured region, or that prolonged stimulation at several distant electrodes in succession would produce *complete* recovery from an injury: and one can scarcely imagine that the injured region would then remain completely recovered so long as oxygen is absent. Thus (*b*) also can be dismissed. We are left therefore with (*c*), which we have assumed in the above discussion of the experiments.

The natural way to regard the injury potential is as a potential difference existing across a membrane surrounding the nerve fibre, the "uninjured" electrode being connected to the outside charge, the "injured" electrode to the inside charge. Accepting this view of the matter, we may express our results as follows:

(*a*) The membrane of the nerve fibre may be temporarily depolarized by a short tetanic stimulus;

(*b*) it may be permanently depolarized by a long tetanic stimulus;

(*c*) the building up again of the potential difference involves processes of an oxidative nature and is impossible in the absence of oxygen.

In the injury and action currents, therefore, we are looking at two aspects of one fundamental property of the living nerve cell, its "active membrane potential," the word "active" being used to distinguish it

from other types of membrane potential which may be due to purely physico-chemical causes, differences of concentration, etc.

In crustacean nerve fibres the outside membrane is exceedingly thin, the inside being composed of an apparently homogeneous jelly-like material. A potential difference is maintained across the outside membrane by a "galvanic combustion element" existing either at its surface or in the living material inside (see Straub(3)). The charge involved is continually being dissipated even if the nerve be at rest, just as an accumulator runs down slowly if left standing unused. In a resting condition even a very low oxygen pressure is enough to maintain the potential difference; an insufficient supply of oxygen, however, leads to a fall of potential, as we have found. During the propagation of a stream of impulses, that is, in the rapid discharge of the "active membrane potential," the amount of electricity released may be so great that the capacity of the available battery is not adequate to cope with the sudden demand. The injury potential falls to a low value, or even to zero, and oxidation is not rapid enough to maintain it. Unless oxygen be present to enable the surface layer of the cell to be re-charged, the injury potential and the action potential never rise again to their initial values. In muscle, oxidative recovery restores the capacity for doing mechanical work; in crustacean nerve, apparently, recovery restores to the membrane around the fibre its original electric charge, by which the injury and action currents can be manifested, by which perhaps the impulse is propagated.

Reversible inexcitability not due to stimulation. During the spring, nerves prepared as usual were often found on testing them in the chamber to be completely inexcitable, that is, there was no action current response to stimulation. In August and September, when the phenomenon reappeared, an effort was made to find the probable cause of it, or at any rate to avoid its consequences. It soon became evident that a few minutes' soaking in sea-water immediately after the nerve was prepared could completely prohibit the appearance of this inexcitability, or at least largely mitigate its effect. The mere washing of a nerve in running sea-water for less than a minute often proved to be effective. Some typical cases are shown in Table VII.

TABLE VII. Reversible inexcitability.

Date	Injury potential millivolts	Deflection due to injury potential mm.	Maximum deflection of action current mm.	Percentage "activity"	Remarks
4. ix.	10·99	102	11	11	30 min. after "pulled-out"
	9·13	595	210	35	Soaked in sea-water for 45 min.
	7·86	512	378	73	40 min. interval
5. ix.	7·73	527	5	1	20 min. after "pulled-out"
	5·75	370	117	31	Soaked in sea-water for 15 min. Interval 40 min.

In both the experiments shown in Table VII the nerves were not at first washed with sea-water but were mounted immediately in a wax chamber after preparation. The maximal deflection obtained on stimulating was only a very small fraction of the injury current, 11 p.c. and 1 p.c. respectively. After the first stimulus they were soaked with sea-water *in situ* for 35 and 15 min. respectively. The recovery of response (see column 5) was remarkable. This type of restoration from an inexcitable state, as the result of washing with Ringer's solution, has recently been reported by Dulière and Horton(8) for the case of a dissected frog's muscle. Gottschalk(9) also has found that nerves rendered inexcitable by asphyxia can be recovered in part either by salt solution or by oxygen, but that complete recovery is attained only when the two agents act together. In the present case the sudden onset of the inexcitable state was not due to exhaustion of the nerve, or to lack of oxygen, for the injury potential before the first stimulation was high. We must suppose, therefore, that inexcitability is somehow produced either by the "shock" of preparation, or by removal from a normal environment, or by some unknown rapid change occurring in the body fluid in contact with air. In crustacean blood there are stated to be so-called "explosive" cells (polynuclear leucocytes) which "explode" spontaneously as soon as the blood is taken from the body. The number of these cells varies greatly from time to time and from one individual to another. The products of the explosion of these cells may conceivably be toxic or narcotic. It is difficult, however, to apply such an explanation to the type of reversible inexcitability found by Dulière and Horton in the case of dissected frog's muscle.

SUMMARY.

1. Levin's experiments on crustacean nerve were repeated and his results confirmed.

2. Injury potential in crustacean nerve diminishes with time, as also does the maximum deflection of a galvanometer recording the sum of the monophasic action currents produced by a tetanic stimulus. The two are connected by an approximately linear relation: both tend to vanish simultaneously.

3. The injury potential is diminished by stimulation (Levin's "retention"). A unit fall of injury potential produced by previous stimulation is accompanied by a diminution in the response to a stimulus which is exactly the same as the deflection due to a unit of potential difference applied externally.

4. By avoiding local fatigue through the use of three pairs of stimulating electrodes a nerve can be temporarily totally depolarized by a tetanus: that is, the maximum deflection due to the action current becomes equal and opposite to the deflection caused by the injury current.

5. Absolute total depolarization can be obtained by long-continued stimulation with five pairs of stimulating electrodes: that is, the action current "retention" may be equal and opposite to the injury current.

6. In the absence of oxygen the injury potential falls: on the

readmission of oxygen it recovers. In the absence of oxygen a considerable part of the action current may be permanently "retained."

7. In crustacean nerve action and injury potentials are two aspects of the same fundamental phenomenon, viz. of an "active membrane potential" existing at the surface of the nerve fibre and maintained by oxidative processes. This potential is discharged by stimulation, or spontaneously at rest in the absence of oxygen. It can be maintained, or restored, only if oxygen be present.

In conclusion I desire sincerely to acknowledge my indebtedness to Prof. A. V. Hill for his suggestion of the present work and for his advice and encouragement throughout it. My thanks are due to Dr E. J. Allen for his permission to work at the Marine Biological Laboratory at Plymouth and for the generous supply of the material used. My gratitude is due also to Mr A. C. Downing for installing the galvanometer employed.

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NOTE BY A. V. HILL.

Experiments made at Plymouth, since the Author left England for Japan, on the heat-production of crab's nerve, have shown that some 98 per cent. of this heat occurs *after*, and only 2 per cent. *during* activity. The recovery heat-production is relatively large, being 20 to 40 times as great as in the frog's sciatic: it occupies, at 13° to 18° C., 25 to 30 minutes after the stimulus (5 to 10 seconds). There is no doubt, therefore, that the gradual restoration to its original value of the injury potential diminished by stimulation, involves considerable energy exchanges and is an active recovery process, involving the consumption of oxygen, as Levin and Furusawa have contended. See also Meyerhof and Schulz(10).