THE ACTION POTENTIAL OF SPINAL AXONS IN VITRO*

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It has been established elsewhere that funiculi of the mammalian spinal cord can be isolated for study *in vitro* (35). There they conduct action potentials over their entire length and maintain constant and apparently normal physiological properties for 12 hours or longer. The present study shows that these properties consistently include a large negative after-potential associated with certain of the labile membrane phenomena which have been described in normal peripheral nerve (9, 31) and intact central axons (5). A period of supernormality accompanies the after-potential and corresponds closely both to the form of this potential and to the recovery sequence previously described for dorsal column myelinated axons *in situ* (38). In Fig. 1 is presented the entire monophasic action potential of a dorsal column *in vitro*.

Methods

The data and conclusions presented in this paper are the result of experiments performed on 160 isolated spinal funiculi and 54 excised peripheral nerves or spinal roots obtained from 108 cats. In all cases in which comparisons are drawn between the properties of central and peripheral axons these are based on observations made on pairs of tissues drawn from the same cat and maintained together in the same nerve chamber under identical conditions.

1. Dissection.—Dissection of the spinal cord was performed either under sodium pentobarbital anesthesia (27 mg./kilo) or not less than 1 hour following the termination of ether anesthesia in curarized, spinal preparations. As in the intact cord (38), the conditions of anesthesia produced no significant differences in the properties of tracts *in vitro*.

After laminectomy and incision of the dura, the cord is ligated in the upper lumbar and upper thoracic regions to minimize bleeding during dissection. It is then transected below the thoracic and above the lumbar ties. The piarachnoid is freed from the surface of the dorsal columns for a few millimeters at the transected ends and incised along one line of dorsal root entry. It is then stripped, usually in one piece,

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from the cord dorsum. If the piarachnoid tears, the other end is grasped and the membrane stripped in reverse. Since the resistance to dissection of the tracts imposed by even small filaments of piarachnoid leads to stretch and damage of the preparation, it is essential to be meticulous, although decisive and rapid in the removal of this membrane. Landmarks are then identified at the cut surface of the transection and the bilateral dorsal columns grasped and stripped from the cord in one smooth motion.

Regions of heavy collateralization must be avoided by using thoracic cord. There, lengths exceeding 6 cm. are available which have not been appreciably stretched.

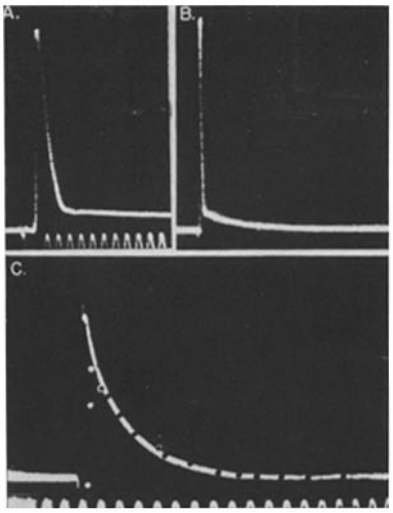

FIG. 1. Dorsal column *in vitro*. A. Spike potential recorded monophasically at 2 cm. of conduction distance (cathode proximal to recording electrodes). B. Slower sweep showing complete action potential. C. Same sweep as B but at 10 times the gain to illustrate the after-potential. Superimposed upon the after-potential in C is the plot of the recovery of excitability. Time for A 2000 C. P. S., for B and C 200 C. P. S.

Dissection and removal of the dorsal columns to the chamber require about 60 seconds from the time of first interference with blood supply; but the dorsal columns are in fact separated from underlying tissue and therefore significantly deprived of CO_2 and O_2 for less than 10 seconds of that time.

The piarachnoid can be peeled similarly from the other funiculi if these are to be isolated. The most convenient tracts to remove are the ventral columns. With these the other parts of the cord are first stripped away from the selected fibers which are protected from stretch by the ventral pia and by the continuous insertion and fixation offered by the ventral roots. After exposing 10 cm. or more, the ventral columns may be grasped and easily lifted away from the ventral pia. They are then subsplit along the ventral commissure to provide unilateral ventral columns of small diameter.

The manner in which the funiculi of the spinal cord may thus be separated is illustrated in Fig. 2.

A careful search through paraffin-fixed methylene blue-stained serial sections of dorsal columns in 3 preparations taken at random from the experimental series has shown no sign of attached gray matter.

Critical to the success of the isolation are avoidance of stretch and speed in dissection and transfer to warm oxygenated physiological solution containing 5 per cent CO_2. As each spinal tract is removed it is placed immediately in a bath of Krebs's (19, 21) or Hastings' (1, 16) solution at 37°C. through which is bubbled a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. From this bath the tissues are transferred to the nerve chamber in a stream of oxygen and carbon dioxide humidi-

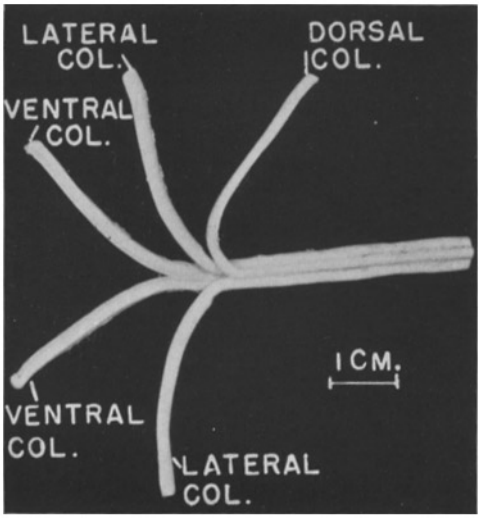

FIG. 2. Thoracic spinal cord partially stripped to illustrate how the various funiculi can be separated in a fresh preparation.

fied at 37°C. Alternatively, stripped tracts can be placed in Krebs's or Hastings' solution at 4°C. and stored at this temperature for many hours prior to study. Since fibers so treated show minor abnormalities of membrane properties after rewarming to 37°C., the procedure has been avoided in this study.

Isolated spinal tracts are capable of conducting impulses immediately upon transfer to the nerve chamber; and when the dissection has proceeded without difficulty, normal spike height and large after-potentials are present. A number of preparations less optimally treated have been in varying degrees of conduction block when first observed, but after 30 minutes of washing recovered their spike and after-potential properties so as to be indistinguishable from those not undergoing transient block. Therefore the tissues were equilibrated as routine with the physiological solutions for 1 hour after dissection and prior to study. The nerve chamber was of the type used by Lehmann (25) but modified to provide for gentle rocking at a frequency of

20 cycles/min. since the high oxygen uptake of spinal tracts apparently causes oxygen supply to be diffusion-limited in the absence of stirring. Action potentials were recorded from the tissue in the gas phase of the chamber either as readings interspersed during a period of constant washing or during prolonged maintenance out of solutions. Repeated controls ensured that washing did not introduce varying degrees of electrical shunting at stimulating or recording electrodes provided solution was drained by capillarity from the preparation as it was taken from the liquid into the gas phase of the chamber. All gases sent to the chamber were first humidified and warmed to 37°C. (the routine temperature of the nerve chamber). It was demonstrated that simple turnover of the gas phase did not disturb the equilibrium of the otherwise closed system.

2. Solutions.—Because the normal environment of central nervous tissue is not known with certainty and may be significantly different from that of peripheral nerve, two different physiological solutions were used: Krebs's solution, an artificial serum, and Hastings' solution, an artificial cerebrospinal fluid similar to Krebs's solution but containing one-half the concentration of Ca^{++} and K^{+}. All solutions were bicarbonate-buffered and contained 200 mg. per cent glucose replacing an isosmolar amount of NaCl.[1] It may be stated that the action potential is quite similar following equilibration in either solution. A pH of 7.23 was used for most experiments since cats either unanesthetized and on a respirator after ether induction, or under barbiturate anesthesia were found to be moderately acidotic (see also references 10 and 25). However, the effect of changing pH to 7.4 has been examined and found to be slight (see Results).

3. Experimental Procedures.—Potentials were led from the spinal tracts *via* bright silver or gelatinized Ag-AgCl electrodes (33) and were amplified and recorded with the equipment described elsewhere (38). Constant current square wave stimulation was used with a pulse duration (0.1 to 0.2 msec.) equal to twice the chronaxie of the columns according to the suggestion of Offner for minimizing power delivery (34). For monopolar recording of the action potential cathodal polarization, heat-crushing, concentrated KCl, or cocainization were used separately or in combination, all with similar results.[2]

In the study of recovery of excitability following impulse conduction use was again made of the current calibration method of Graham and Lorente de Nó (13). Thus excitability values presented here can be compared directly with those obtained *in vivo* (38), but see text.

Changes in "resting" excitability invoked by chemical means were studied as

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[1] The ionic concentrations of these solutions as used in this laboratory were (in mM/ liter) for Krebs's solution: K^{+} 6.0, Ca^{++} 2.55, Mg^{++} 1.24, Na^{+} 138, Cl^{-} 128 $PO_4^{=}$ 1.19, $SO_4^{=}$ 1.20, HCO_3^{-} 17.9. For Hastings' solution: K^{+} 3.32, Ca^{++} 1.25, Mg^{++}, 1.24, Na^{+} 134, Cl^{-} 121, $PO_4^{=}$ 0.48, $SO_4^{=}$ 1.20, HCO_3^{-} 17.9. Both solutions contained 11.1 mM/liter glucose and 2.5 volumes per cent CO_2.

[2] It was often impossible to render the action potential completely monotonic. Even under conditions when it could be ascertained that no activity was being conducted to the distal recording electrode a positive deflection was generally interposed between spike and after-potential. (See Fig. 5 and *cf.* Lorente de Nó, chapters 16, 5, of reference 31.)

routine by simultaneous measurement of variations in current strength required to produce a threshold response, and variation in height of a submaximal (half maximal or smaller) response to a stimulus of constant current strength. Since in the latter case excitability is determined as a function of the number of conducting axons it is necessary to know that the recorded size of the maximal spike remains constant. Therefore, the height of a conducted response to a supramaximal stimulus was measured concurrently. An increase in the amplitude of the response to the constant submaximal stimulus at a time when the amplitude of response to the supramaximal stimulus was constant or falling signified an increase in the number of fibers responding to the stimulus and hence increased tissue excitability.

Stimulating electrodes were oriented longitudinally along the tissue in order to stimulate at the membrane of longitudinal dorsal column parent fibers rather than at collaterals (39). The smaller diameter of collaterals should in addition ensure for them a higher threshold and reinforce this differential response. In several preparations in which threshold was studied as a function of the orientation of stimulating electrodes four to five times as much current was necessary to excite when the electrodes were applied transversely instead of longitudinally. Consequently, "resting" excitability as determined in this way is a measure of excitability of parent longitudinal axons. In order to avoid abnormally depolarized tissue the stimulating cathode when used for testing excitability was never placed closer than 2.5 cm. from a cut end. Conditioning and testing stimuli were delivered as routine through the same pair of electrodes; but, as in intact spinal cord (38), no significant differences were found in the form of the recovery curves when stimuli were applied through separate pairs. Care was also taken to record monophasically the amplitude of all testing volleys so that this parameter would not be a function of changes in wave length of the spike. Preparations were studied either isolated from ground or grounded between stimulating and recording electrodes. Checks for interaction were made as routine whenever stimulating pulses were delivered through more than two stimulators.

4. Oxygen Supply to Spinal Tracts in Vitro.—Essential to the study of isolated central axons is the maintenance of adequate oxygenation by diffusion alone. Using columns initially capable of physiological function, Robert Katzman has kindly provided us with the data in Table I on oxygen uptake in phosphate-buffered Krebs's solution at pH 7.40 in an atmosphere of pure oxygen. The average uptake, 7.1 μl/gm. wet weight/min. is approximately double that generally given for mammalian peripheral nerve (11, 18). It is somewhat higher than the maximum uptake of 5 μl./gm. wet weight/min. given by Holmes (18) for dorsal column snipped out of the rabbit spinal cord. Since the data in Table I (derived from preparations whose diameters were purposefully varied) show no tendency for thinner preparations to increase their oxygen consumption per unit volume it may be assumed that the columns studied in the Warburg apparatus were not diffusion-limited.

By using the formula for diffusion of oxygen into tissue of cylindrical shape derived by Fenn (8), Gerard (12), and Hill (17) the limiting radius for minimally adequate oxygenation was calculated for an external pO_2 of 0.88 atm. (the oxygen tension of 95 per cent O_2, 5 per cent CO_2 corrected for water vapor at 37°C.).[3]

[3] The value used for the diffusion coefficient of oxygen is that given by Krogh for muscle corrected to 37°C. (22).

The limiting radius calculated from the highest value for oxygen uptake given in Table I (8.5) is 0.83 mm. For the average oxygen uptake (7.1) the limiting radius would be 0.905 mm.

The average radius measured in nine bilateral dorsal columns selected at random from those studied in the body of this paper was 0.90 mm. ± 4 per cent error of measurement.

The percentage of tissue volume which would be inadequately oxygenated at any given respiratory rate was also calculated. If a dorsal column of average radius respired at the maximum rate in Table I, 16 per cent of its mass would be inadequately

TABLE I

No.	Tissue	Radius* (±4 per cent error)	Oxygen consumption‡
		mm.	*μl./gm. wet wt./min.*
1	Dorsal column, unilateral thoracic	0.66	6.1 ± 1.4
2	" " " "	0.77	7.1 ± 1.0
3	" " bilateral "	0.87	7.1 ± 1.3
4	" " " "	0.94	7.7 ± 1.3
5	" " " "	0.94	7.6 ± 0.9
6	" " " lumbar	1.08	7.7 ± 1.0
7	" " " "	1.17	7.5 ± 0.8
8	Lateral " unilateral thoracic	0.90	5.9 ± 1.2
9	" " " "	0.90	8.1 ± 1.1
10	" " " "	0.92	6.0 ± 0.8
11	" " " "	0.99	6.3 ± 0.7
12	" " " "	1.00	7.0 ± 1.3
13	" " " "	1.25	8.5 ± 0.8
Average.....		0.95	7.1 ± 1.1

* Calculated from length and volume measurements assuming columns to be cylinders.

‡ Average of readings taken every 10 minutes for 1 hour and 30 minutes after 20 to 30 minutes' equilibration in Krebs's solution (PO_4 buffer, 200 mg. per cent glucose, pH 7.40, 37°C.). Standard deviation calculated using $n - 1$ degrees of freedom.

oxygenated. If the average dorsal column respired at the average observed rate, oxygenation would be adequate for all axons.

Theoretical considerations therefore indicate that adequate oxygenation is possible, but that the margin of safety is not great at 0.88 atm. oxygen. The validity of these calculations can be tested experimentally by observing the effect of graded oxygen removal on an oxygen-sensitive parameter of nerve function such as resting excitability. In Fig. 3 is presented an experiment typical of 8 in which was studied the consequence of replacing 11 per cent of the oxygen in the nerve chamber by nitrogen while keeping carbon dioxide tension constant. The amplitude of the conducted supramaximal response and the excitability (submaximal response) of the dorsal column are seen to be unaffected. The ventral column, on the other hand, shows an immediate change in excitability. It is therefore concluded that in an atmosphere of

95 per cent O_2, 5 per cent CO_2 humidified at 37°C. the average dorsal column at rest is adequately oxygenated with some margin of safety with respect to this sensitive, functional parameter. The average ventral column, however, is probably hypoxic at its center. The presence of gray matter attached to the ventral but not the dorsal columns is undoubtedly significant in this respect.

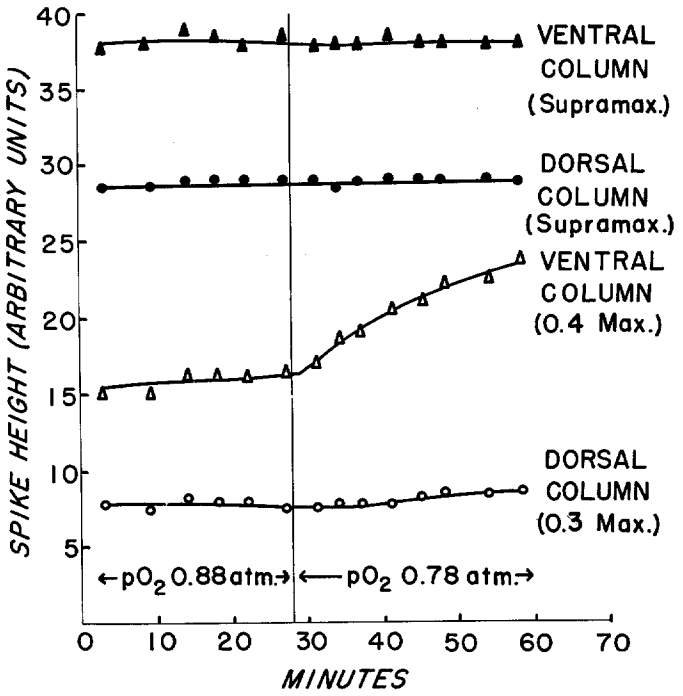


FIG. 3. Effect on excitability and spike height (dorsal and ventral columns studied simultaneously) of replacing 11 per cent of the nerve chamber oxygen by nitrogen while maintaining carbon dioxide constant. Tissue in gas phase throughout. Testing volley size chosen to be maximally sensitive to changes in excitability of both dorsal and ventral columns.

To test the possibility that oxygen uptake might increase sufficiently during activity to cause oxygenation by diffusion to be inadequate, the effect of tetanization at frequencies between 100 and 200 volleys per second was studied in 10 preparations. It was found that supramaximal responses in these tissues never fell more than 5 per cent in the course of conducting over 9×10^4 volleys and showed no change other than recovery of height in the post-tetanic period. One preparation was tetanized for 44 minutes during which time 3.5×10^5 volleys were conducted without any change in amplitude of response. The data therefore show that extreme activity produces neither hypoxia nor any other effect sufficiently intense to alter the Q fraction of membrane potential (31) which alone can be tested under these experimental conditions.

In order to assay the adequacy of oxygenation still further the effect on excitability of increased oxygen pressure was studied. Small increments of oxygen pressure produced no significant change, but at pressures greater than 3 atm. of oxygen severe tissue damage occurred, which could be attributed only to the well known toxic action of oxygen at high pressure. The findings of this investigation are reported elsewhere (36, 37).

5. Stability of Membrane Properties in Vitro.—The stability of membrane properties and requirements for maintenance of a steady-state condition of central axons *in vitro* over a period of hours cannot be predicted *a priori*. In general, excised ventral columns show a slow increase in excitability when maintained in the gas phase of the nerve chamber. The change in excitability is completely reversed by washing and occurs without accompanying change in the height of the conducted maximal spike potential. Dorsal columns are extremely stable either showing no drift at all when unwashed for 30 to 60 minutes or drifts far slighter than ventral columns. The most likely explanation of this difference seems to lie in the absence of gray matter from excised dorsal columns and its presence in ventral columns.

Since glutamate is the only amino acid metabolized to any extent by brain (19) and because of the observations of Krebs, Eggleston, and Terner (20) upon its effect on K^+ turnover of brain slices, the effect of replacing 5 mM/liter chloride ion by glutamate was studied in 30 isolated spinal tracts. Glutamate in this concentration did not alter significantly the form of the after-potential or recovery cycle of excitability (see Fig. 7). Neither was the excitability drift of ventral columns in the gas phase nor the response to anoxia changed significantly.

RESULTS

1. The Spike Potential of Central Axons

The form of the spike potential of central axons in a nerve chamber is similar to that described for A fibers of peripheral nerve (9, 10) (see Figs. 1, 4, and 5). In ventral column in which temporal dispersion is slight spike constants have been measured after 0.5 cm. conduction. The rise time is 0.16 msec. and the duration 0.47 msec. (average of 11 preparations). The average height of a maximal spike potential after 0.5 cm. of conduction is 20.3 ± 9.7[4] mv. in dorsal columns (19 preparations) and 19.4 ± 6.1 mv. in ventral columns (10 preparations). The average maximal conduction velocity of ventral columns is 96 m./sec. (82 to 118 m./sec., Fig. 4, bottom right). This compares favorably with 97 m./sec. (80 to 130 m./sec.) obtained from a study of eight animals *in vivo*. Velocities calculated from the records of Lloyd (28) lie in the same range. Perfunctory examination of conduction in dorsal columns indicates that velocity decrements in a manner typified by Fig. 5. The initial velocity of 55 m./sec. may be compared with values of 58 to 69 m./sec. reported by Lloyd and McIntyre (29). The decrement is consistent with that seen following dorsal root stimulation *in situ* (9).

[4] One standard deviation throughout unless indicated otherwise.

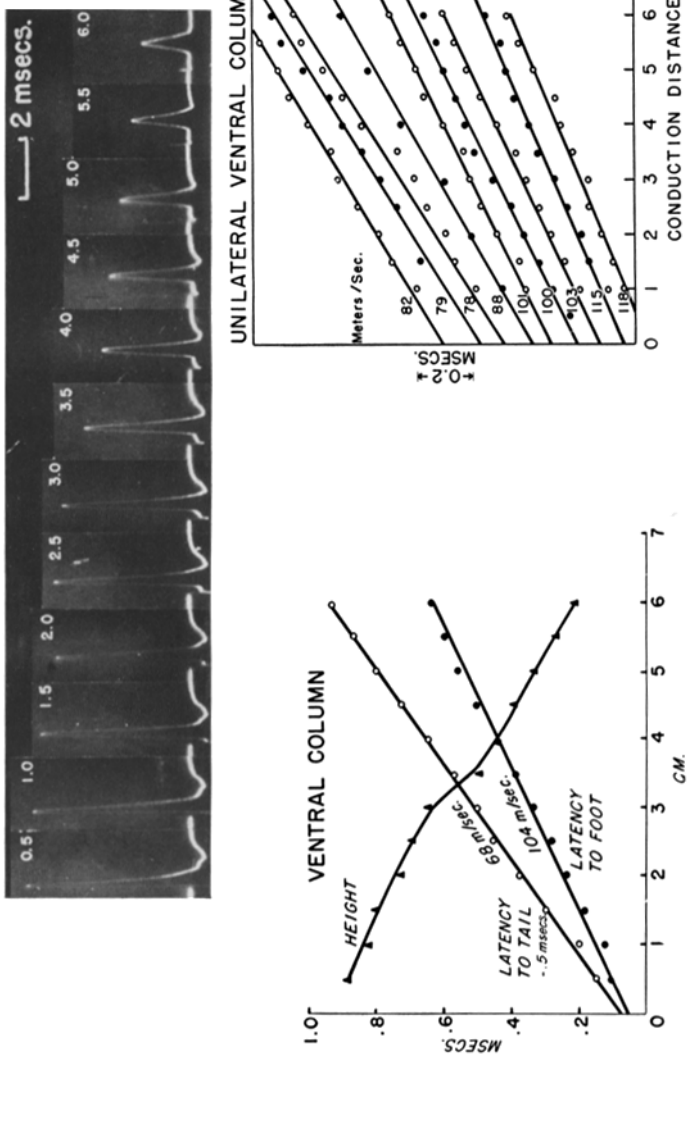


FIG. 4. Ventral column *in vitro*. *Upper*. Spike potentials recorded at the indicated distances (centimeters) from stimulating cathode (recordings slightly biphasic in this case). *Lower left*. The latencies to the foot and tail of the ventral column spike above are plotted against conduction distance (0.5 msec. should be added to ordinate to obtain latency for the latter). In addition, there is plotted the spike height as a function of conduction distance. A break in this curve at 3.0 to 4.0 cm. has often been observed, suggesting a segregation of the fiber population into long pathways and short propriospinals (28). *Lower right*. Ventral column conduction velocities obtained in 9 different preparations.

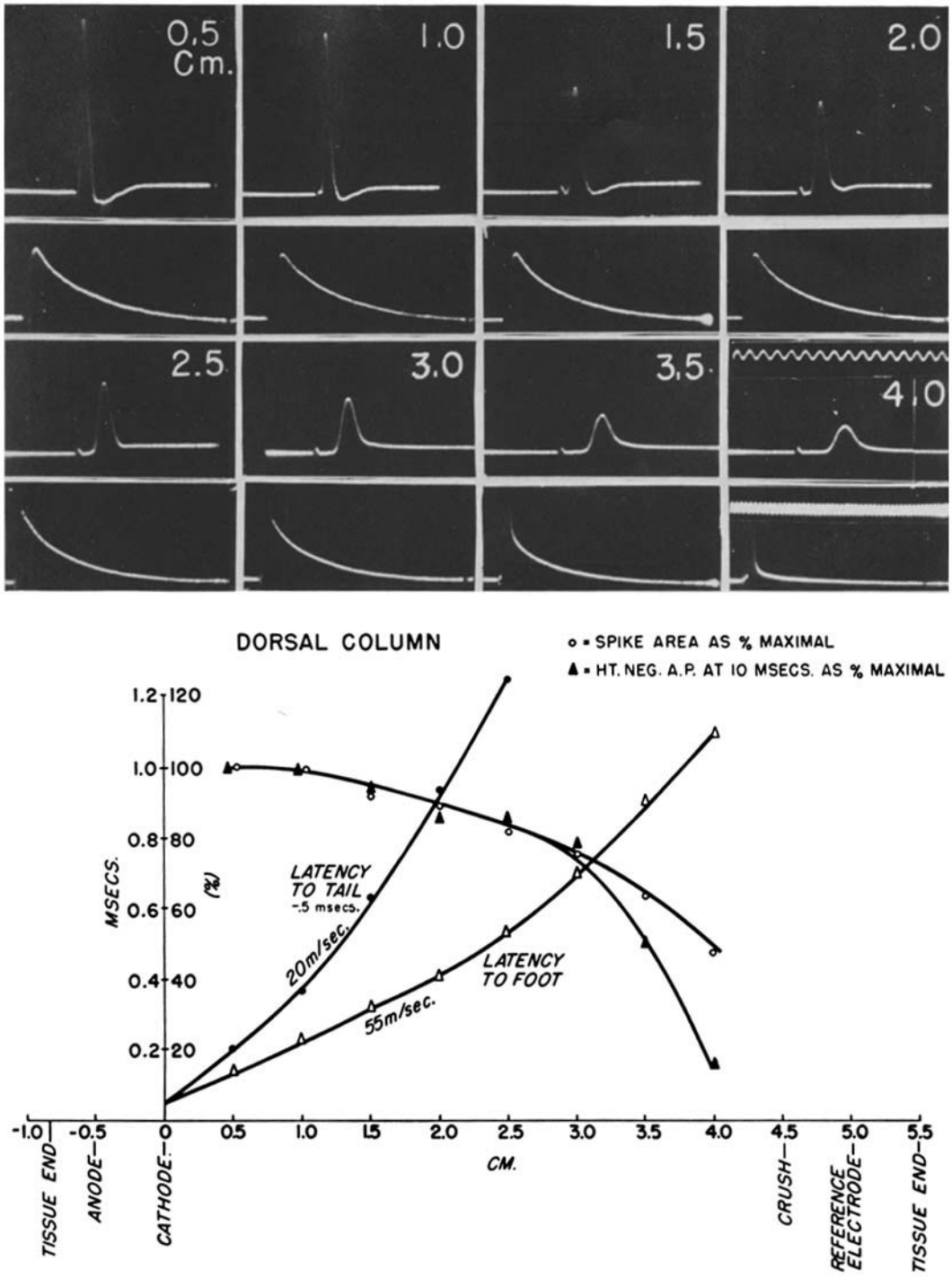

0.5 Cm.
1.0
1.5
2.0
2.5
3.0
3.5
4.0
DORSAL COLUMN
o = SPIKE AREA AS % MAXIMAL
▲ = HT. NEG. A.P. AT 10 MSECS. AS % MAXIMAL
MSECS.
(%)
LATENCY TO TAIL
-.5 msecs.
20m/sec.
LATENCY TO FOOT
55m/sec.
CM.
TISSUE END
ANODE
CATHODE
CRUSH
REFERENCE ELECTRODE
TISSUE END

FIG. 5

The change in form of the action potential which occurs with increasing conduction distance is presented in Fig. 4 for ventral and in Fig. 5 for dorsal columns. Whereas in peripheral nerve the area of the spike is reasonably constant for every distance of conduction, in spinal funiculi it decreases with increasing distance since the constituent fibers vary in length and not all extend throughout any preparation. Nevertheless, the maximum spike area for a given conduction distance is constant wherever it is tested over the length of the tissue. In addition, despite the use of stimulating currents of high intensity and long duration, there has not yet been identified electrical sign of fibers conducting at velocities slower than 15 m./sec. in dorsal column or slower than 50 m./sec. in ventral column. Consistent with this, the height of the spike potential grows as a smooth sigmoid function of stimulus current strength and the after-potential is constant in form throughout the growth of the spike.

The lack of electrical sign of small fibers is noteworthy since such fibers are plentiful in histological preparations of the tracts of human cord (15) and presumably therefore in the cat. While it may be that dissection has selectively blocked the small fibers without significantly altering even the labile properties of large fibers (see sections 2 and 3), it is equally probable that many small fibers conduct only short distances and consequently have not been detected. It is conceivable indeed that many of these fibers under 3 to 4 micra in diameter may be collaterals of parent fibers which pass obliquely across the columns. Whatever the situation, if the spectrum of fiber diameters were as smoothly distributed in the dorsal columns of the cat as in the human (15), an easily identified discreet potential elevation would not be anticipated.[5]

2. The After-Potential of Central Axons

(*a*) *Evidence for an L Fraction in Vitro.*—It has been demonstrated that dorsal column axons in the intact spinal cord support a large L fraction (5).

[5] In studies on the rabbit optic tract *in vivo* Bishop has also reported (2) an absence of potential sign of the many small fibers contained therein.

FIG. 5. *Upper.* Dorsal column spike potentials monophasically recorded at the indicated distances of conduction. Time for these is 1000 C. P. S. in the oscillogram at 4.0 cm. Below each spike at a slower sweep and 10 times the amplification are the slow potentials recorded at the same conduction distances. Time for these is 1000 C. P. S. *Lower.* The length of the above dorsal column is plotted on the abscissa with 0 set at the stimulating cathode. On the ordinate are plotted; (1) latency to foot and tail of the spike, (2) after-potential height at 10 msecs., (3) spike area. After-potential height parallels spike area until 1.5 cm. from the region of crush where demarcation currents produce a selective loss of L fraction and a more rapid fall in after-potential than spike height.

At distances within 2 cm. of the stimulating cathode a positive deflection is seen at the tail of the spike. It is not due to activity under the reference electrode.

Evidence for this derived from the presence of a large phase of supernormality during recovery from conduction, and the appearance of supernormality following extrinsically applied cathodal polarization (DCV) (38). In addition there was found the characteristic enhancement of resting excitability under the influence of such agents as potassium ion, citrate ion, and hypoxia (5).

When central axons are studied *in vitro*, a similar phase of markedly enhanced resting excitability is found in each of the following conditions: *hypoxia* in the presence of maintained CO_2 tension (Fig. 9), *hypocarbia* in the presence of maintained O_2 tension (Fig. 10), *Ca^{++} removal* (Fig. 6), *H^+ removal* (Fig. 6), or upon *application of K^+* (Fig. 6), *Na citrate addition* (Fig. 19), or under the influence of *cathodal currents*. Since the excitability of longitudinally oriented parent axons is being tested in each of these situations (see Experimental Methods), it may be concluded that parent myelinated fibers also support a large L fraction *in vitro*. Accordingly, the existence of a negative after-potential due to activity in parent axons would be predicted *in vitro* as *in vivo*.

(*b*) *Post-Spike Potential and the Recovery of Excitability.*—For the above reasons it is not surprising to find the spike potential in excised central axons followed by a large slow negative potential which is accompanied by supernormality. A representative *in vitro* potential and its particular excitability curve are reproduced together in Fig. 1 which in addition provides the complete oscillogram of the dorsal column action potential. Fig. 7 presents the compiled data from 20 preparations showing the excitability changes in dorsal columns following a single conditioning volley. It may be observed in these figures that both potential and excitability correspond in form to the exponential recovery of excitability (half-time = 7.5 msecs.) previously described for intact spinal axons (38). In addition each is unusually large compared to similar values in other nerve tissue. The potential has a peak value of 8.2 $\pm$ 0.75 per cent (standard error of the mean) of spike height in 16 preparations at 0.5 cm. conduction distance.[6] The absence of a positive post-spike potential is noteworthy and is consistent with the absence of subnormality both *in vivo* and *in vitro*.

For the purpose of further identification of the post-spike events in excised tissue their behavior following repetitive stimulation at frequencies similar to those employed *in vivo* (38) is presented in Fig. 18 (potential) and Fig. 8 (excitability). Subnormality and development of a positive potential result from short trains of conditioning stimuli at moderate frequencies in a manner similar to that *in situ*. Further, just as the component of excitability change intrinsic to intact dorsal columns was constant throughout their longitudinal extent (38), the slow potential and its associated supernormality are constant over the length of excised dorsal columns except near cut ends (Fig. 5, upper and

[6] This value is probably conservatively low being derived from preparations in which supernormality averaged 15 per cent less than that found *in situ* (38).

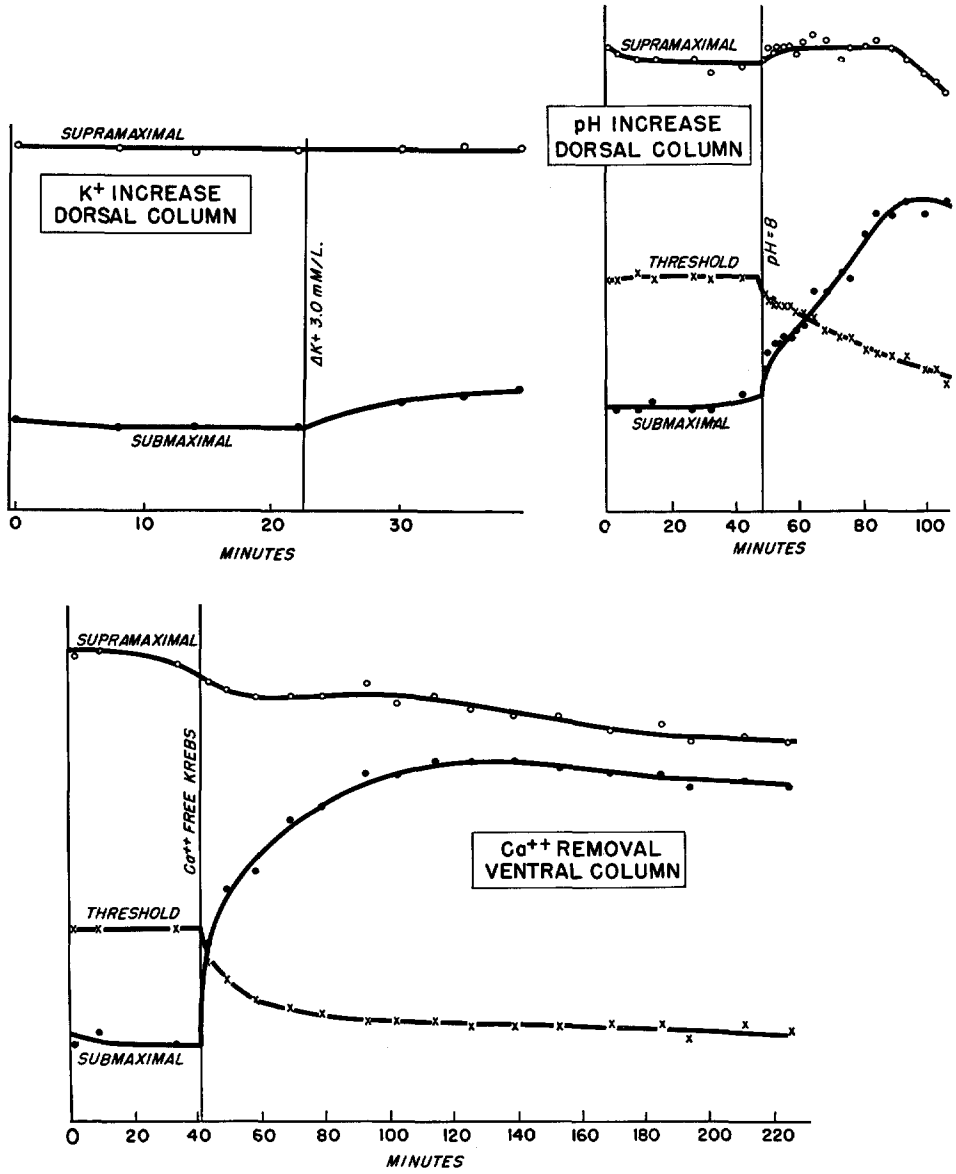


FIG. 6. *Left.* Concentrated KCl was added to increase the K^+ concentration of the Hastings solution from 3.5 mM/liter to 6.5 mM/liter. The increase in height of response is equivalent to that produced by an 11 per cent increase in stimulating current strength. *Right.* pH carried from 7.23 to 8.0 at constant CO_2 tension by replacing sodium chloride with sodium bicarbonate. *Bottom.* Following control period, Krebs's solution was replaced by one containing no calcium but normal in other respects. Note relatively independent behavior of excitability and supramaximal spike height in all cases. All preparations were washed constantly except at the moments when readings were taken. All ordinates are in arbitrary linear units of spike height (increasing upwards) except for threshold curves which are in arbitrary current units.

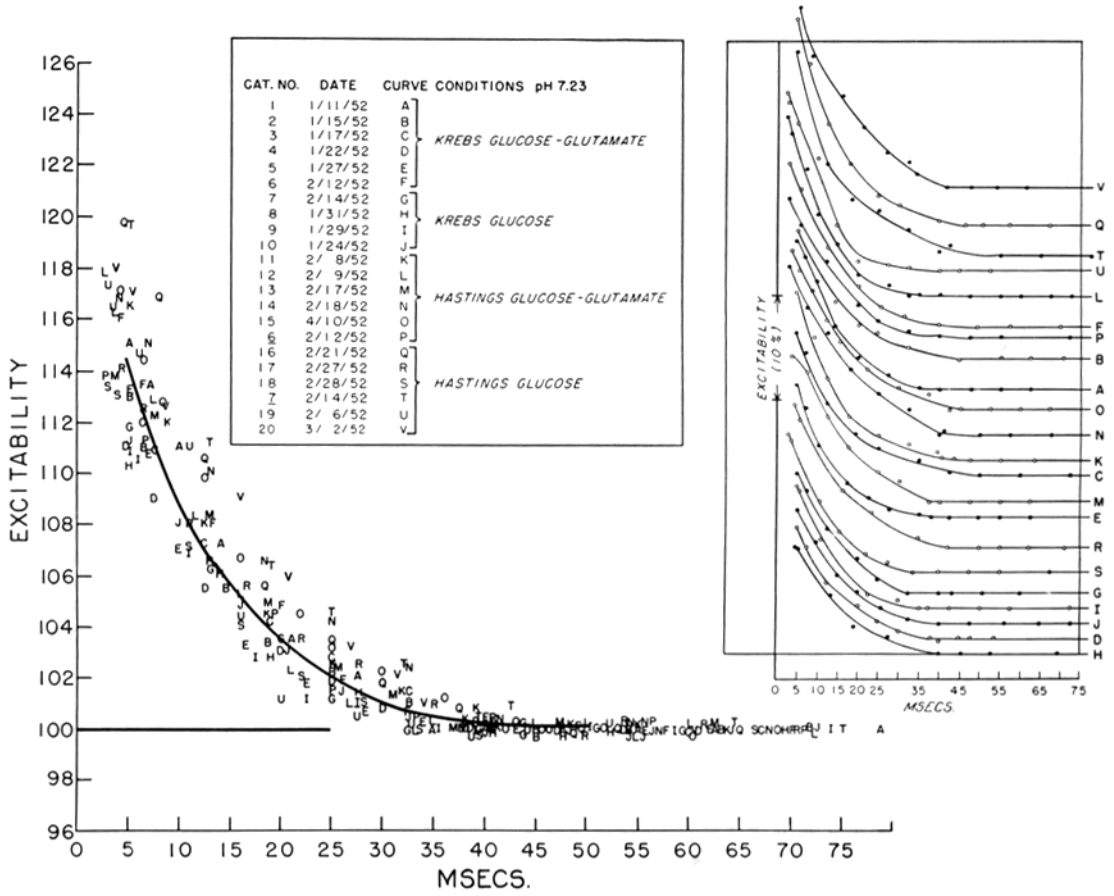


FIG. 7. Dorsal columns *in vitro*. Excitability changes following a single conditioning volley plotted in current calibrated units. The conditions of each experiment are indicated on the figure. Conditioning and testing at same electrodes. On the right are plotted separately the curves from each experiment. In the main figure the points of these curves have been plotted together by letter. Each point represents an average of 6 to 8 individual readings. The line through the points regresses exponentially from the average peak value at 4.5 msecs. with a half-decay time of 7.5 msecs.

lower). Understandably, slow potential height is constant only when compared to spike area (*cf.* circles with filled triangles in Fig. 5, lower) because temporal dispersion, some decrease in active tissue mass, and an increase in inactive tissue mass all occur with increasing conduction distance in spinal funiculi.

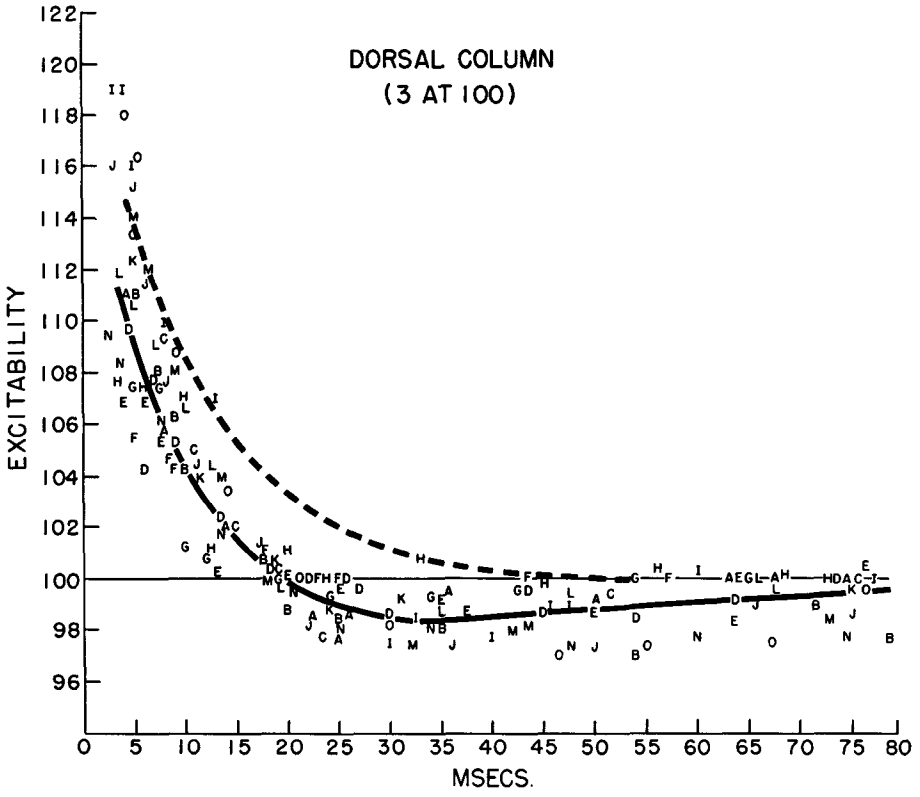
Finally it will be observed that not only the form but the value of peak supernormality *in vitro* (16.3 ± 2.3 per cent in Hastings' solution at pH 7.23 and *in vivo* (15.8 per cent ± 4.0 per cent) is quite similar.[7] However, some precaution must be observed in comparing the values *in vivo* and *in vitro*.

The excitability curves *in vivo* were derived in volume while those *in vitro* were obtained out of volume. Nevertheless, the current calibrated per cent changes can be directly compared in these two situations since experiments performed *in situ* showed an independence between the form and magnitude of recovery curves and the form and orientation of the stimulating field when the latter was manipulated by changing the orientation and separation of the testing electrodes. Since the after-potentials occur in the absence of significant external currents, changes in external resistance should not alter the load on the membrane (see Lorente de Nó (31)). This has been confirmed in dorsal roots (unpublished observations) in which the absolute value of supernormality is constant in or out of volume.

(*c*) *Origin of the Post-Spike Potential.*—The evidence presented to this point demonstrates great similarity of post-spike excitability and potential *in vivo* and *in vitro*. However, it is not entirely justifiable to conclude, by reason of congruence alone, that they are homologous in origin. Just as an analysis of the consequences of action of intact collaterals on their parent fibers was necessary *in situ* (38), here the possible effects of even a few broken collaterals along the longitudinal extent of excised thoracic dorsal columns must be evaluated.

Two types of effects are conceivable. First, steady depolarization of parent axons might result from injury currents. This effect would not appear to be of outstanding importance in view of the demonstrated existence of a large L fraction *in vitro*. It is informative in this respect to examine the attenuation of the negative post-spike potential in the vicinity of a transverse crush at the end of the dorsal columns. The characteristic attentuation depicted in Fig. 5 (*cf.* circles with filled triangles), signifies that demarcation current resulting from damage to parent fibers oriented longitudinally is far greater than possible

[7] In 10 dorsal columns studied in Krebs's solution, the average value of peak supernormality was somewhat smaller than that found in 11 dorsal columns studied in Hastings' solution (12.6 per cent ± 1.9 *vs.* 16.3 per cent ± 2.3). The half-time was somewhat longer in Krebs's solution (8.1 msecs. ± 1.7) than in Hastings' (7.5 msecs. ± 1.6). The recovery of excitability of excised axons thus corresponds better to that found *in vivo* when studied in an artificial cerebrospinal fluid, but the differences seen in artificial serum are not greatly outside the range of experimental error.


DORSAL COLUMN
(3 AT 100)
EXCITABILITY
MSECS.

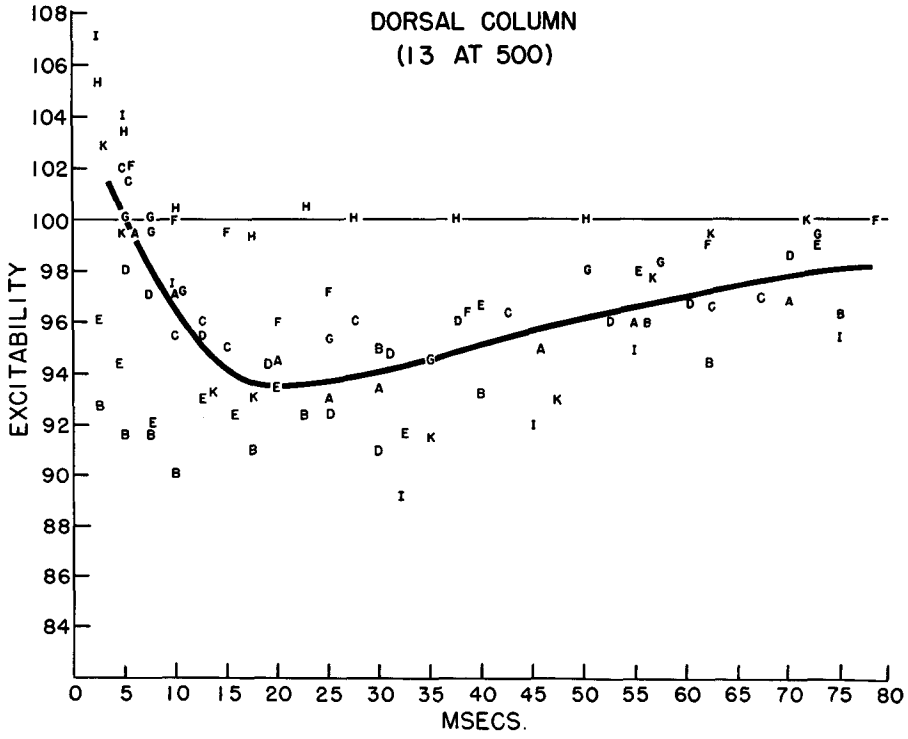

DORSAL COLUMN
(13 AT 500)
EXCITABILITY
MSECS.

FIG. 8

depolarization arising from broken collaterals distributed through the body of the tissue mass. The absence of significant collateral injury currents is entirely consistent with the known paucity of collaterals in thoracic cord and their relatively small diameter (4). (See Lorente de Nó (31) on the minor contribution of C fibers to injury currents in mixed nerves.)

A second action of broken collaterals may be conceived. Prolonged post-spike activity existing in collaterals as an "ending potential" could induce depolarization of parent axons mimicking a true after-potential. Experimental evidence can be obtained that the similarity of post-spike phenomena *in vivo* and *in vitro* is not fortuitous in this sense. It is possible to test the level of resting excitability of the parent dorsal column axons with longitudinally arranged stimulating electrodes (see Experimental Methods) without interference from action currents in the collaterals. By appropriate environmental manipulations membrane properties can then be modified while studying changes in the level of resting excitability of parent fibers as well as the post-spike potential whose origin is in question. In this manner one may determine whether the post-spike potential and the resting membrane properties of parent axons show dependent or independent behavior.

In Figs. 9, 10, and 11 are plotted portions of experiments typical of eight in which either oxygen or CO_2 removal was the experimental tool.

With either hypoxia or hypocarbia the height of the slow potential recorded oscillographically and the magnitude of the L fraction of the parent fibers, as indicated by changes in resting excitability, paralleled each other but varied independently of the spike parameters. The first change, a change in resting excitability occurred simultaneously with the first change in slow potential height, while spike amplitude remained unchanged for long periods. Throughout recovery from hypoxia (Fig. 11) or hypocarbia the correspondence between L fraction of parent axons and slow potential persisted. Overshoots in the height of the slow negative potential that were produced by washing the tissue in Krebs's solution (Fig. 11, 22 minutes) also paralleled overshoots of excitability although once again spike height remained unchanged.[8]

[8] The dependence in central axons of the post-anoxic overshooting of membrane potential upon washing has been a frequent finding. This is in marked contrast to the situation in peripheral nerve, in which no such dependence exists. A reasonable interpretation of this phenomenon in the light of present information would be that

FIG. 8. Dorsal column *in vitro*. Recovery of excitability following repetitive stimulation plotted in current calibrated units. Individual letters refer to points derived from separate preparations as in Fig. 7. Dotted line is the exponential curve representing single pulse data from Fig. 7. Solid lines represent approximate averages of the plotted data. See also Fig. 18 A, B, C for representative potential as well as excitability changes.

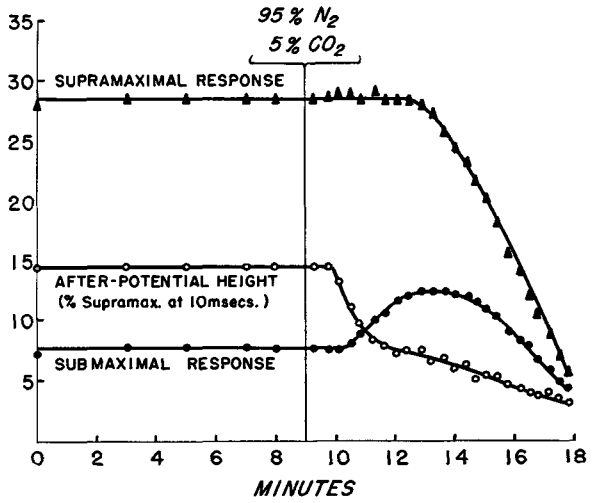


FIG. 9. Effect of hypoxia upon spike height (supramaximal response), excitability (submaximal response), and amplitude of slow potential recorded monophasically at 1 cm. of conduction. (Slow potential height read at 10 msecs., divided by supramaximal spike height and the quotient plotted on the ordinate.) In Fig. 13 are traces of after-potentials from this experiment plotted at several selected times.

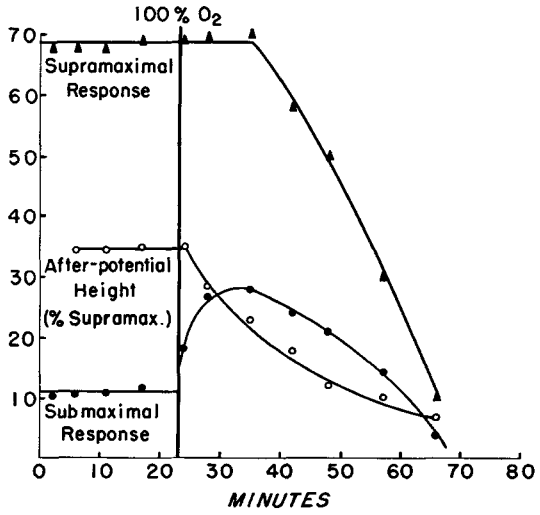


FIG. 10. Effect of CO_2 removal plotted as in Fig. 9. After-potentials of this experiment are traced in Fig. 14, upper. Preparations in Figs. 9 and 10 were in the gas phase of chamber throughout. Note that the related behaviors of resting excitability and after-potential are independent of changes in spike height. The extreme dependency of spike upon the presence of CO_2 in the atmosphere is demonstrated.

At no time in this investigation has it been possible to vary the slow potential without a corresponding change in L fraction of the parent fiber, nor has a change in L fraction of parent axons ever been accomplished without the expected change in slow potential. Consequently, it is concluded that the post-spike potential and the resting properties of the parent fiber membrane show

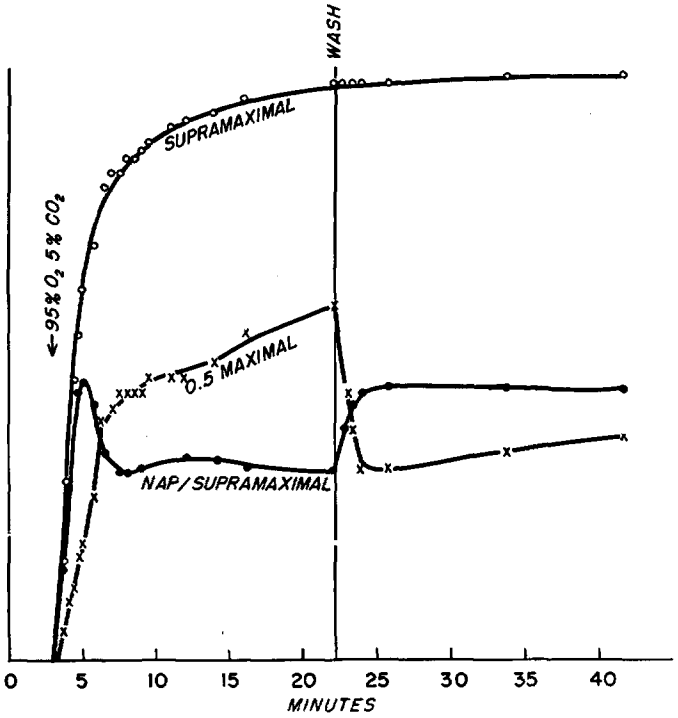


FIG. 11. Production of a post-hypoxic overshoot of membrane potential by washing tissues in Krebs's solution. Note (1) the lack of effect of washing on spike height and (2) the concurrent changes produced both in resting excitability and after-potential height.

dependent behavior. The simplest interpretation of this is that the post-spike potential originates in parent fibers.

By way of résumé the following evidence permits the identification of the negative post-spike potential as an event intrinsic to the longitudinal fibers of the dorsal columns. There is assignable to parent dorsal column axons, both excised and *in situ*, a large L fraction capable of supporting a negative after-potential with attendant supernormality. A dependent relation exists between

washing removes some depolarizing substance (probably potassium) liberated into the tissue spaces.

post-spike potential and the L fraction of excised parent fibers. There is satisfactory correspondence in form, amplitude, spatial distribution, and behavior to repetitive stimulation[9] between the post-spike recovery process in the excised preparation and intact longitudinal axons. Accordingly, this sizeable potential

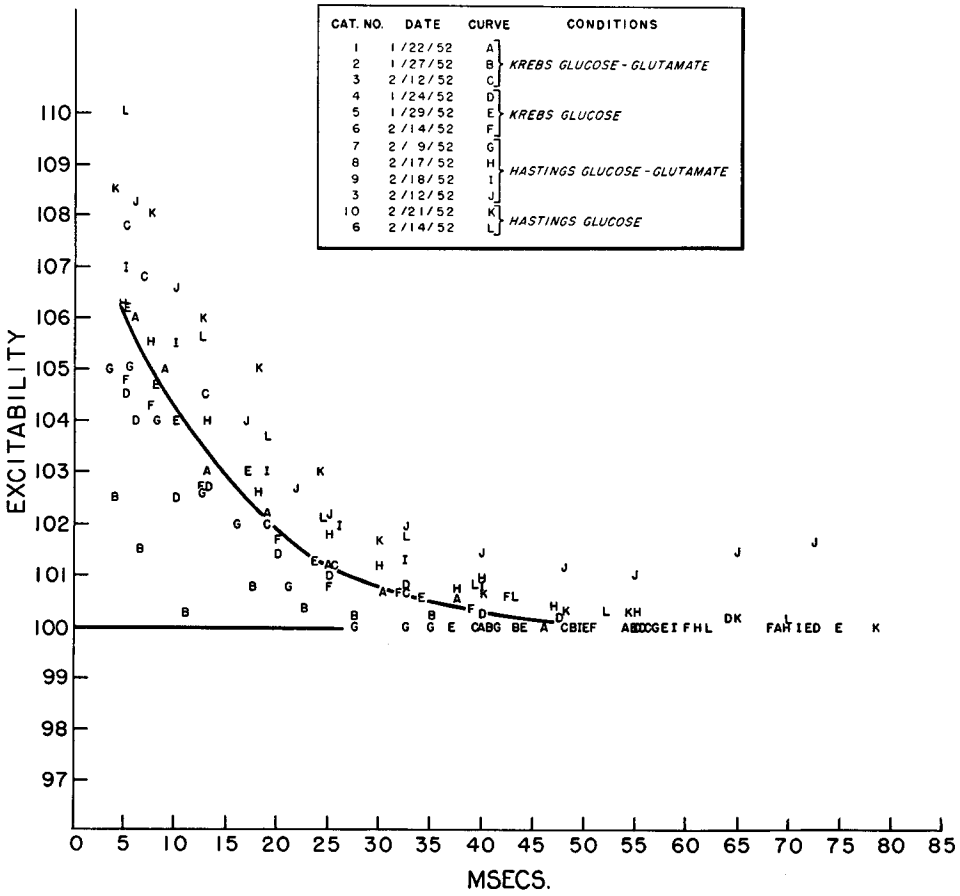


FIG. 12. Ventral columns *in vitro*. Recovery curves following a single conditioning volley. Data plotted as in Fig. 7.

is assigned an origin in parent axons and is labelled a negative after-potential in the sense of Gasser (9) because it is accompanied by supernormality.

[9] The summation of subnormality and production of a positive after-potential with repetitive stimuli that occur *in vitro* are to be contrasted with the summation of supernormality and residual negativity which appear in the vicinity of a cocaine block (30).

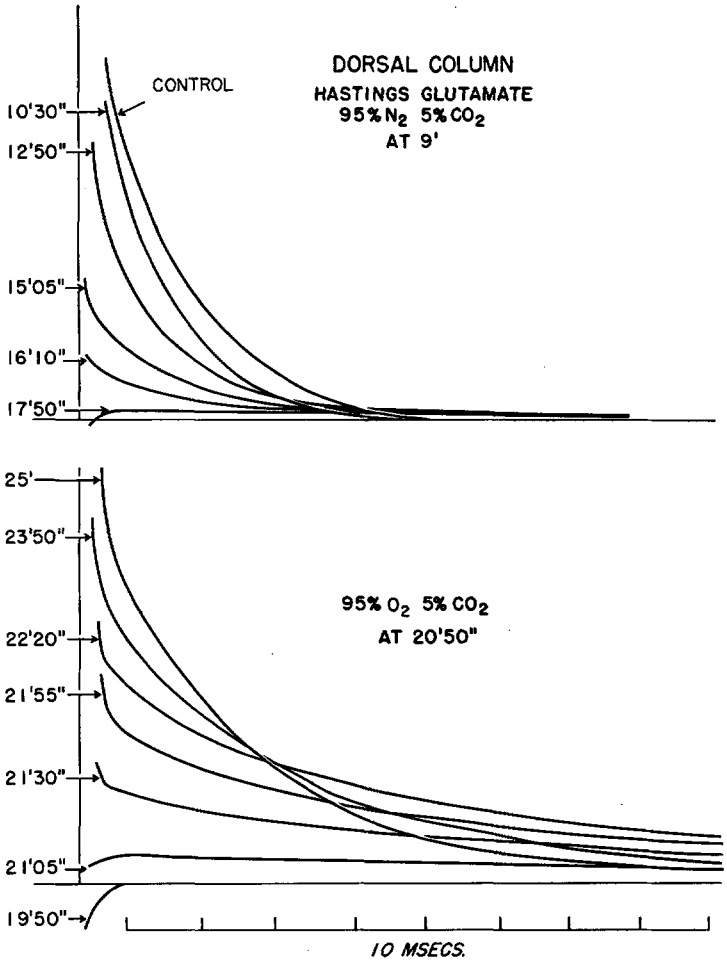
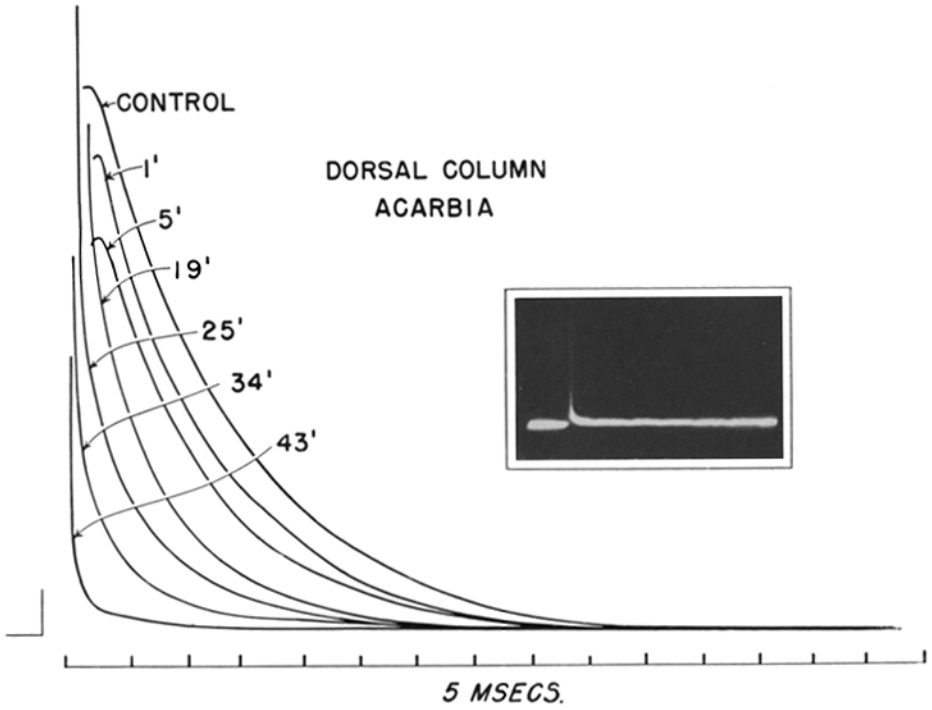


FIG. 13. After-potential of a dorsal column traced from photographic records obtained at the indicated times during hypoxia and recovery therefrom. The increase in duration of after-potential seen both during and after hypoxia is to be noted. The relationship between after-potential height (as per cent of accompanying spike potential) and resting threshold is plotted for this same experiment in Fig. 9.

The After-Potential of Ventral Column

The after-potential of the ventral column, like that of the dorsal column, consists solely of a negative phase of exponential form; however, certain differences are present. First, the dorsal column negative after-potential is large (8.2 per cent) while that of the ventral column is much smaller (1 to 3.5 per cent). The supernormality of the ventral column is also consistently smaller (Fig. 12).


CONTROL
1'
5'
19'
25'
34'
43'
DORSAL COLUMN
ACARBIA
5 MSECS.

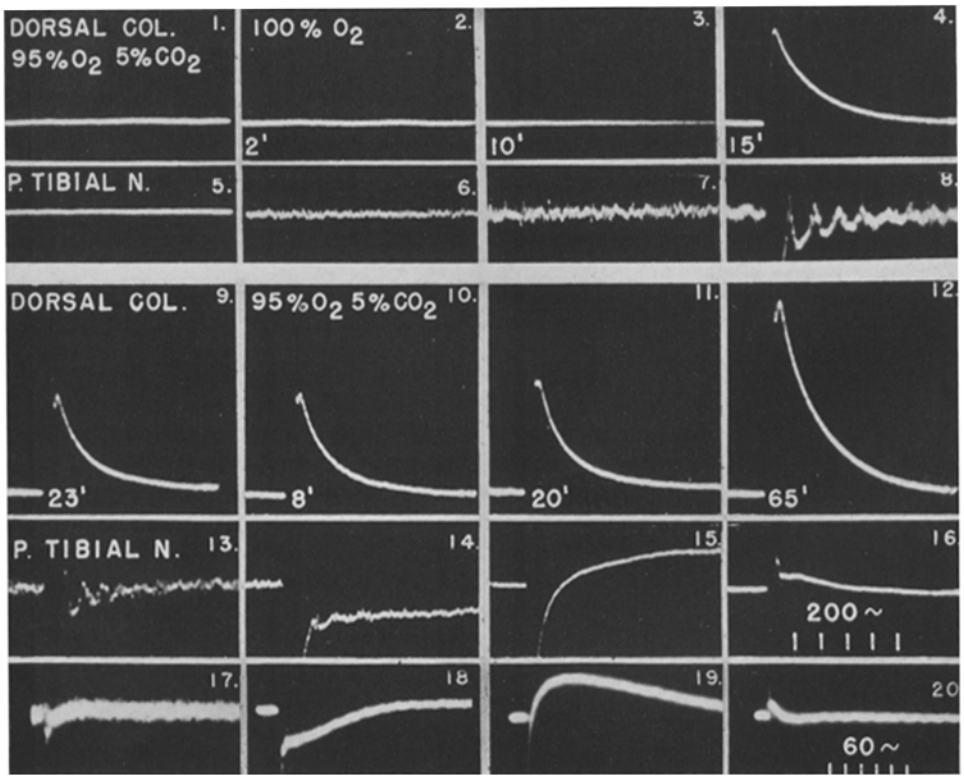

DORSAL COL. 1.
95%O2 5%CO2
100% O2 2.
3.
4.
2'
10'
15'
P. TIBIAL N. 5.
6.
7.
8.
DORSAL COL. 9.
95%O2 5%CO2 10.
11.
12.
23'
8'
20'
65'
P. TIBIAL N. 13.
14.
15.
16.
200~
17.
18
19.
20
60~

FIG. 14

Second, the half-time of exponential decay is longer in ventral (9.3 ± 1.3 msecs.) than in dorsal columns. Third, ventral column is even more resistant to the loss of its negative after-potential on repetitive stimulation than is dorsal column. These differences between dorsal and ventral column may result from the presence of gray matter and a greater tissue radius in the ventral column. The mild accompanying hypoxia and possible leakage of such substances as K^+ from intracellular to extracellular compartments of both gray and white matter would act to depress the negative after-potential. However, no amount of washing has been successful in producing after-potentials in ventral columns comparable in magnitude to those of the dorsal columns. In addition, studies of the electrotonus of dorsal and ventral columns have revealed significant evidence of differences in their fundamental membrane properties (unpublished observations).

3. The Response of Central Axons to Altered Environmental Parameters

It has been indicated previously (section 2) that removal of Ca^{++}, H^+, oxygen, or CO_2, as well as the addition of citrate, K^+, or O_2 at high pressure (36, 37) elicits in central axons a phase in which the resting level of excitability is enhanced. When severe or prolonged all these manipulations produce depression of excitability and diminution of the conducted response. This behavior is qualitatively similar to that of peripheral axons (31, 25–27). Nevertheless, significant differences exist in the response of central and peripheral axons to altered environments.

Consistent with their higher metabolic rate, spinal tracts are more sensitive than peripheral nerve to oxygen removal. The sequence of changes which lead to conduction block has occurred three times more rapidly in the former in five pairs of tissues studied simultaneously. In addition, the spike potential of

FIG. 14. *Upper.* After-potential tracings during removal of CO_2 obtained from same preparation as that of Fig. 10. Numbers indicate minutes after CO_2 removal. Insert: Photograph of the after-potential at 43 minutes to show lack of spontaneous activity although the negative after-potential is absent. *Lower.* CO_2 removal and restoration studied concurrently in a dorsal column and a posterior tibial nerve. Dorsal column: 1 to 4, 9 to 12; posterior tibial nerve: 5 to 8, 13 to 20. Times indicate number of minutes at which records were taken following CO_2 removal or its restoration. In all cases time in minutes is the same for dorsal column and posterior tibial nerve below it. 17 to 20 same times as 13 to 16 but at slower sweep to emphasize the great variation in after-potentials of peripheral nerve during recovery from CO_2 removal. Time lines for 17 to 20 in record 20, in record 16 for all others. In records 1 to 3, 5 to 7 the effect upon noise level of CO_2 removal is shown. In records 4 and 8 and in all subsequent ones a maximal spike was introduced to show after-potential as well as base line changes. Note the oscillations of peripheral nerve in records 8, 13, and 14. Tissues in gas phase throughout. In order to equalize the effects of differences in the external conductors, gains were adjusted so that maximal spikes in both tissues gave equal deflections.

central axons appears far more dependent on the presence of CO_2 than the spike of peripheral nerve as can be seen by the rapid failure of conduction in Fig. 10. Finally, unlike peripheral nerve in which the positive after-potential may become more prominent with all the above ionic or gaseous manipulations, central axons normally display no positive after-potential and have not developed one under any of these conditions in over 70 experiments. To illustrate this Fig. 13 presents the effect of hypoxia and recovery therefrom upon the after-potential of dorsal column. At no time does a positive after-potential appear even though resting excitability overshoots the control value. Similarly the result of carbon dioxide removal and restoration is plotted in Fig. 14 (upper and lower respectively). Again, the lack of any sign of positivity is to be noted;

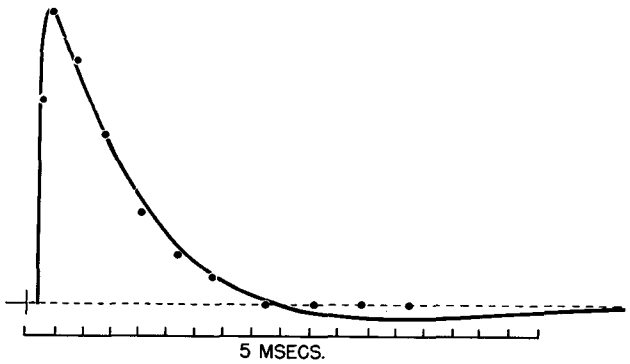


FIG. 15. After-potential tracing and recovery of excitability (dots) in a preparation with a positive deflection following the negative after-potential. Note absence of corresponding subnormality.

this is in sharp contrast to the situation in peripheral nerve in which oscillatory phenomena of both high and low frequency are observed as routine (Fig. 14, lower) (23–27, 31).

Although central axons do not normally exhibit a true positive after-potential following a single volley, a number of preparations *in vitro* have exhibited a positive deflection following the negative after-potential. As demonstrated in Fig. 15 the positivity is not associated with subnormality. While description of the origin of this positivity will require detailed knowledge of the extracellular potential field, it would appear to belong to a class of potentials developed along the longitudinal resistance of the system without associated change in membrane potential (see chapter XVI, pp. 436 *ff.* of reference 31).[10]

The composition of the fluid normally bathing central axons is unknown,

[10] A true positive after-potential and subnormality following a single volley have been observed in central axons only under one set of conditions; namely, in the period of recovery following prolonged cooling at 4°C. when they are exhibited transiently.

and the artificial serum and cerebrospinal fluid used in this study are clearly only crude approximations. Nevertheless, there is only minor effect upon the after-potential when the concentration of important cations and temperature are varied within the range that could conceivably be encountered in the normal animal.

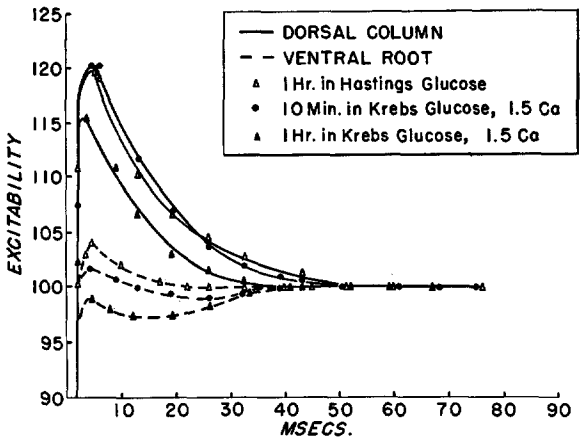


FIG. 16. Typical recovery curves of excitability in dorsal column and ventral root following a single conditioning volley. The two tissues were treated identically in the same nerve chamber and studied concurrently after reaching the steady state in Hastings' solution. After washing in Krebs's solution (containing 1.5 times usual Ca^{++} concentration) for 10 min., and 1 hour (new steady state), additional excitability curves were derived. Note that the ventral root curves swing across the resting level of excitability (100) from almost complete supernormality to total subnormality while the dorsal column only changes in magnitude of supernormality. (Comparable changes occur in peripheral nerve in which greater lengths of tissue permit the testing cathode to be placed further from cut ends.)

In eight dorsal columns studied both at pH 7.23 and 7.42 the negative after-potential was but slightly shorter and smaller at the higher pH. At pH 7.23 there was an average of 16.2 per cent peak supernormality and a half-regression time of 8.2 msecs., while at pH 7.42 the values were 15.5 per cent and 7.5 msecs., respectively. There may be observed in Fig. 16 the effect upon dorsal column and ventral root of changing K^+ and Ca^{++} concentrations through the entire range encompassed by artificial cerebrospinal fluid on the one hand and artificial serum on the other. Ventral root, like peripheral nerve loses the supernormality present in artificial cerebrospinal fluid and gains a large amount of subnormality in artificial serum. By contrast, dorsal column supernormality remains constant in form although changing slightly in amplitude and duration.[11]

[11] Fig. 16 also affords a ready comparison of the amounts of supernormality present

The result of increasing temperature from 35° to 39°C. was a decrease of 20 per cent in duration and height of the negative after-potential with corresponding changes in supernormality. The result of all the above observations is to indicate that uncertainties concerning the precise values of pH, temperature, K^+, or Ca^{++} in the *in vitro* environment of central axons are of little concern in determining the tissue characteristics studied.

On the other hand, it was found that peripheral nerves in full equilibrium with Krebs's solution (30 to 60 minutes of continuous washing) did not present the normal negative-positive after-potential sequence seen *in vivo* or *in vitro* when washing is minimized. There was no negative after-potential and no

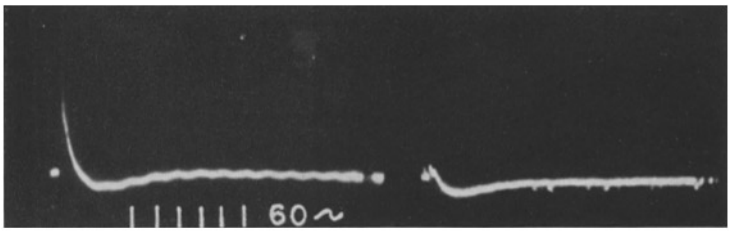


FIG. 17. Posterior tibial nerve. *Left.* After-potential immediately on transfer to nerve chamber. *Right.* After washing in Krebs's solution for 1 hour. After-potential changes were accompanied by corresponding changes in recovery curves of excitability.

absolute supernormality but only after-positivity and subnormality in 21 nerves studied.[12]

Fig. 17 shows the after-potential of a posterior tibial nerve immediately after transfer to the nerve chamber (left) and after washing in Krebs's solution for 1 hour (right). The after-potential changes were accompanied by corresponding changes in the recovery curves of excitability. Fig. 18 emphasizes the differences between the recovery processes of central and peripheral axons in equilibrium with Krebs's solution.

Thus the form of the after-potential of peripheral nerve and spinal root is easily varied and changes greatly when equilibrated with Krebs's solution whereas the after-potential of central axons demonstrates more stereotyped

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in central and peripheral axons under a variety of ionic conditions. Excitability was tested in the ventral root 2.0 cm. from each cut end.

[12] In 1937 Lehmann observed similar potential changes with extensive washing. While 10 to 20 minutes of equilibration and only occasional washing thereafter have been routine for his studies (25), he also observed that longer periods of equilibration led to a loss of negative after-potential (personal communication). For a detailed discussion of the difficulties inherent in the analysis of these changes see Lorente de Nó, chapter XVI, pp. 436 *ff.* of reference 31.

behavior. With moderate variations in all the environmental parameters studied, the latter shows only minor quantitative changes and subnormality is never observed. With little doubt, it is this property of central axons which is

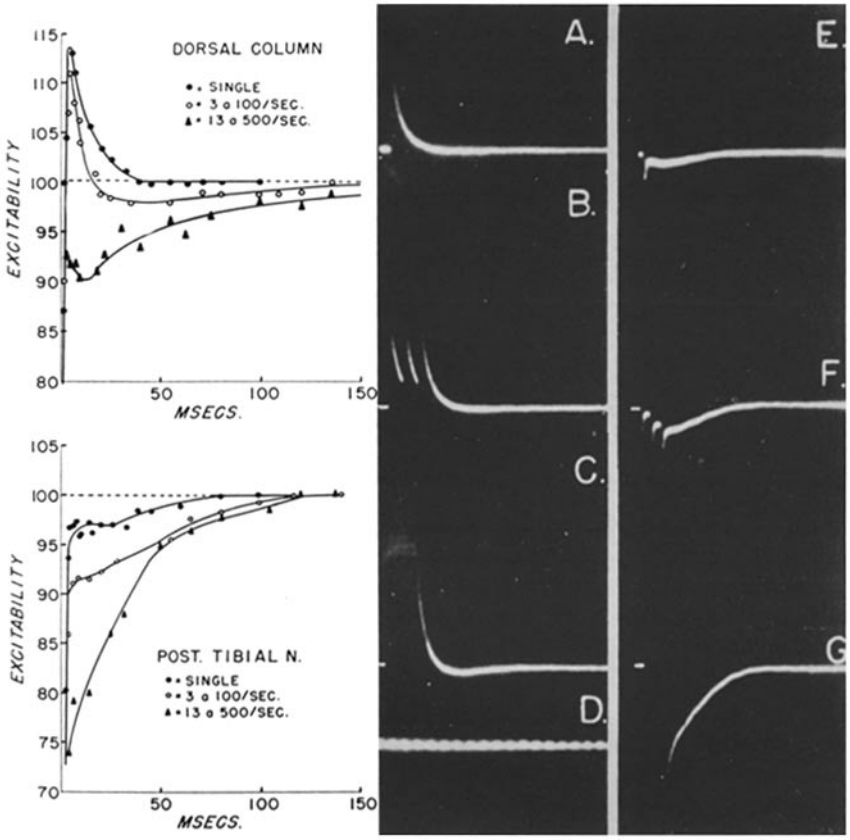

FIG. 18. After-potential of a dorsal column (A, B, C) and a posterior tibial nerve (E. F, G) studied simultaneously after equilibration with Krebs's glutamate solution for 1 ½ hours. A and E, single pulse; B and F, 3 pulses at 100 per second; C and G 13 pulses at 500 per second; D, time = 100 C. P. S. Accompanying excitability curves are on the left. For recording the after-potentials maximal volleys were used and gain set to make the spike potentials equal in both tissues. Thus relative sizes of slow potentials may be directly compared.

responsible for the preservation *in vitro* of an after-potential so nearly like that observed *in vivo*. This fact, the general normality of the tissue *in vitro*, and the correspondence in magnitude and form of the excitability cycle *in vivo* and *in vitro* provide strong argument that the after-potential recorded *in vitro* is representative of the after-potential in the intact spinal cord.

4. On the Rhythmic State in Central Axons

The rhythmic state (31, 3), with its attendant spontaneous activity, has not been induced in central axons by any variation in the concentration of constituents normally present in their environment. Even when maximum excitability results and the negative after-potential has been completely obliterated no sign of spontaneous firing occurred in 74 preparations in which pH or K^+ was increased or Ca^{++}, O_2, or CO_2 removed.

The effect of CO_2 removal upon the base line activity in dorsal column and peripheral nerve is presented in Fig. 14, records 1 to 8. In Fig. 19 this situation is demonstrated with respect to the action of increased potassium ion concentration on both excitability and base line noise in ventral column. The absence of firing contrasts sharply with that appearing in peripheral nerve or root in

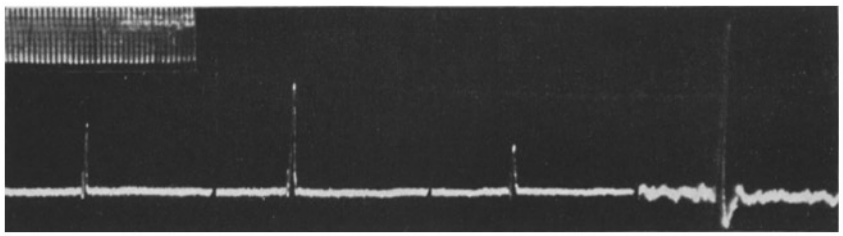

FIG. 19. Effects on excitability and noise level of addition of droplets containing potassium ion or citrate ion at the stimulating cathode to a ventral column *in vitro*. Excitability change is indicated by alteration in height of response to weak stimuli of constant current strength. From left to right, control, 10 mM KCl in Krebs', 30 mM KCl in Krebs', concentrated Na citrate.

which rhythmicity is easily incited by any of the above maneuvers (Fig. 14) (3, 5, 7, 23–27, 31).

The unique stability of central axons in the face of excess potassium ion is not peculiar to the excised state but has been found true of intact axons as well (5). A study of these characteristics in dorsal columns *in situ* demonstrated, nevertheless, that the membrane properties responsible for rhythmic activity existed in central axons and that the mechanism could be activated by introduction of citrate ion into their environment (5).

In the excised state dorsal and ventral columns also consistently show marked spontaneous firing following the local application of sodium citrate (Fig. 20). In addition, when the preparation is stimulated under the influence of citrate, synchronization of the spontaneous firing results in the appearance of periodic oscillations with a frequency (360 to 480) in ventral columns which is similar to that found in dorsal columns *in situ* (5) and twice that in peripheral nerve (see Fig. 14, No. 8, Fig. 20).

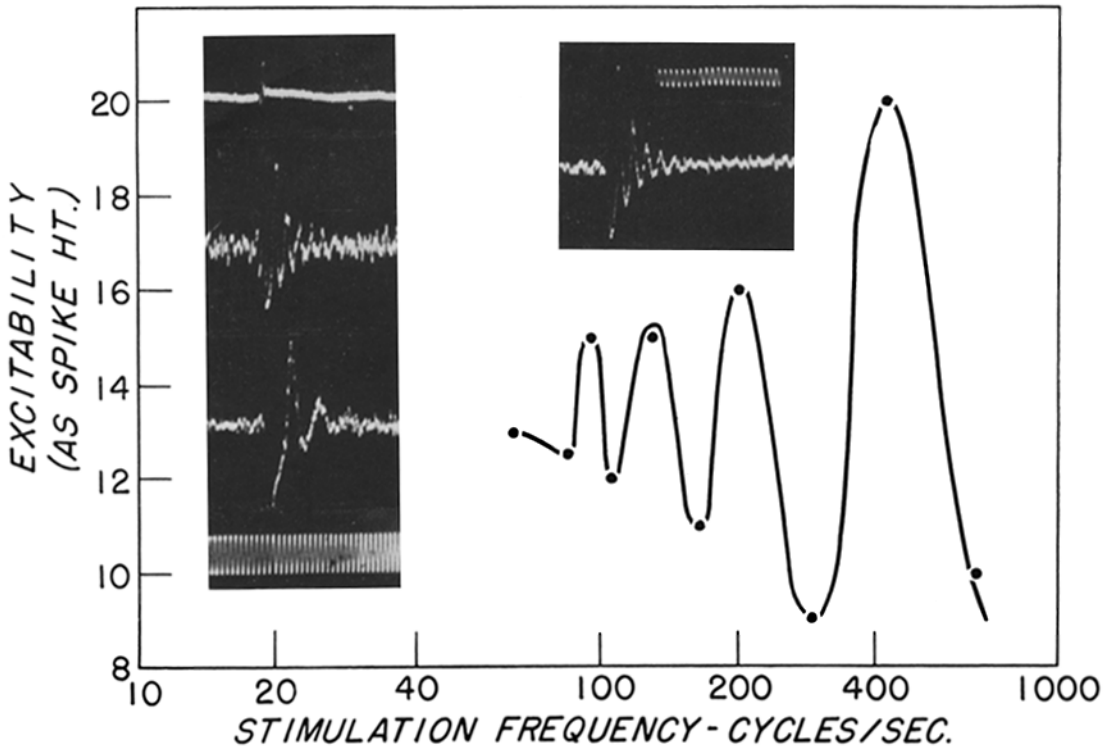

FIG. 20. Frequency of oscillation of ventral column and peripheral nerve *in vitro*. *Left hand inset.* Top to bottom, ventral column control; ventral column after being touched with a drop of concentrated Na citrate; posterior tibial nerve in same chamber after being touched with a drop of concentrated Na citrate. Time for all three is 1000 cycles. Note that the frequency of oscillation of ventral column is double that of peripheral nerve. (Also see Fig. 14, No. 8 for comparable oscillatory behavior of peripheral nerve upon CO_2 removal.) The *right hand inset* shows in another ventral column the regularity and frequency of oscillation and amount of spontaneous firing observed with citration. Time 1000 cycles. *Graph.* Submaximal stimulating pulses of constant current strength were delivered at various frequencies to the citrated ventral column of the right hand inset and the amplitude of response was plotted as a function of frequency. Peaks of increased excitability occur at frequencies whose periods correspond to multiples of the fundamental period of oscillation of the ventral column (2.5 msecs.); *i.e.*, at 2.5, 5., 7.5, and 10 msecs.

TABLE II

Properties of Central and Peripheral A Axons

	Central axons*	Peripheral A axons*
Spike		
Duration, *msecs.*	0.47 V	0.4–0.5 (9, 14)
Rise time, *msecs.*	0.16 V	0.14–0.16 (9, 14)
Conduction velocity, *m./sec.*	15–118 D, V	5–100 (9, 14)
Negative after-potential‡		
Peak value, per cent of spike height	8.2 D	3–5 (10), 6 R (6)
Value at 4.5 msecs. (time of peak supernormality)	4.2 D	2 R (6)
Duration, *msecs.*	40 D	15 (10, 13), 22 R (6)
Area as per cent of spike area	350 D	150 R (6)
Positive after-potential‡		
Peak value, per cent of spike height	None D, V	0.2 (10, 14)
Supernormality, current calibrated in per cent following single volley *in situ*	16 D (38)	3.5 (13), 7.2 R (6)
Form of after-potential upon CO_2 removal, Ca^{++} removal, addition of K^+	Stable D	Non-stable
Spontaneous firing with CO_2, H^+, Ca^{++} removal, K^+ addition, or hypoxia	None D, V	Marked
Frequency of rhythmicity after citrate$^-$ addition at 37°C., *cycles per sec.*	340–400 D (6)	200
Order of susceptibility of spike		
To oxygen removal	1	2
To carbon dioxide removal	1	2
To oxygen at 8 atm. pressure	1 (36, 37)	2
Dependence of posthypoxic overshooting on washing preparation	Marked V	None
Oxygen uptake $mm.^3\ gm^{-1}\ min.^{-1}$ wet weight (phosphate buffer)	7.1 D, V, L	2–4.7 (11,18)

* Numbers in parentheses indicate references. D refers to data derived from dorsal column, V to that from ventral column, L from lateral column, and R from dorsal root.

‡ Following single volley.

DISCUSSION

Interpretation of the action potential recorded from isolated spinal funiculi requires a more stringent examination than is necessary for peripheral nerve since the geometry of spinal funiculi is less simple. Specifically, not only must the axons of the funiculi be shown to have normal resting properties, but one must also be certain that activity which is assigned to the longitudinal axons does in fact originate in them and not in severed collaterals. In the preceding sections certain questions relevant to these problems were investigated.

In addition it must be emphasized that funiculi of the spinal cord are complex tissue systems. At the present time neither the metabolic nor the electrical

role of the large numbers of oligodendroglia which envelop central axons can be evaluated separately from the role of neural elements. Therefore certain of the results reported here can now be assigned only to action in the entire dorsal column "system" whether this action be purely axonal or "oligo-axonal". In particular the stability of central axons when environmental parameters are varied might conceivably be related to a buffering effect of the surrounding oligodendroglia.

Since the properties of central myelinated axons differ markedly from those of their peripheral axonal extensions and probably from those of their own collaterals, it is likely that there exist electromotive double layers at junctional regions along these cells about which extracellular currents would flow during activity (see (6)).

In Table II are compiled and offered for comparison a number of the properties of axons of the mammalian central and peripheral nervous systems as currently understood.

SUMMARY

Despite the trauma of dissection and special metabolic requirements, the physiological properties of funiculi of the mammalian spinal cord can be studied *in vitro*. They are adequately oxygenated by diffusion at 0.88 atm. pO_2 and remain in a functionally normal state for over 12 hours.

The internal consistency of several kinds of data presented in this and the foregoing papers (5, 38) serves to characterize certain properties of central myelinated axons whether excised or *in situ*. (1) Spinal tracts support a large spike potential *in vitro* whose form, duration, and velocity are comparable to those of alpha fibers *in vitro* and spinal tracts *in vivo*. (2) Properties consistent with a large L fraction are found in central axons whether excised or *in situ*. (3) Following conduction there has been identified post-spike supernormality with exponential time course (7.5 msecs. half-time) which is the result of activity intrinsic to parent fibers of dorsal columns. The supernormality is similar in form and magnitude both in excised and intact funiculi. (4) In excised funiculi the action potential of parent axons includes a large negative after-potential whose form and duration correspond satisfactorily with this supernormality. This potential appears not to result from activity arising in broken collaterals. (5) Central axons, excised or intact, fire spontaneously in the presence of citrate ion, and when synchronized by stimulation develop periodic oscillations at about 400 C.P.S. but show no such behavior in the presence of excess potassium ion.

Certain characteristics peculiar to central axons indicate that they occupy an extreme position in the spectrum of properties encountered in conducting tissues. Dorsal column myelinated axons differ from their peripheral counterparts, even though they are parts of the same cell, in the following ways. The

maintenance of the column spike potential is more critically dependent on CO_2 and the entire tissue mass has a higher oxygen consumption. The negative after-potential is much larger and the positive after-potential, non-existent following a single volley, is more difficult to develop by repetitive stimulation. Unlike peripheral nerve, central axons are not incited to spontaneous activity by manipulation of certain constituents normally present in their environment. However, when induced by the application of citrate the resulting rhythmic behavior has twice the frequency of that in peripheral nerve. In general, the recovery process in central axons is more invariant than that in peripheral axons when they are subjected to similar changes in their artificial environments.

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