resistance to radiation, but the vanishing of the threshold shown by our data is a sufficiently significant phenomenon to warrant looking for it in other systems which are affected by lower dosages (*5*).

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Nerve-End Recording in Conducting Volume

Abstract. When the end of a freshly cut nerve is drawn into a tube by means of a hydraulic device that serves as a holder and as an electrode, monophasic positive records of action potentials are recorded. A trailing positive phase develops, with time, after the cut. After-potentials can also be recorded by this method.

Potentials comparable in size to those recorded conventionally in oil or air may be obtained from the end of a nerve which has been drawn into a small glass tube. In this method the entire nerve is at all times completely immersed in Ringer's solution, and the potential drop occurs between a wire inserted inside the tube and an indifferent lead in the surrounding medium. This method has been used, in principle, for stimulation but apparently not for recording from nerve (*1*).

The device shown in Fig. 1 provides precise control of the position of the nerve end in its holder. The tubing, completely filled with Ringer's solution, constitutes a hydraulic pressure system. The contained fluid can be forced in or out, and the nerve moving along with it may be fixed at any point by means of screw controls *A* and *B*. These coarse and fine controls apply pressure through inserts in the Plexiglas block holding the control bulb.

Electrodes were constructed of silver wire (Birmingham and Stubs gauge 22) inserted through a length of about 15 cm of polyethylene tubing (inside diameter, 0.034 in.; outside diameter, 0.050 in.). At one end the wire extended about 1 cm beyond the tube. At the other, the wire was bent upon itself and sealed to the edge of the Plexiglas stopper *C*, which was inserted into the control bulb constructed from 10 cm of amber latex tubing (inside diameter, 0.125 in.). A second wire attached outside the end of the tube served as the indifferent lead.

Air bubbles were eliminated from the system by drawing Ringer's solution through the rubber tube and then replacing the terminal plug *D*. The polyethylene tube *E* was mounted on a manipulator constructed from rod-end bearings. Holding tubes *F* were constructed of melting-point glass tubing (inside diameter, 0.8 to 1.0 mm; outside diameter, 1.0 to 1.5 mm); the smooth cut ends were fire-polished to provide the desired size of aperture. The tubes were sorted by means of a series of brass wires of standard gauge (B & S 18 to 36). A tube with opening of appropriate size could be quickly selected by comparing the nerve diameter with the standard wires. The glass tubes were readily slipped over the silver wire and into the polyethylene tubing as needed. The silver wire was chloridized for about 2 cm at the tip before the glass tube was attached.

Records were obtained from exsected sciatic nerve or spinal root of *Rana pipiens* and *R. catesbeiana*. The preparation was grounded via the metal tubes used for circulating the water that maintained a temperature of 15°C in the bath. The end of the nerve was usually about 1 cm from the end of the wire in the tube, but changes in this distance had no observable effect on the record.

Several electrodes for stimulation and recording could be used simultaneously. A switching arrangement allowed any combination to be selected. The use of multiple indifferent leads did not seem to complicate the records. Each indifferent lead was placed near the glass tube of its companion electrode. The nerve end, oriented close to the tube opening, was drawn into the tube by release of pressure, by the fingers directly or via one of the screw controls.

Injury current was maximal immediately after the nerve had been cut with sharp scissors. The current declined with time, presumably due to narrowing of the cut end during outflow of axoplasm and the spreading of myelin over the cut end (*2*). Action currents at the distal, "healed" end and at the freshly cut proximal end of the sciatic nerve of a frog are shown in Fig. 2. Action currents were monophasic positive immediately after the cut, whether the nerve was pulled into the tube for a few hundred microns or for several millimeters (*3*). With time, after the nerve was cut, a trailing negative phase developed unless the end was left in the tube, in which case the monophasic positive record persisted (*4*). After-potentials lasted about 0.5 second and were initially positive-going, while a later negative phase developed during a train. These positive and negative phases appear to correspond, respectively, to the negative and positive after-potentials conventionally recorded with external electrodes. With passage of

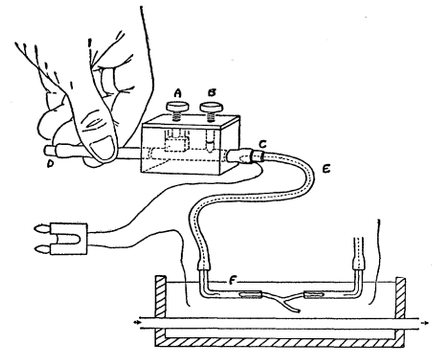

Fig. 1. Hydraulic holding and recording device for nerve end.

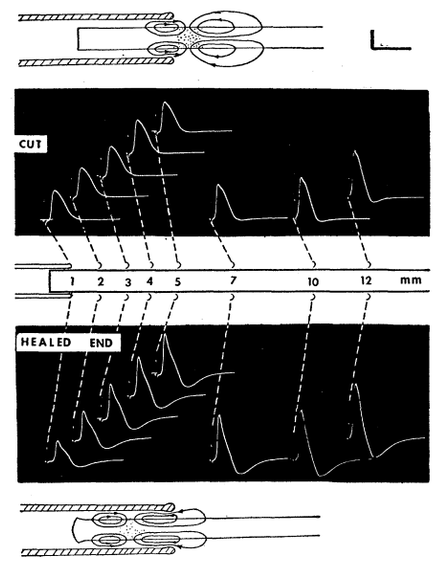

Fig. 2. Action currents recorded by means of the hydraulic holding device. The sciatic nerve of a bullfrog was prepared 8 hours before the recording was made. Upper records are from the proximal end of the freshly cut nerve, which had been drawn into the tube for the distances indicated. Diagram (top) shows the net direction of current flow outward near the nerve end. Lower records are from the distal (healed) end of the nerve, which had not been touched since the cut was made, 8 hours previously. Diagram (bottom) shows the net current flow inward during the negative phase. (Top, right) Calibration: 2 mv, 2 msec.

time after the cut, the after-potentials became smaller. At a healed end, after-potentials were absent or were negative in sign. The negativity increased during a train and was followed by a positive phase.

The sign of spike (positive with respect to the indifferent lead) and possibly the sign of the after-potentials and the injury currents recorded at the freshly cut end by this technique appear to be consistent with the sign of membrane resting and action potentials recorded by intracellular electrodes. The use of the technique may therefore result in less confusion than prevails when conventionally, externally obtained negative-upward records are compared with the intracellularly obtained observations. Less length of nerve than is ordinarily required is adequate with this method, and the inconvenience and deleterious effects of air and oil are eliminated (*5*, *6*).

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6. This research was supported by grant No. B-927 from the National Institutes of Health, U.S. Public Health Service.

27 June 1960

Three γ-Globulins in Normal Human Serum Revealed by Monkey Precipitins

Abstract. Precipitating antibodies specific for three normal human γ-globulins of relatively slow electrophoretic mobility were prepared in monkeys and demonstrated by immunoelectrophoresis in conjunction with absorption techniques in which two myeloma globulins were used as absorbents.

Monkeys were selected for the preparation of antibodies to human serum proteins because antibodies prepared in a more closely related species might be more discriminating for minor antigenic differences among the serum proteins than antibodies prepared in a more distantly related species (*1*). Rhesus monkeys were immunized with normal human γ-globulin prepared by cellulose ion-exchange chromatography (*2*). Doses of 5.0 to 50 mg of γ-globulin were injected subcutaneously or intramuscularly. The first dose was emulsified in complete Freund's adjuvant; subsequent doses in incomplete adjuvant or in saline were given at monthly or biweekly intervals. Sera were analyzed by immunoelectrophoresis in agar gel used in conjunction with absorption techniques as previously described (*3*).

Figure 1 is a photograph of a stained immunoelectrophoretic agar plate showing the precipitin bands which appear when a monkey antiserum (E235) against normal γ-globulin reacts with the electrophoretically separated globulins of normal human serum, myeloma serum Br, myeloma serum Ro, and a mixture of the two myeloma sera (*4*). Figure 2 is a photograph of a stained plate showing the precipitin bands which result when the same monkey antiserum (E235) is absorbed with each myeloma serum (Br and Ro). The electrophoretic patterns of Br (1:16 dilution in saline), normal human serum (undiluted), and Ro (1:16 dilution in saline) are shown superimposed on the results of double diffusion. The left trough had been filled with E235 absorbed with Br; the right trough, with E235 absorbed with Ro.

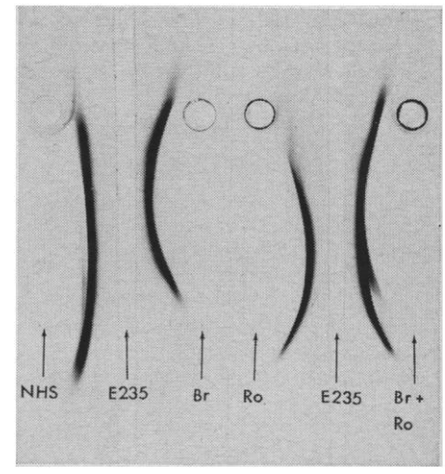

Fig. 1. The precipitin bands which appear when monkey antiserum E235 reacts with the electrophoretically separated globulins of normal human serum, myeloma serum Br, myeloma serum Ro, and a mixture of the two myeloma sera.

After E235 is absorbed by either myeloma serum (Fig. 2), the antibodies remaining no longer react with the myeloma serum used for absorption but do react with normal serum to yield a long and short precipitin band. The two long bands are asymmetrical with respect to the electrophoretically separated "slow" γ-globulins, indicating that the unabsorbed antibody in the left trough reacted with a γ-globulin (closer to the anode) of faster average mobility than the unabsorbed antibody in the right trough; these two γ-globulins are designated γ-A and γ-B, respectively (*5*). The two shorter bands are symmetrical and represent reactions of an unabsorbed antibody specific for a third γ-globulin which is designated γ-C. The precipitin reaction patterns in Fig. 2 were also obtained with γ-globulins which were considered free of macroglobulin (*2*). Thus, γ-A, γ-B, and γ-C are presumably 7S γ-globulins.

The antibody to γ-A prepared by absorption of E235 with Br was found to react also with Ro; thus, γ-A and Ro have an antigenic determinant (designated as X) in common. Antibody to γ-B prepared by absorption of E235 with Ro was found to react also with Br; thus, γ-B and Br have an antigenic determinant (designated as Z) in common. In Fig. 1, the coalescence observed when the mixture of Br and Ro react with E235 suggests the presence of antibody to an antigenic determinant (designated as Y) common to Br and Ro. That Br and Ro have an antigenic determinant in common was also indicated by other monkey antisera which were completely absorbed by either Br or Ro. Finally, the antigenic determinant on γ-C is designated as W.

Still other monkey antisera, T710 and E221, in their reactions with normal γ-globulins, yielded long, broad precipitin bands which showed splittting at the cathode or anode end, respectively, and coalescence at the anode or cathode end, respectively (*6*). Each fork of the split band could be shown to coalesce with a band formed with one of the myeloma globulins (*6*, *7*). These results suggested that γ-A and γ-B correspond in two of their antigenic determinants with the two myeloma globulins Ro and Br. Thus, γ-A and Ro have determinants X and Y, while γ-B and Br have determinants Y and Z.

Accordingly, the reaction of unabsorbed E235 and normal serum, which results in the long, broad band (Fig. 1), represents the superimposed reactions of anti-X, anti-Y, and anti-Z with γ-A (XY) and γ-B (YZ), and, as would be expected, the antibodies appear to react with a γ-globulin of intermediate mobility between γ-A and γ-B. When E235 is absorbed by Br (YZ), it should contain anti-W and anti-X (Fig. 2). Since anti-Y has been removed, the reaction of anti-X with γ-A (XY) is slightly closer to the trough than when unabsorbed E235 is used (Fig. 1), and the separation of this band from the short band due to anti-W with γ-C (W)