XII. COMMUNICATIONS BIOPHYSICS*

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A. CONTOUROGRAPHIC DISPLAY IN INCORPORATING HIDDEN LINES

A contourographic display[1, 2] is, essentially, a raster-type display in which the beam is both intensified and deflected vertically in proportion to the instantaneous amplitude of one variable. Raster-based displays have been used to good advantage in examining noisy data for long-term trends, as well as for the detection of

*This work was supported principally by the National Institutes of Health (Grant 5 PO1 GM14940-05), and by the National Aeronautics and Space Administration (Grant NGL 22-009-304), and in part by the National Institutes of Health (Grant 5 TO1 GM01555-05), Boston City Hospital Purchase Order 10656, and by a grant from B-D Electrodyne Division, Becton Dickinson and Company.

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occasional transients.[3-5]

As well as the usual application of observing variations in the beat-to-beat rhythm of the electrocardiogram, we have found the contourogram to be particularly useful in detecting rare, atypically shaped beats.[6] We have regularly incorporated a grid in our displays of electrophysiologic data. The vertical grid is formed by blanking the oscilloscope beam periodically as it moves uniformly from left to right. The horizontal grid is formed by periodically adding a constant to the X and Y components of the signal forming the undeflected raster. In addition to facilitating the temporal location of an event, this grid strikingly enhances the three-dimensional appearance of the display. The key to simplicity in implementing this grid is the fact that it is formed by blanking the oscilloscope. Thus hidden lines are truly hidden. The grid seems particularly useful in examining structures that are rather smooth and lack abrupt transitions. Oblique presentation also seems to enhance the three-dimensional appearance of the picture.

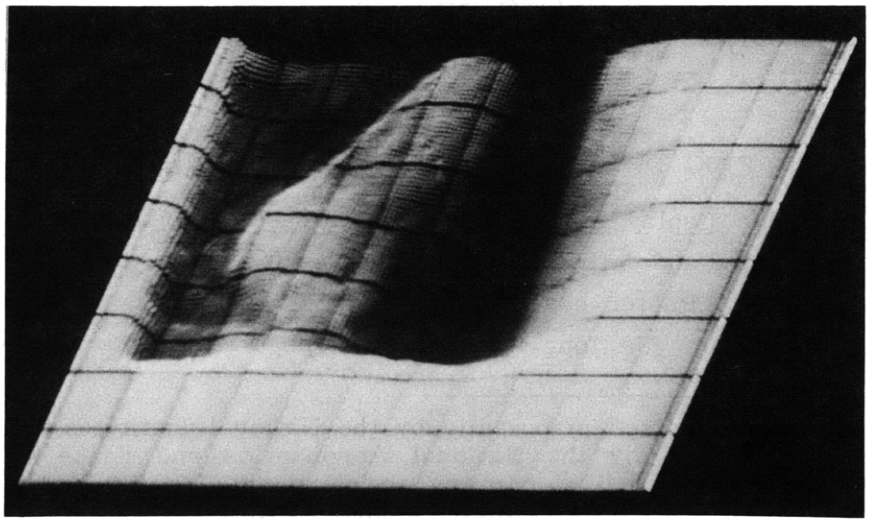

Fig. XII-1. Rescan of a single frame from a motion picture of a fluoroscopic image of the left ventricle of the human heart. This frame was scanned while the ventricle was relatively uniformly filled with the radio-opaque substance. The oscilloscope beam is deflected vertically in proportion to the intensity of the spot. Thus high spots are brighter and the resultant image looks something like a side-lighted mountain range. The rectilinear grid is an important feature of the display which has been added to enhance the three-dimensional appearance of the presentation.

Application of this same technique to raster displays of data that actually originate from a three-dimensional source, such as an x-ray image or micrograph, results in a similarly striking picture. Figure XII-1 is an example of the process applied to a

single frame of a cine-angiogram of a human heart. The actual scanning process is rather elaborate and employs a high-resolution scanner interfaced to a PDP-9 computer (Digital Equipment Corporation, Maynard, Massachusetts). The resulting signal, however, is equivalent to that of a low-speed television camera. Clearly, it is possible to process television signals in the same manner. We intend to pursue this rather simple process of image enchancement by developing a television display that includes a contourographic mode. Provision will also be included for both logarithmic and linear processing of the video signal. Initial application will be in the area of image-enhancing fluoroscopy. Scanning micrographs appears to be another useful application of the technique.

S. K. Burns, I. T. Young

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B. DIGITAL MATCHED FILTER FOR ELECTROCARDIOGRAMS

In the detection or recording of electrocardiogram signals from patients, unwanted muscle noise or power line interference often obscures the desired signal. A notch filter will eliminate the 60-Hz noise but a more complicated filter is needed to suppress the spurious muscle signals. The use of a filter whose impulse response is matched to the desired transient will yield the maximum signal-to-noise ratio under random-noise conditions. One such filter was partially designed by R. A. Collesidis[1] and P. B. Jergens.[2] The design was completed and preliminary evaluation was made by the author.

The filter is a digital system which has two recirculating memories to implement convolution (Fig. XII-2). The input signal is sampled at up to 5 kHz (528-Hz sample rate is used for electrocardiograms), and 256 contiguous 4-bit samples are stored in a MOS shift register. These samples are multiplied with corresponding samples

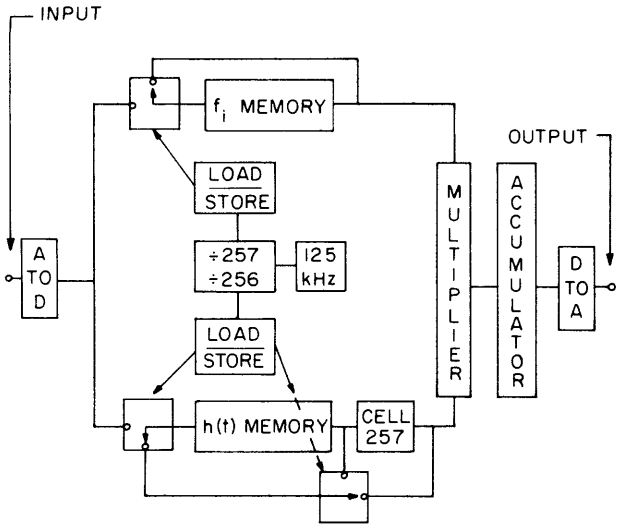
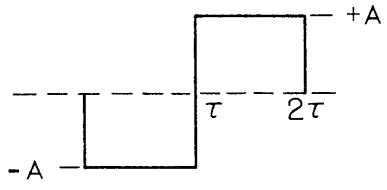


Fig. XII-2.

Samples of the input signal are converted to 4-bit digital representations gated to either the $f_i(t)$ or h(t) memory, depending upon the "load-store" condition. The memories are clocked by a 125-kHz clock that provides the 8-μs shift period. Cell 257 of the h(t) memory is bypassed in the "load h(t)" mode, which allows h(t) to be loaded in a manner similar to f(t). Outputs of the memory are multiplied and accumulated until 257 operations have been completed. The digital result is converted back into analog form. The ÷257,256 control allows $f_i(t)$ to be viewed repeatedly on an oscilloscope. Normal operation uses the ÷257 position.

of a similarly stored impulse response template, and the products are accumulated to form the output at that time. The entire contents of each memory is completely circulated once in each sample period so that the output appears in "real time." (That is, in the time between samples, the last 256 samples of the input are convolved with the 256 samples of the stored impulse response.) Precession of the input signal with respect to the stored h(t) is accomplished by the addition of one extra memory cell and the accumulation of 257 products during each sample period.[2]

The multiplication is implemented with a combinatorial multiplier which generates a 7-bit signed number. A clocked 8-bit arithmetic unit and 8 bits of counter overflow, combined with D-type flip-flops as storage, form a 16-bit accumulator. The sign of the 7-bit product determines whether it is added or subtracted from the previous accumulator value. Another set of flip-flops is connected as an output memory and is strobed after all 257 operations have been performed. The template memory was loaded with a pulse illustrated as follows:



The input was a low-frequency square wave to simulate a train of steps. Since, for a linear filter, the impulse response is the derivative of the step response, the impulse

response of the filter, as shown in Fig. XII-3, is indeed the one that we programmed. The average value of the impulse response is zero, resulting in a zero dc response that is shown on the output as a return to zero after each transient.

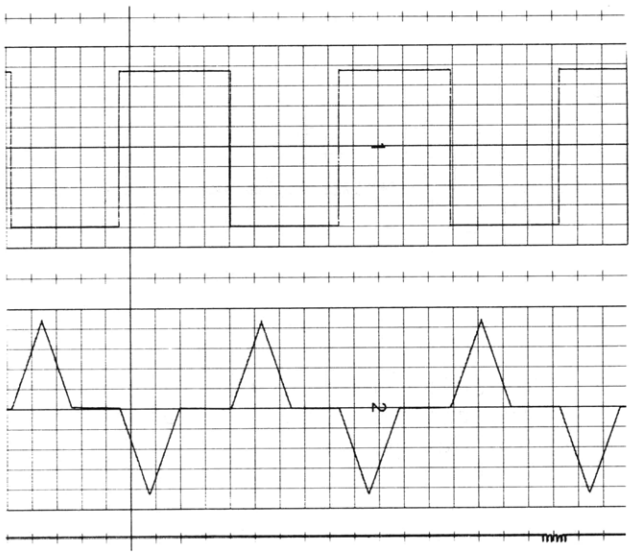


Fig. XII-3. Step response with stored test template.

The filter was programmed with an electrocardiogram waveform such as the one shown in Fig. XII-4. The signals used were from test tapes on which muscle noise was deliberately generated along with the electrocardiogram signal. As can be seen from Fig. XII-5, substantial reduction of this kind of noise is possible. The filter also offers good 60-Hz suppression, as shown in Fig. XII-6.

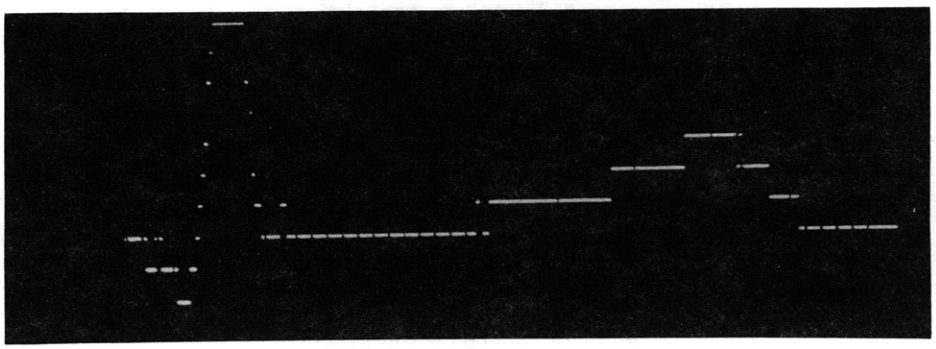


Fig. XII-4. A noiseless electrocardiogram as recorded in memory. The QRS complex and the "t" wave are clearly visible. The signal was amplified past clipping so that more of the "t" wave would appear.

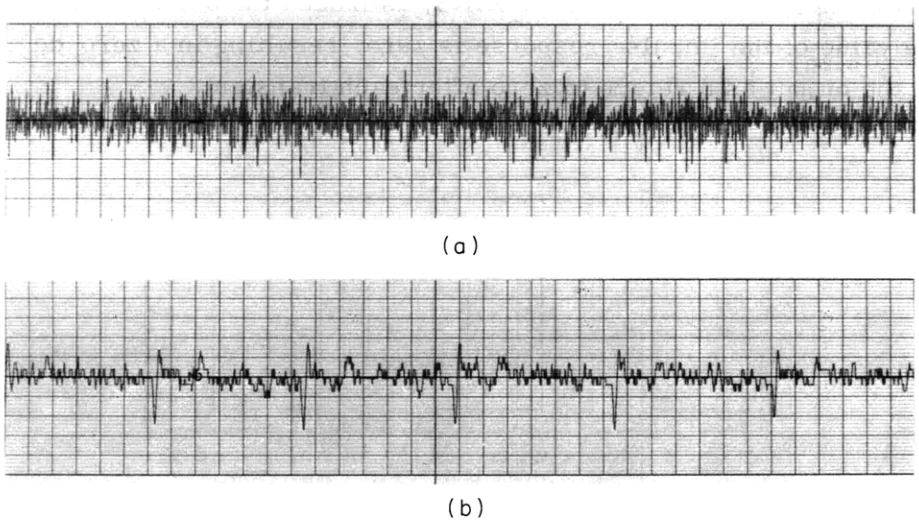


Fig. XII-5. Result of filtering a very noisy electrocardiogram signal through the filter programmed with a normal transient as in Fig. XII-4. (a) Input. (b) Output.

Since the impulse response is programmed by a captured transient signal, the filter is easily programmed, even when the available signal sources are free from noise for short periods. Because it is easy to program, and has an error rate of approximately 1%, the filter is a very useful device for the detection of low-frequency transient

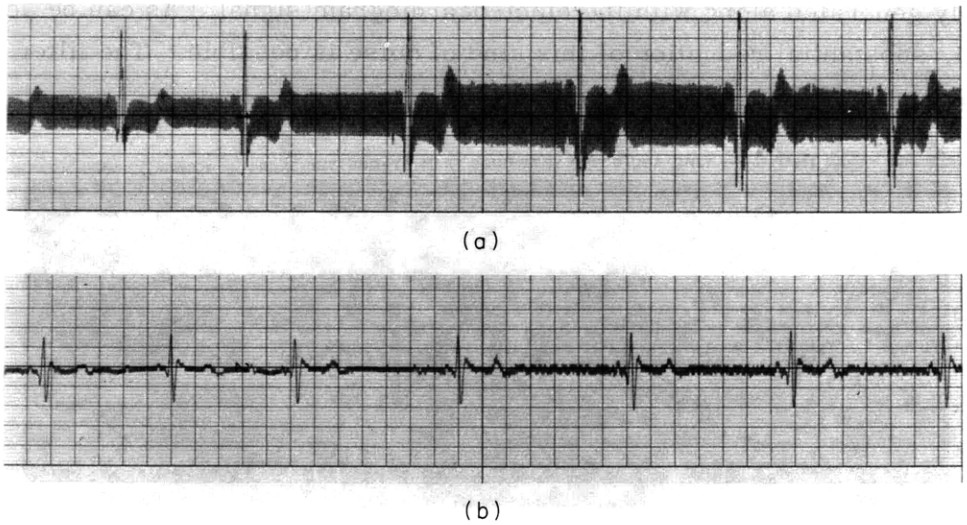


Fig. XII-6. (a) Input. Electrocardiogram signal with 60-Hz power line interference.
(b) Output. Power line interference noise is markedly reduced.

signals. The filter is also useful as a demonstration device for convolution, Fourier principles, and digital-filtering techniques.

Future effort on this filter will be to reduce the amount of hardware and increase the operating efficiency. Since the filter involved the efforts of many people, interfacing was necessary, which would not be the case in a better-planned system. Redesign of the system would also include a method of automatically storing the impulse response for easier programming.

P. L. Smith

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C. VECTORCARDIOGRAPHIC DISPLAY AND MEASUREMENT SYSTEM

The fundamental theoretical basis of vectorcardiography is Einthoven's "dipole theory" which states that the electrical field generated by the heart is equivalent to that produced by a simple dipole with fixed origin and time-variant magnitude and direction. Resulting potentials are obtained from the body surface by means of multiple recording leads that are algebraically combined to produce three signals that are more or less orthogonal. These signals represent the projection of the equivalent electrical vector onto the axes of an orthogonal coordinate system. In standard clinical vectorcardiography, pairs of these signals are used as horizontal and vertical deflection of an oscilloscope beam that thus displays a point representing the projection of the vector on the frontal, horizontal, and sagittal plane. The QRS complex, representing ventricular depolarization, occurs in approximately 80 ms. Hence direct writers are unfeasible and to get hard copy of a record requires an intermediate photographic process.

Extending the work of Sheridan,[1] we have developed a vectorcardiographic display and measurement system using our general-purpose programmable Electrophysiologic Data Processing System. This system is capable of acquiring and displaying vectorcardiograms in an extremely flexible manner. It has three-dimensional perspective, and can make a quantitative examination of vectorcardiographic data.

Operation of the system includes 3 basic processes: signal acquisition, scalar display, and vector display. Having acquired a signal, the operator can readily switch

between scalar and vector display modes; and in the vector mode, he has the option of selecting a three-dimensional perspective presentation that may be viewed with special glasses. Normal presentations are on an oscilloscope, but all displays may be presented on an X-Y plotter for permanent records.

In the signal acquisition process, the X, Y, and Z signals are samples by a standard clinical vectorcardiograph machine (1000 samples/s). The samples are then stored in the system's core memory. Front-panel knobs allow establishment of a threshold on one of the channels. When the signal exceeds this threshold, an adjustable number of additional samples are stored and these, together with the previously acquired points, form the 1000-point record that will be displayed. This phase also has provisions for calibration and normalization of the three leads.

The scalar display mode presents the stored data on the oscilloscope and allows the user to select a portion of the vector complex to analyze and display as a vector loop. The selected portion is delimited by visible cursors and the time interval between these cursors may be displayed numerically. The first cursor selects the E-point, usually the initial point of depolarization, and this point determines the origin of the orthogonal coordinate system.

In the vector mode, the selected complex is displayed on the oscilloscope in three-dimensional form with provision for rotating the viewing position by means of front-panel knobs. The standard clinical views, frontal, horizontal, and sagittal, may be

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CURSOR WIDTH   462
MEAN VECTOR:   58, 105
AREA:  25321
POINTER ANGLES:  72, 122
MAGNITUDE:   267 , SAMPLE NO.   254
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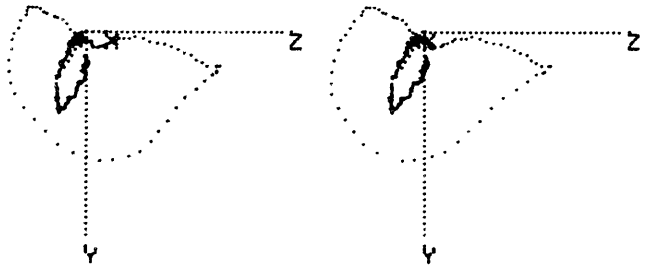

Fig. XII-7. Normal horizontal plane vector loop.

selected by means of front-panel switches. Available numerical data include the projected area delimited by a displayed loop, and the mean vector of the loop. A visible pointer representing the instantaneous vector may be superposed and positioned by the operator to any displayed point. The angle, magnitude, and latency (relative to the E-point) of this vector may be displayed.

Other features include adjustable magnification of the vector loop and optional display of 1-mV calibration marks on the three orthogonal axes. By allowing examination of a magnified view of only the initial portion of the loop, the system provides more detailed information on the important initial forces of depolarization than can be obtained by standard vectorcardiograph techniques.

Recent trends in clinical vectorcardiography[2, 3] indicate that the full potential of this technique for improved diagnosis will depend on quantitative analysis of vectorcardiogram data. The VCG Display System provides a unique combination of flexible quantitative capabilities, as well as the stereo three-dimensional perspective display of the vector loops. Preliminary clinical trials, conducted with data from selected patients at Boston City Hospital who had a variety of abnormalities, suggest that the system enhances the qualitative differentiation of the vector loop, as well as quantitative analysis. In these cases several characteristics of potential diagnostic value were studied. The relative planarity of normal loops compared with abnormal loops could be readily appreciated and quantitated. In certain cases of myocardial infarction, stereo viewing

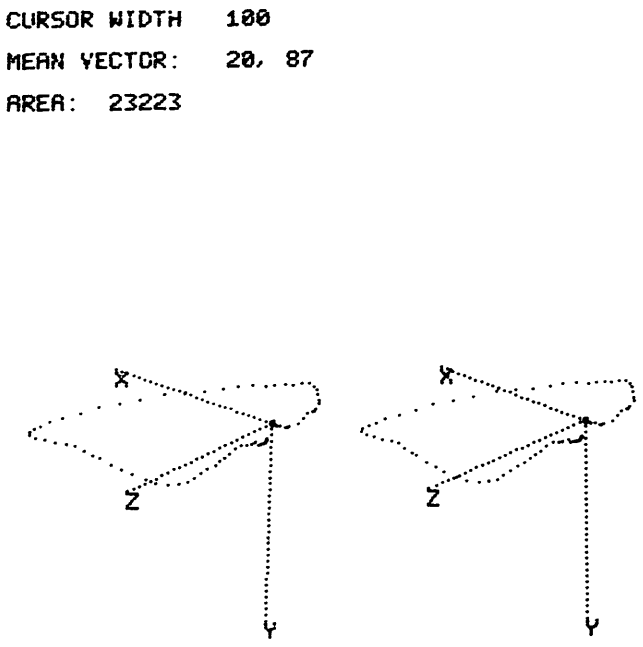

Fig. XII-8. Rotated three-dimensional vector loop.

of the loop, with examination from several viewpoints, disclosed alterations that are not ordinarily found in the standard planes. The three-dimensional capability provides a more unified representation of the cardiac dipole than is obtained by using 3 orthogonal planar component views, and may assist in revealing new criteria relevant to diagnosis.

Examples of the output from the VCG Display System, recorded on an X-Y plotter, are shown in Figs. XII-7 and XII-8. Figure XII-7 shows a standard horizontal plane vector loop from a normal person. Data specifying width of the vector complex, the projected planar area, the mean vector, and the instantaneous vector (254 ms from the E-point) are presented at the top of the display. Figure XII-8 shows a rotated view of the same person's vector. These figures are normally viewed with stereo glasses, but some observers are able to achieve the three-dimensional effect by crossing their eyes.

The VCG Display System may be used to analyze vectorcardiogram data recorded from astronauts and scientists aboard NASA's SkyLab Project. It should aid in the analysis by allowing changes of the vector in normal subjects to be readily observed and quantitatively measured.

B. Gaiman, R. Rifkin, S. K. Burns

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D. CRYOPROTECTIVE ACTION OF DIMETHYL SULPHOXIDE (DMSO) ON HOMARUS AXONS

1. Introduction

The cryoprotective ability of DMSO in simple cellular systems is well known,[1,2] but attempts to revive vertebrate nervous tissue frozen below -10°C have been only partially successful.[3,4] The work of Pascoe[5] furnishes an exception; he reports complete recovery of desheathed rat superior cervical ganglia treated with 15% glycerol and kept at -76°C for 24 hours or less; untreated ganglia could not withstand a 5-min exposure to temperatures below -15°C. Menz[4] reports, however, that rat cutaneous nerves treated with 15% glycerol or DSMO show no recovery after 5 min at -10°C.

Pribor and Nara[3] report limited cryoprotection in DMSO-treated frog sciatic nerves

kept at -10°C for 5 min after the external Ringer's solution was completely frozen. The action potential regains 20-55% of its total value for 5-15% DMSO-treated nerves, but there is no recovery at all for glycerol-treated nerves or nerves with intact perineural sheaths.

We report here the preservation of the electrical response in DMSO-treated Homarus nerves frozen for as long as 2 h at temperatures between -20°C and -30°C. These experiments follow a preliminary study of the effects of DMSO on Homarus nerve response at room temperature,[6] and are part of an investigation of cryoprotection in invertebrate nervous tissue.

2. Methods

The electrical response of each Homarus ventral nerve is recorded before and after a 30-min treatment with a solution of 5-10% DMSO in artificial seawater. The dissection and recording procedures are similar to those reported previously.[6]

The fiber is placed on a glass slide, covered with a small amount of DMSO solution and placed on a copper block in the freezer compartment of a standard refrigerator. A thermometer is kept in contact with the block to monitor the temperature (-20°C to -30°C). After an hour in the freezer, the nerve is thawed in DMSO solution at room temperature, and is left in artificial sea water for 90 min before further testing. Departures from this procedure are noted below.

3. Initial Results

There is no observable electrical response in untreated Homarus ventral nerves frozen for 30 min or more at -20°C to -30°C independent of the presence or absence of the perineural sheath. In contrast, prior treatment with 5-10% DMSO solution is found to exert a consistent cryoprotective effect on the electrical response of both sheathed and desheathed nerves.

In our first experiments the perineural sheath was removed before treatment with 10% DMSO. Figure XII-9b and c show the electrical response of such a nerve 20 min after a 40-min exposure at -20°C. Typically the compound action potential (CAP) is distorted, delayed, and attenuated (note scale change). The stimulus threshold is greatly increased. Nonetheless, the nerve continues to respond to electrical stimulation.

It is difficult for us to determine, at this time, the nature of the observed freezing damage, since we have yet to identify which axon populations are being recorded at each stage of the procedure. For example, a large delay can result from a decrease in the conduction velocity of individual axons, from the preferential survival of small-diameter fibers, or from both. DMSO toxicity, mechanical strain, and osmotic stress may also weaken the nerve before freezing, or may damage it during thawing. In later

experiments we attempted to minimize these extraneous effects by leaving the perineural sheath intact, and by using only 5% DMSO.

4. Further Results

It is possible to stimulate and record the CAP of Homarus ventral nerves without removing the perineural connective sheath, although the stimulus threshold is thereby

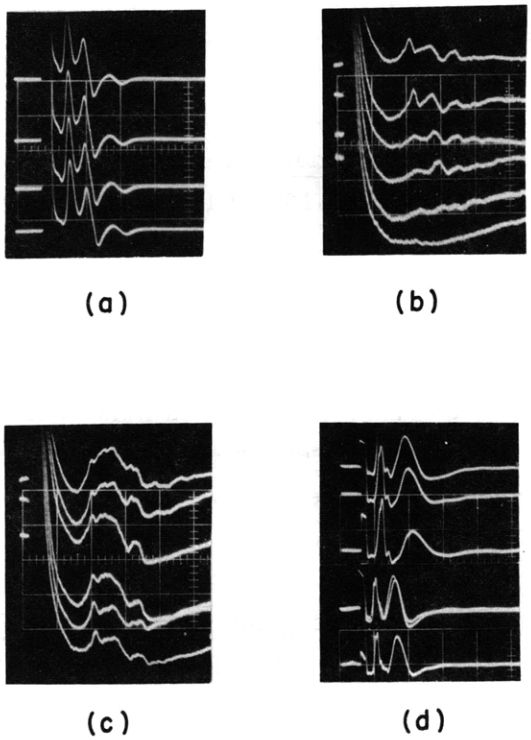

Fig. XII-9. (a)-(c) Cryoprotection in desheathed DMSO-treated Homarus nerves, at −20° C. The compound action potential is recorded.
(a) Desheathed nerve before treatment. Stimuli: 41-47 μA; scale: 1 mV/cm, 1 ms/cm.
(b) Same nerve after 40 min in 10% DMSO, 40 min at −20° C, and 20 min in room-temperature artificial sea water. Stimuli: 125-175 μA; scale: 0.1 mV/cm, 2 ms/cm.
(c) Same as (b). Increased stimulation 185-260 μA.
(d) Another nerve, perineural sheath intact, before (two lower traces) and after (upper traces) 10 min in 10% DMSO. Stimuli: 150, 220 μA and 100, 135 μA; scale: 2 mV/cm, 2 ms/cm.

increased (Fig. XII-9d). DMSO affects the CAP of sheathed nerves in the same characteristic manner as reported previously[6] for desheathed nerves, although the observed changes in Fig. XII-9d are somewhat smaller.

A CAP response can also be obtained from DMSO-treated sheathed nerves kept

for an hour at -20°C (Fig. XII-10). Again the threshold is greatly increased (35 μA → 360 μA), as is the delay between stimulus and response signals. The threshold is reduced by putting the stimulating and recording sets of electrodes closer together (1 cm → 0.5 cm), a procedure that has no effect on the threshold of normal, fresh nerves. Of course, decreasing the propagation distance automatically reduces the observed delay.

Reducing the freezing temperature from -20°C to -30°C (Fig. XII-10c and d and

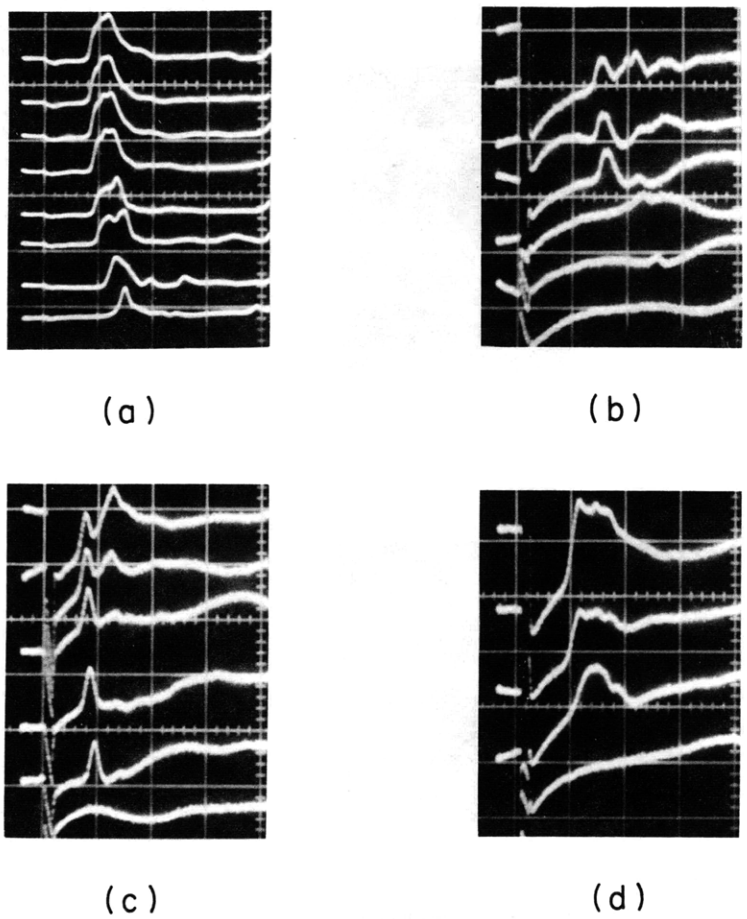

Fig. XII-10. Cryoprotection in sheathed DMSO-treated Homarus nerves at -20°C. Time scale: 2 ms/cm.
(a) Response of nerve treated 30 min with 5% DMSO. Stimuli: 35-88 μA; scale: 2 mV/cm.
(b) Same nerve exposed 60 min at -20°C; thawed 90 min at room temperature. Stimuli: 235, 260, 300, 360, 430, 500 μA; scale: 0.2 mV/cm.
(c) Same as (b), with closer electrode configuration (see text). Stimuli: 180, 220, 260, 300, 360, 430 μA; scale: 0.2 mV/cm.
(d) Another nerve, treated 30 min with 5% DMSO, exposed 30 min to -24°C; thawed 90 min. Stimulus: 185-260 μA; scale: 0.1 mV/cm; close electrodes.

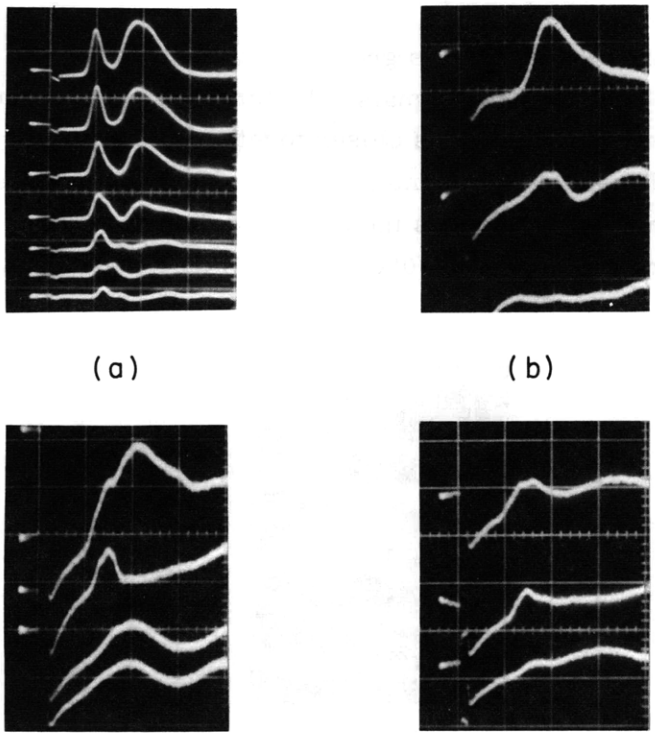

Fig. XII-11.

Effects of DMSO concentration on cryoprotection for 1-hour exposure at -30°C. (a)-(d) use a close electrode configuration (see text). Time scale: 2 ms/cm.

(a) Response of sheathed Homarus ventral nerve after 30-min treatment with 5% DMSO. Stimuli: 150 μA-450 μA. Scale: 2 mV/cm.

(b) Same nerve exposed 60 min at -30° C. Stimuli: 485, 560, 630 μA; scale: 0.2 mV/cm.

(c) Response of nerve similarly treated with 10% DMSO. Stimuli: 450, 500, 560, 630 μA; scale: 0.2 mV/cm.

(d) Response of nerve similarly treated with 2.5% DMSO. Stimuli: 450, 500, 560 μA; scale: 0.2 mV/cm.

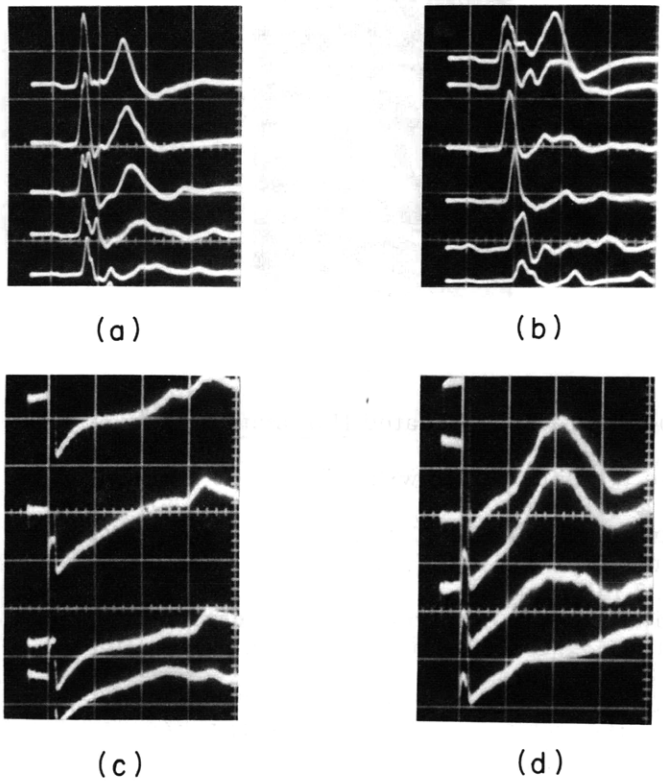

Fig. XII-12.

DMSO-induced cryoprotection for 2-hour exposure at -30°C. Time scale: 2 ms/cm.

(a) Response of sheathed Homarus ventral nerve before treatment. Stimuli: 100-180 μA; scale: 2 mV/cm.

(b) Same nerve treated 30 min with 5% DMSO. Stimuli: 68-135 μA; scale: 2 mV/cm.

(c) Same nerve exposed 120 min to -30°C. Stimuli: 485, 520, 560, 610 μA; scale: 0.2 mV/cm.

(d) Same as (c) using closer electrodes. Stimuli: 485, 520, 560, 610 μA; scale: 0.2 mV/cm.

Fig. XII-11b) has little effect on the overall level of response (~10-20% original CAP height), but the stimulus threshold is further increased. The post-freezing CAP becomes increasingly smooth, and all evidence of individual peaks disappears at -30°C, the lowest temperature obtainable with our present apparatus.

Cryoprotection at -30°C has been found over a range of experimental conditions. DMSO provides comparable cryoprotection in a 5-10% concentration (Fig. XII-11b and c), but 2.5% DMSO is considerably less effective (Fig. XII-11d) and is often ineffective. The effects of a 2-hour exposure to -30°C (Fig. XII-12) differ little from those of a 1-hour exposure (Fig. XII-11), but we have yet to obtain a response from nerves kept 3 hours at that temperature. Unfortunately, one of the most important variables, the freezing rate, cannot be controlled with our present apparatus,[7] but work on a feedback-controlled freezer is already underway.

I. M. Asher, S. Carney

Footnotes and References

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7. The uncontrolled cooling rate, as measured with a thin calibrated resistor, decreases typically from 45°C/min (first 30 s) to 20°C/min (next 20 s) to 6°C/min (next 180 s).