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Nerve conduction as a means of estimating early post-mortem interval

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Summary. Methods in current practice for ascertaining time of death are largely based on the cooling of the body after death and are somewhat unreliable. A theoretical relationship is known to exist between the decline in the properties defining nerve conduction and time after death caused by the gradual cessation of metabolic activity in nerves. A number of such properties were measured in rats during life and after death. In most cases the relationship was found to be inconsistent. The chronaxie of the strength duration curve for the sciatic nerve was, however, found to increase consistently and reproducibly in a linear fashion over the first 90 min after death to a plateau value which was maintained beyond 135 min. These findings are discussed as the possible basis of a forensic method of determining the duration of the "post mortem interval" within the first few hours after death.

Key words: Early post mortem interval – Time of death – Nerve conduction – Strength duration curve – Chronaxie

Zusammenfassung. Die in der Praxis angewandten Methoden zur Bestimmung der Todeszeit basieren hauptsächlich auf der Abkühlung des Körpers nach dem Tod und sind nicht immer ausreichend zuverlässig. Eine theoretische Beziehung zwischen dem Absinken der Nervenleitfähigkeit und der Dauer des postmortalen Intervalls ist bekannt. Die Veränderung der Leitfähigkeit ist dabei durch das allmähliche Erlöschen der Stoffwechselaktivitäten bedingt. Einige für die Nervenleitfähigkeit bedeutsamen Parameter wurden an lebenden und verstorbenen Ratten gemessen. Die meisten dieser erlaubten jedoch keine eindeutige Aussage. Aus der Reizzeit-Spannungs-Kurve des nervus ischiaticus wurde die Chronaxie bestimmt. Sie stieg während der ersten 90 Minuten im Tod ständig und reproduzierbar in linearer Form an, um dann in ein Plateau überzugehen. Diese Ergebnisse werden als Basis eines Modells zur Bestimmung der Todeszeit im früh-postmortalen Intervall diskutiert.

Schlüsselwörter: Früh-postmortales Intervall – Todeszeit – Nervenleitfähigkeit – Reizzeit-Spannungs-Kurve – Chronaxie

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Introduction

The methods which are in use for estimating the post mortem interval are unreliable, inaccurate and only useful in approximately the first 18 h after death, (DiMaio and DiMaio 1989; Mason 1983). The error in such an estimation may be as much as 10% and to make matters worse the size of the error varies in a non-linear fashion with time. Therefore the need exists to find some time-related quantity which changes after death in an accurately measurable way.

Knight (1982) observed with reference to calculation of time since death from rectal temperatures: "these investigations have all succeeded mainly in demonstrating the extreme inaccuracy of the temperature method, but it remains the only one where errors can be measured in hours and not days". The measurement of the time dependant increase in potassium concentration in the vitreous humour of the eye, (Sturner and Gantner 1964), is not practical for on-the-spot diagnosis.

Besides accuracy, estimation of time of death demands a method which is simple and does not require cumbersome apparatus to be moved to, or operated at the scene of death. The provision of a significant volume of control data is crucial to test the method's accuracy and reproducibility. Observations are bound to be somewhat empirical and very time-consuming to collect. Transcutaneous stimulating and recording of peripheral nerves is non-invasive and its possible application and adaptability to estimate the "post mortem interval" was investigated. The present experiments set out to discover if there were any properties of nerve conduction that changed in a quantifiable manner after death such that they could be used to specify the "post mortem interval".

It should be stressed that this preliminary investigation addresses itself only to the relationships between a number of electrophysiological properties of dying nerves and does not seek to comment on the technological developments required to make the technique available for practical forensic use. Nor does it seek to relate findings made by this method to particular causes of death. Care has been taken to use only simple electrophysiological processes which with appropriate technological help could be envisaged as measurable with portable, battery-operated instrumentation.

The investigation was centred around the measurable properties of nerve conduction (Hodgkin and Huxley 1952; Hodgkin 1964). In the living, resting nerve the kinetics of the intra- and extracellular ion concentrations establishes a dynamic equilibrium between the concentration gradient (mainly established by potassium ions), and the electrochemical gradient resulting from charge separation. This steady state based on differential permeability defines the membrane potential which is the basis for any measurable changes produced if the membrane undergoes depolarization to produce an action potential. The differential permeability and hence concentration gradient is maintained by ion pumps in the cellular membrane which are powered by energy derived from ATP breakdown by membrane ATPase enzymes. At the time of death enzymes are inactivated so that ATP production ceases and the pumps run out of energy. The resulting final concentration of ions and hence the plateau post mortem membrane potential is established by the steady state attained when the concentration and electrochemical gradients are so run down as to establish equilibrium at a value of zero volts.

It may be supposed that the attainment of this final state is not instantaneous after death but is arrived at in a time-dependant manner which will be related to the running down of the pumps. Eventually the membrane will become inexcitable but until that time it is reasonable to suppose that any measure of its excitability will show a decay which is time related. It is important therefore to establish to what extent the measurement of the properties of nerve excitability are constant in life and reproducible in the changes which they show after death.

Materials and methods

Under the experimental conditions described below, indices of excitability and nerve function which were measurably constant before death were estimated before and after death in the rat sciatic nerve.

The "strength duration curve" is essentially a measure of the excitability of a nerve at the site of the stimulating cathode. In the normal nerve the voltage required for excitation bears an approximately exponential relationship to the stimulus duration (Glasby et al. 1988; Glasby 1991). Two features of the curve are described. Rheobase, V_0, is that voltage required to cause excitation at very long durations, (where the curve is asymptotic or essentially flat). This value depends to some extent on the preparation as well as on the nerve itself and hence is a poor index of function. Its value lies in its use for standardizing strength duration curves so that one may be compared to another (Glasby et al. 1988). Values of voltage and duration obtained experimentally are plotted against one another and the value of rheobase obtained from the flat part of the curve. The curve is then replotted with the ordinate V/V_0 where V is the experimentally obtained voltage and V_0 is the rheobase. Families of curves standardized in this way may then be compared. In order to contain all curves within the same values for the abscissa, the values of duration are expressed logarithmically so that a standard scale of -2 to $+2$, (in $\log_{10}$ ms), can be used.

Chronaxie is "the time required for a stimulus of twice rheobasic voltage to be effective for a constant response" (Lapique 1926). It is independent of the preparation and thus a good index of nerve excitability.

For the general case where the standardized strength duration curve has been plotted as a scatter graph relating experimentally obtained data points of:

V/V_0 against $\log_{10}$ duration

The computer has fitted an equation to the curve in the form $y = f(x)$ or:

$$y = a\,e^{-bx}$$

When $V/V_0 = 1$, chronaxie $= 2$, (conditions of standardized curve)

Therefore solving for x when $y = 2$:

$$y = a\,e^{-bx}$$

$$2 = a\,e^{-bx}$$

$$-b\,x = \ln(2/a)$$

$$x = -\ln(2/a)/b$$

From the semilogarithmic graph where $y = 2$, $x = \log_{10}$ chronaxie:

Therefore:

Chronaxie = antilog x (ms)

From the specific example in Fig. 6, (below):

$$y = 1.5009\,e^{-1.2005x}$$

$$2 = 1.5009\,e^{-1.2005x}$$

$$-1.2005x = \ln(2/1.5009)$$

$$x = -0.2391$$

Chronaxie = antilog (-0.2391) = 0.5766 (ms)

Twelve 300 g male Sprague Dawley rats were anaesthetized with a mixture of Hypnovel and Hypnorm anaesthetic (2.5:1 v/v). At room temperature the right sciatic nerve was then exposed for its entire length in the buttock and thigh and cleared from adherent connective tissue. A bipolar low-impedance palladium stimulating electrode was applied to the most proximal part of the nerve with its cathode lying distally. This was used to deliver square-wave stimulating pulses from a Dagan 9200 Omnipulse stimulator and 9250 Stimulus isolator (Dagan Corporation, Minneapolis, Minn USA) and synchronized TTL triggering impulses to a Gould 4074 digital storage oscilloscope and Gould 270 waveform processor. An additional synchronized TTL pulse was delivered to one of the Y-inputs of the oscilloscope as a timing reference marker for the stimulus.

Recordings were taken from the distal end of the nerve segment using an identical electrode placed as far distally along the nerve as was possible. The recording electrode was connected to a Neurolog NL104A differential pre-amplifier and NL125 filter (Digitimer, Welwyn Garden City, UK.). The gain on the pre-amplifier was set at 2000 and the low and high frequency cut-offs at 20 Hz and 5 kHz respectively. The output from the filter went directly to a Y-input of the oscilloscope and a second unfiltered output was taken to a Neurolog NL705 Root Mean Square Integrator with the time constant for integration set at 20 ms. The output of the integrator was subsequently fed to a further Y-input of the oscilloscope. The NL705 provides a rectified RMS-integrated value for compound action potentials which is a function of the power of the input signal. Outputs from the filter (NL125), RMS-integrator (NL705) and a TTL pulse from the stimulator were also delivered to a digital audio tape recorder allowing storage and play-back facilities for detailed off-line analysis at a later time. The Gould 270 waveform processor allowed signal averaging of both the compound action potential and its RMS integral over a number of stimulus shots. In all of the present experiments 16 shots were averaged. This represents a number sufficient after averaging to give a good signal-to-noise ratio without unduly tiring the nerve. The circuit was earthed by connecting a piece of silver wire situated in soft tissue between the electrodes to the earth pole of the differential amplifier. The output from the oscilloscope and waveform processor could be sent to a digital x-y plotter in order to produce permanent records.

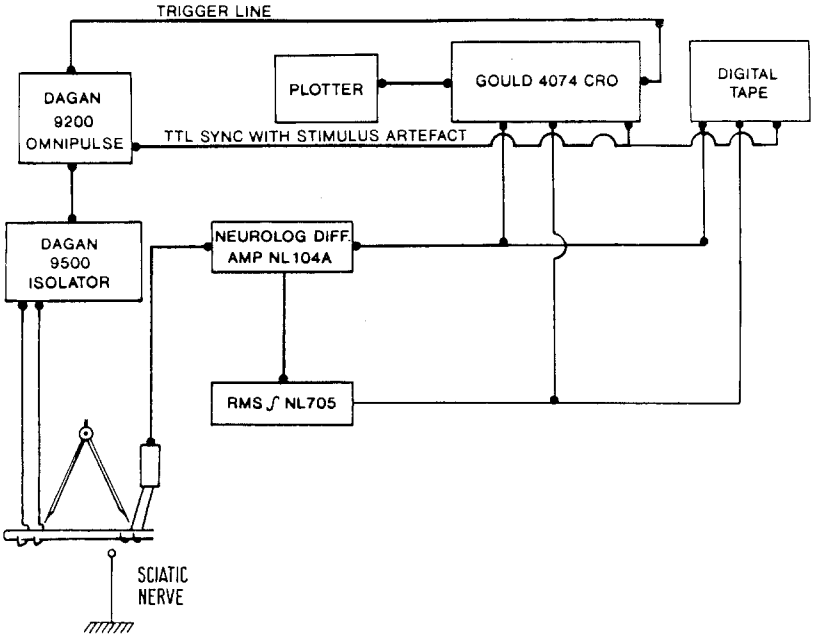

Fig. 1. Circuit diagram for nerve stimulation and recording of compound action potential and its root mean square integral

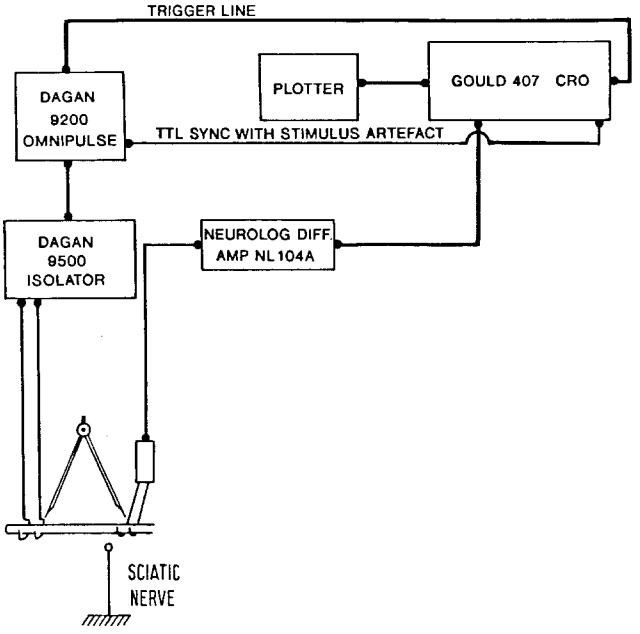

Fig. 2. Circuit diagram of apparatus used to determine the strength duration curve of rat sciatic nerve

For the first series of experiments (Fig. 1) the compound action potentials and their RMS integrals were recorded in 5 rats. Stimuli of 2 V and 500 μs duration were delivered to the nerve at a rate of 1 Hz and the outputs recorded. Recordings were made while the rat was alive to give control values for the compound action potential and RMS-integral.

The rat was then killed by cervical dislocation, time of death was noted and recordings were made every 5 min for 60 min after death. Throughout the experiment the nerve was coated in liquid paraffin to prevent drying out. Rectal temperature was also measured with a thermistor probe while the rat was alive and every 5 min after death.

In the second series of experiments (Fig. 2) the excitability of the nerve was measured as its strength duration curve in 7 rats. The recording setup was identical to that described above except for the omission of the integrator and signal averager. Successive stimuli of fixed duration but increasing voltage were delivered to the nerve until a definitive all-or-nothing action potential was first seen on the oscilloscope and at this point the voltage was noted. This procedure was repeated for a range of durations of 0.05–4.00 μs while the rat was alive and the entire process repeated again at each of 5, 45, 90 and 135 min after death. Values for voltage, (V) and duration, (D), were fed into the computer and a raw strength duration curve plotted. This allowed rheobase, (V_0) to be determined. V/V_0 and $\log_{10} D$ were then calculated and a strength duration durve plotted for each rat at each time point before and after death. Using the curve-fitting programme the equation for each curve was ascertained and the value of chronaxie for each time point in each of 7 rats was calculated. This allowed a graph to be constructed for the change of mean ± standard error of the mean value of chronaxie at each time point for the population of rats before and after death.

Results

The results showed that an index of nerve excitability quantified in the form of chronaxie, related linearly to the "post mortem interval" over the first 90 min after death. Thereafter the value remained constant. The compound action potential and its power integral bore no simple mathematical relationship to time after death.

Figure 3 is an example of the direct print-out from the oscilloscope showing the measurements that were made. When mean ± standard error of the mean values for the averaged RMS integral of the compound action potential was plotted against time after death the relationship depicted in Fig. 4 was obtained. This shows an inconsistant relationship between the power of the compound action potential and time, and is thus of little help as a determinant of "post mortem interval".

Since the "raw" averaged action potentials had been saved it was possible to do further "off-line" analysis of these measurements. The signals were electronically differentiated to look at rates of change versus electrical

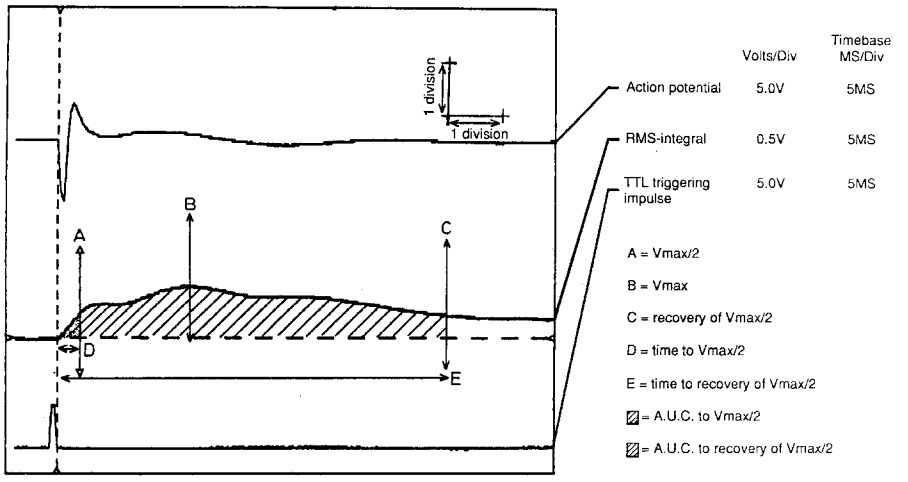

Fig. 3. An example of the plotter output in an experiment to determine compound action potential and its RMS integral. (AUC = area under curve)

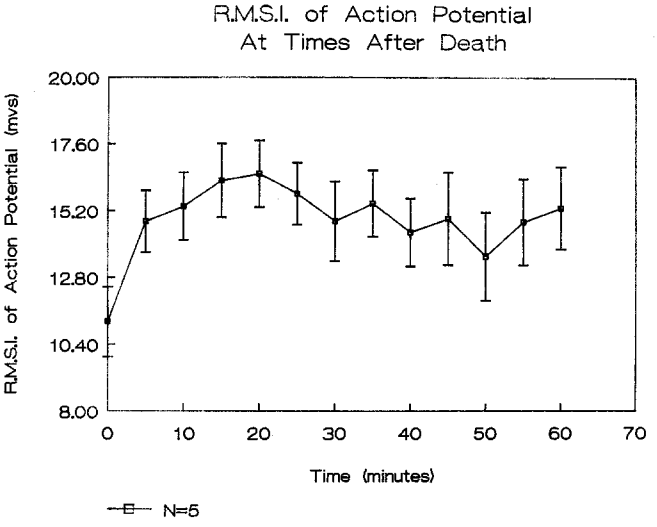

Fig. 4. The relationship of mean ± standard error of the mean values of the RMS integral of the compound action potential in five rats immediately before death, (t = 0) and for 70 minutes after death by cervical dislocation. There is no obvious simple mathematical relationship to be seen

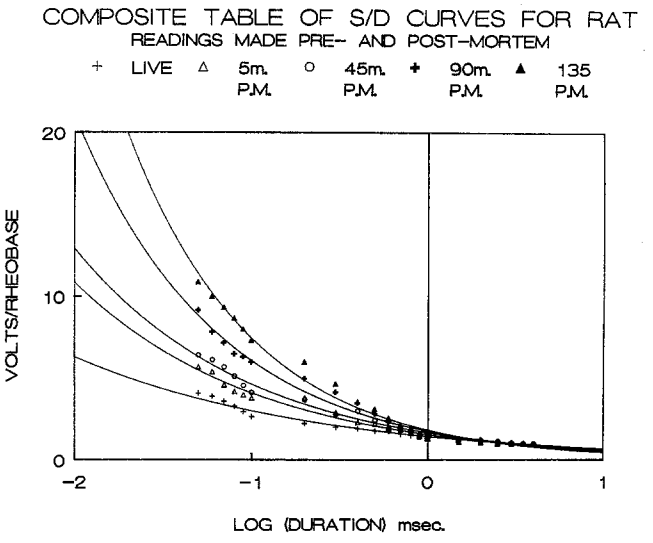

Fig. 5. A family of standardized strength duration curves for a single rat taken immediately before death and at 4 post-mortem time points. It can be seen that the nerve becomes progressively less excitable with time after death

activity; this also showed no clear relationship with time. The power spectrum of each averaged compound action potential was obtained by applying a fast Fourier transform (Hanning window) to the wave form to examine the amplitudes of its component frequencies. These however were found to be inconsistent and therefore again showed no clear relationship to "post mortem interval".

The strength duration curve, though more difficult and time-consuming to measure, indicated a relationship between nerve excitability and "post mortem interval". This would be expected from a theoretical consideration. Figure 5 is an example of the family of curves obtained for a single rat at different times after death. Each curve is standardized by dividing the voltages obtained by the rheobasic voltage thus allowing comparisons to be made [7]. As "post mortem interval" increased, the number of volts required to reach threshold must be increased for a given stimulus duration or the duration must be prolonged for a given stimulus amplitude. In other words the nerve becomes less excitable with time. This feature was consistent for all of the rats studied ($N = 7$).

For each strength duration curve in the family the value of chronaxie was obtained. Figure 6 is an example of a standardized strength duration curve plotted as a scatter graph and fitted with a "best fit" line using a computer programme. The curve fit shows an exponential relationship and the programme provides the equation of the curve. When this equation is solved for $y = 2$, (since rheobase, $V_0 = 1$ and chronaxie $= 2\,V_0$), the value of chronaxie, (0.5766 ms), was obtained for this time point. All curves were thus analysed and the mean values ± standard error of the mean values for chronaxie plotted against time (Fig. 7). Under the standardized experimental conditions discussed above, the relationship between chronaxie and "post mortem interval" was found to be linear over the first 90 min and to reach a

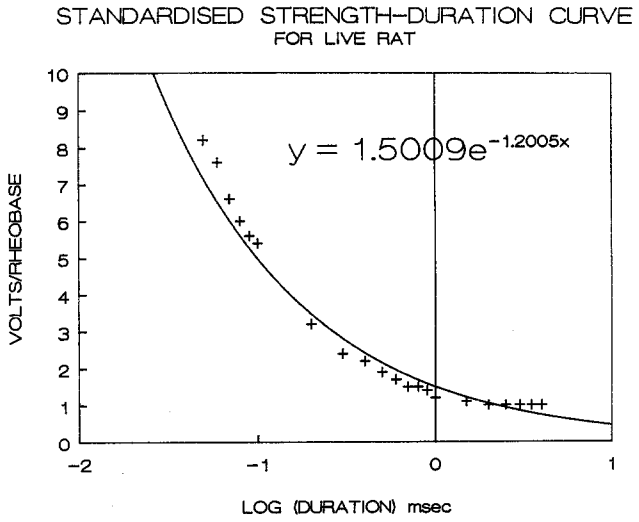

Fig. 6. An example of a best fit line applied to a standardized strength duration curve of a live rat. The equation for this line is $y = 1.5009\,e^{-1.2005x}$ which would correspond to a chronaxie value of 0.5766 ms

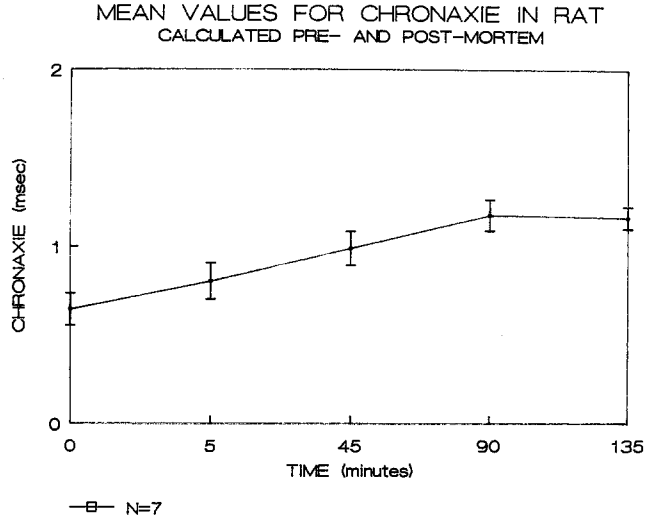

Fig. 7. Mean ± standard error of the mean values of chronaxie determined for 7 rats before death, ($t = 0$) and over the subsequent 135 minutes. The relationship is linear for the first 90 minutes and attains a plateau value thereafter

plateau value thereafter. The bars for standard errors of the mean are large (Fig. 7), and may surpass the change in the means seen between two consecutive experimental points. This is a reflection of the small sample sizes used here. Larger samples would give more precise values but the trend is clearly established as a principle needing further refinement in terms of numbers.

Discussion

Of the various indices of nerve function studied in the rat sciatic nerve under the standardized experimental conditions described the only one found to show a reproducible, simple mathematical relationship to "post mortem interval" was that of the chronaxie of the strength duration curve. All other measurements were inconsistent and hence unreliable. Though tedious to measure using the technique described here, it is likely that measurement of chronaxie could be a useful indicator of the "post mortem interval".

It is necessary to recognize that there may be a considerable difference between rats and humans in the time taken for their nervous tissue to lose function. This is likely because of the considerable difference in metabolic rates. A human cadaver cools down at a rate of approximately 1.5°C per hour, averaged out over 6 h (Spitz and Fisher 1980; Hutchins 1985; DiMaio and DiMaio 1989). In the present experiments it was noted that the rat cooled down at a rate of about 6.6°C per hour, almost four times faster than a human. It is probable therefore that in the human, chronaxie may be a useful measurement of "post mortem interval" for a longer period of time and, in any case, the first 90 min after death currently represents a period in which it is particularly difficult to obtain information from cooling (Hutchins 1985).

There is a considerable gap still to be bridged, between the demonstration of a reproducible and linear relationship between chronaxie and "post mortem interval", and the translation of such findings into a useful forensic technique. Not least is the difficulty in obtaining human data. However these techniques are used daily in clinical electrophysiological studies and the "hardware" for transcutaneous recording from, for example, the median nerve is available and well tested. Making the apparatus portable and battery powered should not be beyond the technology which produces battery operated nerve stimulators for surgical use and programmable electronic calculators with digital displays.

There is the additional problem of the neurological state of the individual immediately prior to death and the effects of, for example, alcohol and medication upon the measurements. Also the mode of death may affect the result just as in the case of post-mortem temperature measurement. Clearly, as with any technique designed to establish post mortem interval a painstaking acquisition of control values would have to be undertaken to establish standards in cases where the mode and time of death were precisely known. The present findings nevertheless have significant implications for the development of a method of determining "post mortem interval" from characteristics of nerve function and should provide a basis for further study.

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