RESEARCH PAPERS

THE ASSESSMENT OF CONDUCTION ANAESTHESIA

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Experiments are described which involve observation of the rates of decline in the compound action potentials of frog sciatic nerve produced by local anaesthetics. The percentage reduction and regression, with time, of action potentials are assessed for each of four concentrations of lignocaine hydrochloride. The results are analysed for variance. It appears that percentage reduction of action potential gives a better assessment of conduction anaesthesia than the regression of potential with time. The percentage reduction of action potential bears a linear relationship to the logarithm of the concentration of local anaesthetic applied to the nerve.

Conduction anaesthesia has been considered to involve the penetration of nerve sheaths by anaesthetic agents, resulting in reversible paralysis of the nerve (see Sollmann, 1917; Sinha, 1936; Bülbring and Wajda, 1945). The isolated frog sciatic nerve has become accepted as a standard preparation for its measurement since the experiments of Bennett, Wagner and McIntyre (1942).

In the present work it was thought that the measurement of the action potentials of such nerves, as absolute voltages, would be a direct assessment of activity in a given nerve trunk. By observation of the extent of changes effected in the potentials by local anaesthetic, it was hoped to investigate the relationship between the concentration of drug applied and the conduction anaesthesia produced.

Experimental

Preparation of the Nerve

Sciatic nerves of pithed frogs (*Rana temporaria*) of either sex were dissected under frog Ringer solution (as modified by Starling) from the ninth root to the knee, each end being tied with silk thread. They were then stored overnight (approximately 16 hr.) at 5°, allowing the nerves to stabilise after any injury during dissection. The frogs were not less than 25 g. in weight, giving a length of nerve of 4 to 6 cm.

Apparatus

The apparatus consisted of an enclosed cabinet, the front of which could be raised. In the cabinet, suspended from a perspex beam (B), were two stimulating (SS), one earth (E) and two recording electrodes (R′R′) (Fig. 1), all of 20 s.w.g. platinum wire. The recording electrodes which were at a fixed distance apart (12 mm.), could be adjusted relative to the

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other electrodes in order to accommodate any variation in the lengths of nerves used.

A notch (N) in one end of the perspex beam and a pulley system (P′P″) at the other end allowed the nerve, ligatured at both ends, to be held in position over the electrodes, aided by tension applied to the nerve from a 1 g. weight. This prevented the position of the nerve on the electrodes from changing during application of the drug, or washing.

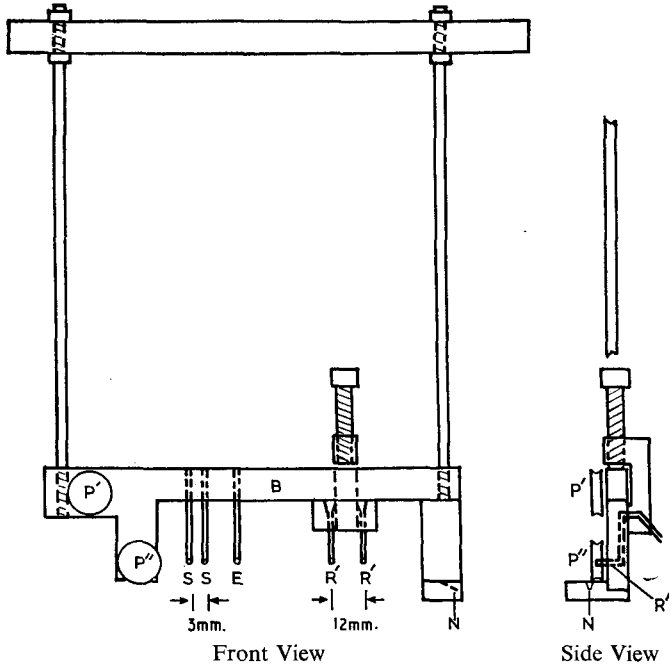

FIG. 1. Arrangement of electrodes in apparatus. B, Perspex beam. SS, Stimulating electrodes. R′R′, Recording electrodes. E, Earth electrode. Pulley system (P′P″) and notch (N) allowed nerves, ligatured at both ends, to be held in position over the electrodes.

A perspex trough, volume 45 ml., by means of which the nerve could be washed with Ringer solution, and a perspex cup for the anaesthetic, volume 0·3 ml., were incorporated in the enclosed cabinet. Both could be manipulated from outside the cabinet. The cup had V-shaped notches cut in opposing faces. This enabled the nerve to pass through the meniscus of a constant volume of anaesthetic solution, which therefore surrounded the nerve without the nerve touching the sides of the cup.

In the cabinet, humidity was maintained at saturation throughout experiments by means of (15 cm. × 30 cm.) chromatographic paper suspended from beams at either end of the cabinet, dipping into perspex troughs containing water. Humidity, which was measured by a paper hygrometer, was initially raised to saturation by blowing water vapour

into the cabinet. The temperature of the cabinet was raised to, and maintained at, 24° by table and overhead heaters.

After placing the nerve in position, the stimulating electrodes were connected with a stimulator, the recording electrodes were connected with the input of the recording apparatus and the fifth electrode was led to earth. All leads were "screened".

Electronic apparatus. A block diagram of this apparatus is given in Fig. 2. The rate of firing of the stimulator and of the sweep generator was controlled by the master oscillator. After delays imposed by the

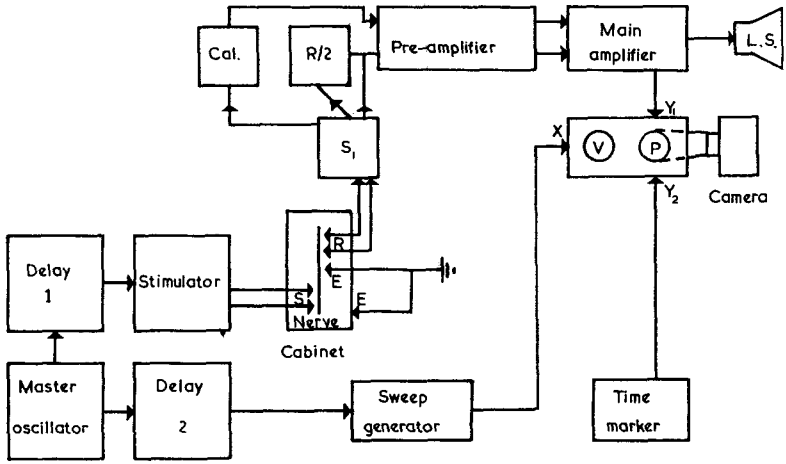

FIG. 2. Block diagram of apparatus. S, Stimulating electrodes. R, Recording electrodes. E, Earth electrodes. S_1, 4-pole, 3-way switch. R/2, Variable parallel resistance. Cal, Calibrator. L.S., Loud speaker. X, Y_1, Y_1, Plates of double beam cathode ray tubes V and P.

delay circuits (1 and 2), the stimulator and sweep generator were triggered. Normally delay 2 was in the zero position so that the traverse of the spot across cathode ray screens V and P began immediately after the firing of the master oscillator. The time at which the stimulus fell was governed by the setting of delay 1. The stimuli were square wave voltage pulses of 0·1 msec. duration, their amplitude being variable between 0 and 12 V. When stimulating the nerves, a pulse just supramaximal for the action potential was delivered every 10 sec.

The potentials picked off from a stimulated nerve were led to a 4-pole, 3-way switch (S_1) and passed to a pre-amplifier by twin screened cables either directly or after passing a variable parallel resistance R/2. The switch also permitted a calibrator (cal.) to be connected with the pre-amplifier. The main voltage amplification was performed in the second or main amplifier. The output of this amplifier was connected to one of the Y-plates of a double beam cathode ray tube and to a small audio-amplifier which fed a loud speaker (L.S.). The latter provided an aural indication of the nervous activity. The other Y-plate of the cathode ray

tube was connected with a time marker giving pulses of 0·1, 1·0 or 10 msec. intervals so that the recorded phenomena could be timed accurately.

The display unit consisted of two double beam flat faced cathode ray tubes, each presenting the same information. One tube (V) was used for visual examination of the trace; the other (P) was attached by a metal cone to a camera in which traces were recorded on 35 mm. film, negative voltages being recorded upwards.

The calibrator was a device whereby a one mV pulse of varying duration could be controlled manually. The voltage was obtained by dividing off the required potential by an appropriate resistance from a 1·5 V cell. The value of the mV was controlled by regulating the current flowing through a resistance of one ohm to one mA.

The calibrator made it possible to assess, in terms of volts, the values of the action potentials observed on the face of the oscillograph screen. This assessment involved recording the Y-plate deflection achieved by the mV pulse on the oscillograph screen.

Thus the value of the standard mV could be determined directly, irrespective of the amplification used in any particular experiment.

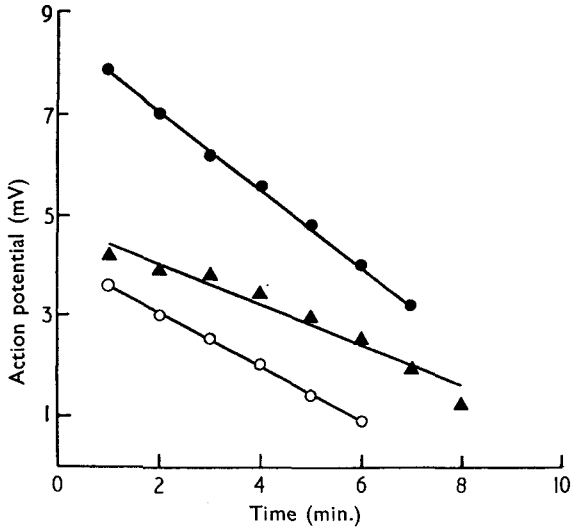

FIG. 3. Decline in action potential in three frog sciatic nerves, following the application of 5 mM lignocaine hydrochloride.

Experimental Procedure

The sciatic nerve was mounted and its action potential rendered monophasic by burning. The time marker was photographed to serve as future reference for temporal estimates. The sweep speed having been adjusted remained set throughout the experiment. The stability of the preparation was tested by recording twenty control action potentials at 10 sec. intervals after adjusting the shock strength of the stimulus. The

drug in the anaesthetic cup was brought up to surround the nerve completely at the point of application and the time was noted. Recording continued until the action potential was reduced to 80 to 90 per cent of its original value when the anaesthetic was removed and the time again noted. The nerve was allowed to rest, surrounded by Ringer solution and the degree of recovery at subsequent 15 min. intervals was ascertained until nearly complete.

RESULTS

The effects of four different concentrations of lignocaine hydrochloride (10, 5, 2·5 and 1·25 mM) were recorded on the action potentials of four different groups of sciatic nerves. Each determination at a given concentration involved a separate nerve, the voltage being measured with the aid of a calibrator.

TABLE I

EFFECT OF LIGNOCAINE HYDROCHLORIDE ON THE ACTION POTENTIAL OF FROG SCIATIC NERVE, MEASURED AS THE REGRESSION (b) IN mV/MIN., OR AS THE "PERCENTAGE REDUCTION" (p) OF POTENTIAL. p IS EXPRESSED IN MIN.$^{-1}$ × 100

Concentration (mmolar)	1·25	2·50	5·00	10·00	Ringer
pH	7·20	7·15	7·10	6·90	7·30
Number of determinations	16	16	15	14	8
Mean value of b	0·15	0·43	0·53	0·84	0·04
Variance of b	0·0062	0·0150	0·0970	0·1112	0·03
Mean value of p	2·84	6·68	10·69	15·66	—
Variance of p	3·58	2·97	5·46	9·01	—

As the local anaesthetic blocked conduction the action potential was found to decline linearly with time (Fig. 3). It was thought that the criterion of the effectiveness of a drug as a conduction anaesthetic could be taken as the slope of the line relating voltage and time for a given nerve. The slope was expressed as a regression coefficient (b) in mV/min., the line having been calculated by regression analysis (Saunders and Fleming, 1957). The mean value of b was determined for each concentration of drug used and the variance (S^2) of the observations about each mean value was determined (Table I). Ringer controls are included in this Table.

TABLE II

CORRELATION COEFFICIENTS (r) OF THE MEAN CONTROL ACTION POTENTIALS WITH THE MEAN RATES OF DECLINE IN POTENTIAL PRODUCED BY LIGNOCAINE HYDROCHLORIDE

Concentration (mM)	1·25	2·50	5·00	10·0
Number of determinations (N)	16	16	15	14
r_e—calculated	0·02	0·53	0·92	0·84
r_t—theoretical	0·50	0·50	0·52	0·54
N–2 degrees of freedom	—	—	—	—
P = 0·95	—	—	—	—

For significant correlation r_e should be greater than r

However, for each concentration of drug, the mean value of b has been correlated with the mean value of the control action potentials recorded from the nerves (Table II). From Table II it may be seen that at the 10, 5 and 2·5 mM concentrations such a correlation was significant.

Therefore it was decided also to determine for each nerve the "percentage reduction" (p) in action potential as given by the fraction:

$$p = \frac{b}{\text{Initial Action Potential}} \times 100.$$

Thus, p was a measure of the fall-off of potential in unit time as related to the control action potential. However as b, for a given nerve, may be measured by dividing the control action potential by the time taken by the drug to produce a 100 per cent block in conduction, then p can be considered as being a measure of the reciprocal of "100 per cent block time". The variances of p about the mean for each concentration of lignocaine were determined (Table I).

An estimate of whether b or p gave the best measure of conduction anaesthesia was required. Hence, it was necessary to compare the variance of results calculated by these different means at each concentration of lignocaine.

For reproducible results it was desirable that the "spread" of results about a mean should be minimal. As the "spread" is proportional to the square root of the variance, $\sqrt{S^2}$/mean value of b should be a minimum.

To compare regression with "percentage reduction", the null hypothesis that there was no difference in the Variancy ratio $\frac{S^2b/mean^2b}{S^2p/mean^2p}$ was made. The hypothesis was tested by determining whether or not the ratio lay within the range 0·38–2·60 as indicated by the "F value" derived from tables for n–1 degrees of freedom at a probability level of 95 per cent.

TABLE III

VARIANCY RATIOS OF REGRESSION (b) AND PERCENTAGE REDUCTION (p) FOR ACTION POTENTIALS IN NERVES TESTED WITH LIGNOCAINE HYDROCHLORIDE

Concentration (mM)	1·25	2·50	5·00	10·0
Number of determinations	16	16	15	14
Variancy ratio—$\frac{S^2bv/mean^2bv}{S^2pv/mean^2pv}$	0·64	1·22	7·31	4·25

For there to be no significant difference in the variances of regression and "percentage reduction", the Variancy ratio should lie within the range 0·38–2·60. Values greater than 2·60 show that regression has a larger variance.

The results (Table III) obtained suggested that at the 10 and 5 mM concentration p and b are significantly different, the "percentage reduction" of action potential giving less "spread" or variance than regression.

The "percentage reduction" also bears a linear relationship to the logarithm of the concentration of drug applied (Fig. 4). Hence this measure would appear to be preferable in assessing conduction anaesthesia.

Discussion

One of the most sensitive devices for the study of activity in a nerve trunk was introduced by the application of the cathode ray oscillograph to electro-physiological work by Gasser and Erlanger in 1922. As local anaesthesia involves a reversible block of nerve conduction it was to be

expected that some of the effects of local anaesthetics on nervous tissue could be observed and possibly assessed from oscillographic recordings. Such assessments were in fact attempted by Bennett and others (1942) and Bennett and Chinburg (1946). The limitations of this approach to the screening and bioassay of local anaesthetics seemed to lie in the fact that the method can be applied only to conduction anaesthesia.

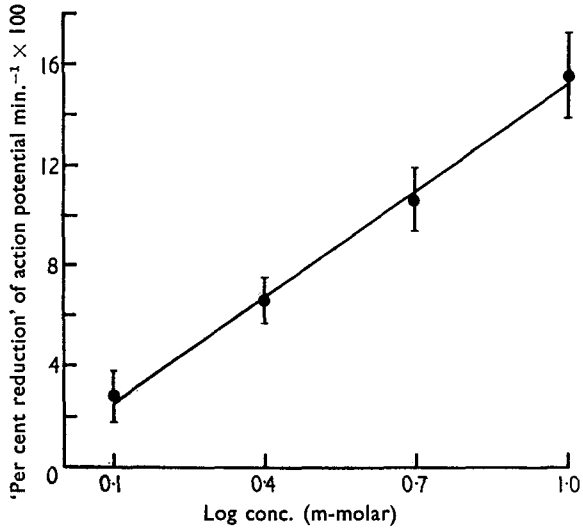

FIG. 4. "Percentage reduction" of action potential produced by lignocaine hydrochloride in frog sciatic nerve: mean value ● and limits of error of mean. The line relating "percentage reduction" and log concentration was determined by regression analysis (Saunders and Fleming, 1957).

The work of Bennett and others (1942) involved the use of isolated frog sciatic nerve, a mixed nerve trunk, whereas ideally the effectiveness of local anaesthetics should be assessed on sensory nerve fibres alone. Indeed, as early as 1917 Sollmann had criticised the use of motor nerve fibres in assessing conduction anaesthesia. The use of frog sciatic nerve in such studies has, however, persisted. Results obtained from such experiments are less subjective than those obtained from methods involving intracutaneous injection (as in infiltration anaesthesia) or surface anaesthesia which involve the study of reactions of whole animals (Sinha, 1936).

The stimulus for the present work lay in the fact that the action potential might give a measure of the number of active fibres in a nerve trunk. It has been found that an assessment of conduction anaesthesia could be made which would be capable of statistical analysis. A compound action potential has been the basic measurement in this work. It has not been possible to examine the effects of lignocaine on its possible subdivisions since such resolution requires nerves rather longer than those which were obtained from *R. temporaria.*

The action potential was found to fall off in a linear manner as the nerve fibres were progressively blocked by local anaesthetic. As this regression, in a given nerve, was related to the control action potential, it was decided to compare results obtained by measuring regression of potential with those obtained from determinations of the "percentage reduction" of potential.

"Percentage reduction" may be considered as a function of the time required by a concentration of drug to produce a 100 per cent block of conduction in a given nerve. It appeared to give more reproducible results than measures of regression.

The "percentage reductions" have been expressed as a dose-response relationship which has been applied for the first time to results obtained from work in conduction anaesthesia involving observations on action potentials. It is established that different concentrations of anaesthetics produce rates of nerve blockade which are significantly different.

Sensitivity of the method is seen from the fact that preparations presented stable potentials during control periods, whilst the smallest concentrations of drug exerted a noticeable effect on these potentials. Similarly there was a difference between the results obtained when a low concentration of drug, and when Ringer solution, was applied to the nerve.

It should be noted that the method employed in the present work, unlike that of Bennett and Chinburg (1946) does not give any indication of the mechanism by which the nerve block is produced. They were able to investigate the mechanism of nerve block by local anaesthetics from a study of resting and demarcation potentials in isolated frog sciatic nerve.

Ultimately, it is likely that the problems of the site and mechanism of action of local anaesthetics on peripheral nerve will be resolved. Much knowledge of these subjects could be gained by a study of stereochemically related substances if their anaesthetic potencies could be compared accurately.

Thus, the present work has described an attempt to provide an objective and reliable method for the quantitative biological comparison of substances which may have local anaesthetic qualities.

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REFERENCES

Bennett, A. L. and Chinburg, K. G. (1946). *J. Pharmacol.*, **88**, 72–81.
Bennett, A. L., Wagner, J. C. and McIntyre, A. R. (1942). *Ibid.*, **75**, 125–136.
Bülbring, E. and Wajda, I. (1945). *Ibid.*, **85**, 78–84.
Gasser, H. S. and Erlanger, J. (1922). *Amer. J. Physiol.*, **62**, 496–524.
Saunders, L. and Fleming, R. (1957). *Mathematics and Statistics for use in Pharmacy, Biology and Chemistry*, p. 157. London: The Pharmaceutical Press.
Sinha, H. K. (1936). *J. Pharmacol.*, **57**, 199–220.
Sollmann, T. (1917). *Ibid.*, **10**, 379–397.