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# PRESSURE REVERSAL OF THE EFFECT OF URETHANE ON THE EVOKED SOMATOSENSORY CORTICAL RESPONSE IN THE RAT

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**1** The cerebral response evoked by stimulation of the forepaw in the rat shows an increase in latency and decrease in the amplitude of its initial components as anaesthetic dose (urethane) is increased.

**2** These changes are reversed if the ambient pressure is increased with helium and the electrocorticogram shows an increase in basic frequency.

**3** The dose of urethane needed to prevent reflex response to tail stimulation is increased as pressure increases.

**4** The implications of these behavioural and somatosensory responsiveness changes as pressure is increased are discussed.

## Introduction

Experimentally, high ambient pressures have been shown to antagonize the effects of anaesthetic chemicals administered to a wide variety of animals (Johnson & Flager, 1950; Lever, Miller, Paton & Smith, 1971; Halsey, Eger, Kent & Warne, 1975; Miller, 1975). This pressure 'reversal' of anaesthesia has been rationalized in terms of the critical volume hypothesis of anaesthetic action in which it is proposed that anaesthetics cause the expansion of certain neuronal membranes and that an anaesthetic state results if this expansion exceeds a certain critical volume (Miller, Paton, Smith & Smith, 1973). Present day theories on the mechanism of anaesthetic action suggest that anaesthetics interact with a hydrophobic site or sites within the nerve cell membrane and cause a conformational change in some membrane component resulting in expansion of the membrane. However, a major problem is that anaesthetics have a wide variety of effects of which only a few can be relevant to the production of the anaesthetic state. The pressure reversal of anaesthetic effects offers the possibility of eliminating certain processes from being implicated in the mechanism of anaesthesia. Previously it has been found that anaesthetics impair the transmission of somatosensory information through the thalamus (Angel & Unwin, 1969). In this paper we show that this effect can be reversed by application of high pressures.

## Methods

For these experiments 30 female Sprague-Dawley rats in the weight range 190 to 210 g were used. These were all anaesthetized, without premedication, with intraperitoneally administered ethyl carbamate (urethane 25% w/v, in 0.9% w/v NaCl solution: saline) at doses which varied from 1.25 to 1.31 g/kg for the time control experiments, the anaesthetic effect experiments and the behavioural experiments (*see below*) to 2.25 g/kg for the pressure reversal experiments.

### *Electrophysiological experiments*

A midline incision approximately 1 cm in length was made in the skin over the cranial vault, the periosteum scraped off the skull and two small holes (1 mm diameter) were drilled into the skull, one over the centre for contralateral forepaw projection area (3.5 mm lateral and 1.0 mm anterior to the bregma; Angel, Berridge & Unwin, 1973) and the other over the posterior cortex. These holes were sufficiently deep to penetrate the cranium but not the underlying dura-mater. Fine insulated silver wires (Diamel coated silver, 0.006 inch diameter, Johnson, Mathey and Co.) with their tips un-insulated and fused into small balls, approximately 1 mm in diameter, were then inserted into the drill holes and fixed in contact with the dura-mater with bone wax (Ethicon W810). The skin incision was closed and covered with a saline soaked gauze pad. The contralateral forepaw was stimulated electrically via two fine pins inserted sub-cutaneously one on either side of the wrist. The animals' temperature was monitored throughout the experiment with a rectal thermistor and kept at 37°C ± 0.5°C.

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The preparation was then placed inside a pressure chamber (100 ATA, 20 litres, A. Pratt and Co. Ltd.) together with a soda lime absorber to remove $CO_2$ and a fan to ensure adequate gas mixing (1 ATA = 101 kPa). The chamber was then closed and the pressure increased to 1.34 ATA with oxygen to give a partial pressure of oxygen of 0.54 ATA. After obtaining preliminary control records and waiting, if necessary for temperature stabilization, the chamber pressure was increased with helium to 100 ATA at a rate of 1 ATA/min pausing for 4 min periods at 17 ATA intervals to record the evoked cerebral response and electrocorticogram. The very deep level of anaesthesia (2.25 g/kg) used at the start of these experiments was chosen to ensure that the animals did not 'awaken' at high pressure. In one case, in which the animal showed signs of behavioural arousal, the experiment was immediately terminated by rapid decompression.

The potentials from the silver wire electrodes were fed into two a.c. coupled amplifiers one with a flat frequency of response from 1 to 100 Hz to record the electrocorticogram, the other flat from 60 Hz to 5 kHz to record the evoked response. Outside these frequency limits the amplifier response was attenuated by 6 dB per octave. Electrical stimuli were delivered to the wrist at a rate of 1/s from a stimulator (Devices, Type 2533) and were 50 μs in duration and 40 V in amplitude. The outputs from the two amplifiers together with a pulse coincident with the stimulus were recorded on a tape recorder (TEAC R-70A) for subsequent analysis with a PDP-11/10 minicomputer (Digital Equipment Corporation) and displayed on an oscilloscope for immediate visual inspection.

*Behavioural experiments*

In these experiments ($n = 6$) the rat was lightly anaesthetized with urethane before pressurization, and extra anaesthetic was added as required while the pressure was increased. Anaesthesia was assessed by observing the response to an electrical stimulus applied to the proximal inch of the tail, and the end point was chosen to be that at which there was a moderate abdominal twitch in response to a 10 V single stimulus. A 'Medicut' 16 G cannula was sewn into the peritoneum, and connected to a 'Perfusor' motor driven syringe (Braun) containing 25% urethane w/v in saline. The syringe could be controlled automatically and was placed in the chamber with the rat. When respiration, heart rate and temperature were maintained at a steady level, chamber pressure was increased with helium at 1 ATA/min and extra urethane was added via the intraperitoneal cannula to maintain a constant depth of anaesthesia.

## Results

*Effect of urethane on the evoked cortical response*

In 12 animals the effect of increasing the dose of urethane anaesthesia was determined. For these experiments the average cortical response to 100 consecutive stimuli applied to the right forepaw at a rate of 1/s was taken at an initial dose of 1.25 g/kg and at 5 min after intraperitoneal injections of urethane to increase the anaesthetic level by increments of 0.25 g/kg. The cortical response can best be described as a wave of surface positivity divided into initial and late positive waves by a surface negative wave or waves (see Figure 1a). At the start of the experiment (1.25 g/kg) the mean latency to the start of the initial positive wave was 3.75 (± 0.14 s.e. mean) ms and the amplitudes of the initial positive (Pi Figure 1a) and initial negative (Ni Figure 1a) waves varied between 14 to 63 μV (mean 32.5 ± 4.27 s.e. mean) and 22 to 117 μV (mean 47.1 ± 8.55 s.e. mean) respectively. The effects of increasing the anaesthetic level with urethane were (a) to increase the mean latency of the response, (b) to decrease the amplitude of the initial positive wave and (c) to decrease the amplitude of the initial negative wave. (These amplitudes were measured from the baseline to the peak of the initial positive wave and from the peak of this wave to the trough of the ensuing negative deflection). For example doubling the dose of urethane (from 1.25 to 2.5 g/kg) gave an increase in latency to 5.90 ms (± 0.25) and a reduction of the initial positive and negative waves to 41.5% (± 3.5) and 20.0% (± 2.5) of their starting values respectively. Figure 1b shows the mean levels of these parameters versus the anaesthetic dose.

*Effect of increasing ambient pressure*

(a) *On the evoked cerebral response* The effects of increasing chamber pressure on this response were the reverse of those seen on increasing the depth of anaesthesia. The mean latency at the start of the 10 successful experiments was 5.75 ms (± 0.56) and at 100 ATA 4.45 ms (± 0.43). Both the initial positive and negative waves increased in amplitude, the latter to 5.13 times (± 0.86) the starting level. The shape of the responses showed the reverse changes to those seen with increasing anaesthetic depth (compare Figures 1a and 2a). The mean size of the initial negative wave of the cortical response versus pressure is shown in Figure 2b. The initial negative wave was chosen as the measured parameter for this part of the experiment because if it becomes very large its latency of occurrence tends to decrease which can have the effect of cutting short the initial positive wave (see Figure 2a compare top and bottom traces on the left hand side).

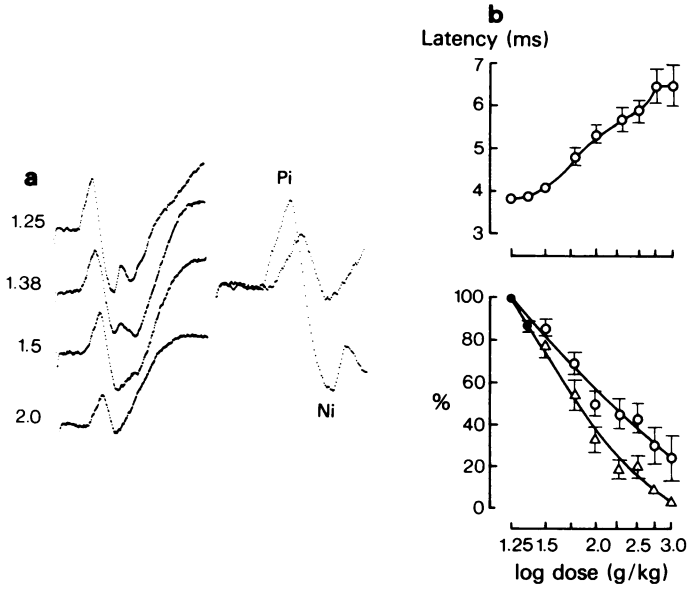


**Figure 1** (a) This shows a series of averaged cerebral evoked responses ($n$ = 100, sweep = 20 ms left, 10 ms right) to stimulation of the right forepaw at a rate of 1/s. On the left are shown 4 averages at various doses of ethyl carbamate (1.25, 1.38, 1.5, 2.0 g/kg from top to bottom) and on the right the averages at doses of 1.25 g/kg and 2.0 g/kg are shown superimposed to illustrate the changes in latencies and amplitudes of the initial positive (Pi) and negative (Ni) waves of the responses. (b) Shows in graphical form the decrease in amplitudes of the initial positive (○) and negative (△) waves of the cortical response, expressed in relative terms, versus the log dose of anaesthetic (Bottom graph) and the mean change in latency versus log dose of anaesthetic (Top graph). Each point represents the mean change from 12 experiments and the vertical lines represent s.e. mean.

In two animals the pressure was decreased from 100 ATA to 68 ATA at a rate of −1 ATA/min. In both cases the evoked cerebral response showed changes indicating an increase in the effective level of anaesthesia.

In one such experiment the cerebral evoked responses were reprocessed to give four averages of 50 consecutive responses each to obtain a measure of the scatter of the results. At the start these gave a mean level of the initial negative wave of 1.00 (±0.09); at 68 and 100 ATA these were increased to 2.02 (±0.16) and 3.28 (±0.16) respectively. Decreasing the pressure from 100 to 68 ATA gave a mean level of response of 2.23 (±0.07). These two measures at 68 ATA are not significantly different from each other but are both highly statistically different ($P < 0.001$) from the means at the start and at 100 ATA.

In all the pressure experiments the initial negative wave appeared to increase as a linear monotonic function of pressure although the slope of this function varied enormously from animal to animal; from a 3.3 fold to a 6.2 fold increase in amplitude comparing starting and finishing values (4.77 ± 0.42).

Since these pressure experiments took place over a 128 min time span it was necessary to eliminate the possibility that the metabolic removal of the anaesthetic was the cause of the apparent reduction in anaesthetic level seen with increasing pressure. To this end, a series of control experiments ($n$ = 12) were performed in which the starting level of anaesthesia was 1.25 g/kg and the evoked cortical responses observed over a 2 h period. In all experiments no statistically significant differences were seen in either the mean latency of response or the mean amplitudes of the initial positive and negative waves of the cortical response (see Figures 2b and 3).

(b) *On spontaneous electrocortical activity* Again the effects of pressure were the reverse of those seen with increasing anaesthetic depth. As the pressure was increased the electrocorticogram changed from a pattern of high voltage low frequency oscillations to one of low voltage higher frequency oscillations (see Figure 4a). In an attempt to quantify this change with a single parameter the electrocorticogram was led to a zero-crossing detector and the mean interval between crossings was determined. Since the distribution of the intervals was negatively skewed when plotted on a linear abscissa and quasi-Gaussian when plotted as a log interval, the geometric mean frequency was taken as the measure of the predominant frequency (see

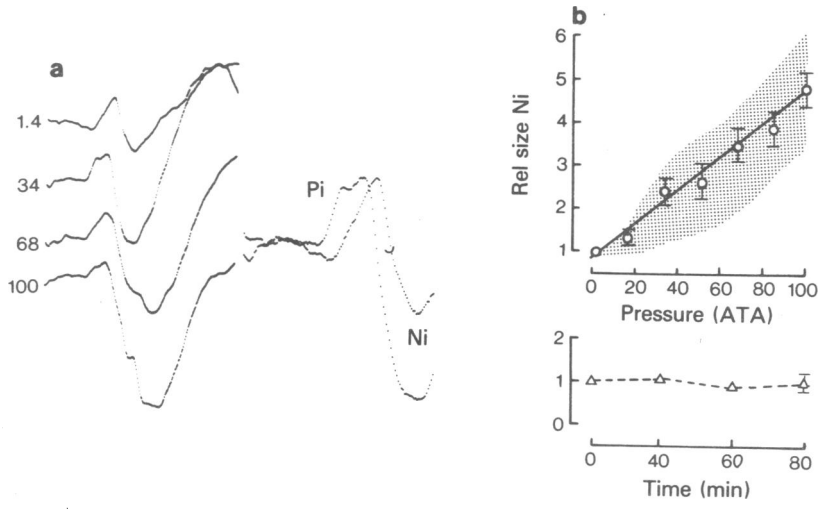


**Figure 2** (a) A series of average evoked cortical responses ($n$ = 100, sweep = 20 ms left, 10 ms right, stimulus to the right forepaw at a rate of 1/s) at various ambient pressures; from top to bottom 1.4 ATA, 34 ATA, 68 ATA and 100 ATA respectively. The expanded averages compare the responses obtained at 1.4 ATA and 34 ATA, to accentuate the differences seen in latencies and amplitudes of the initial components of the cortical responses. (IATA = 101 kPa) (b) (Above) The change in mean amplitude of the initial negative wave (○) of the cortical response (ordinate scale) versus pressure (abscissa scale). Each point is the mean of 10 experiments and the vertical lines represent s.e. mean. The shading encloses the absolute scatter of the results. The line drawn through the points represents the calculated regression for the data ($y = 0.034x + 0.877$, coefficient of linear regression = 0.992; $P < 0.001$). (Below) The symbols (△) represent the mean size of the initial negative wave taken at 40 min intervals in 12 control experiments at normobaric pressure. The horizontal lines above and below the last symbol represent ± 3 times the standard error of the mean (this parameter being contained within the symbols for the first 3 points).

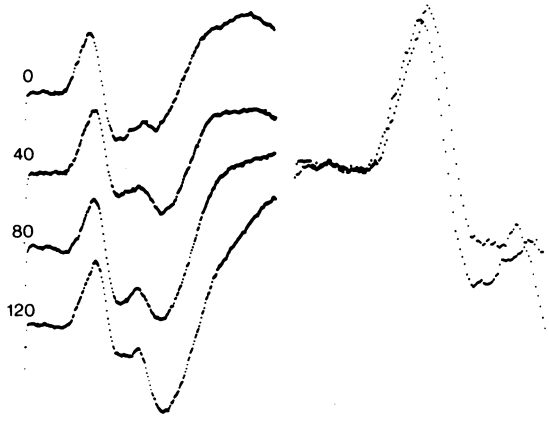


**Figure 3** A series of averaged cortical evoked responses ($n$ = 100, sweep = 20 ms left, 10 ms right, stimulus to the right forepaw, rate 1/s) at 40 min intervals (left) with the animal at 1 ATA breathing room air. The averages on the right on an expanded scale are the starting and finishing responses and show only small variations in latencies and amplitudes of the initial positive and negative waves.

Figure 4b). This was found to increase steadily with pressure but in a non-linear fashion.

(c) *On the behavioural experiments* In this series of rats the initial dose of urethane required to maintain a moderate abdominal twitch in response to stimulating the tail was 1.31 g kg (±0.2). Increasing ambient pressure led to an increase in the amount of urethane required, and results are shown in Figure 5. As with the effect of pressure on the evoked cortical response,

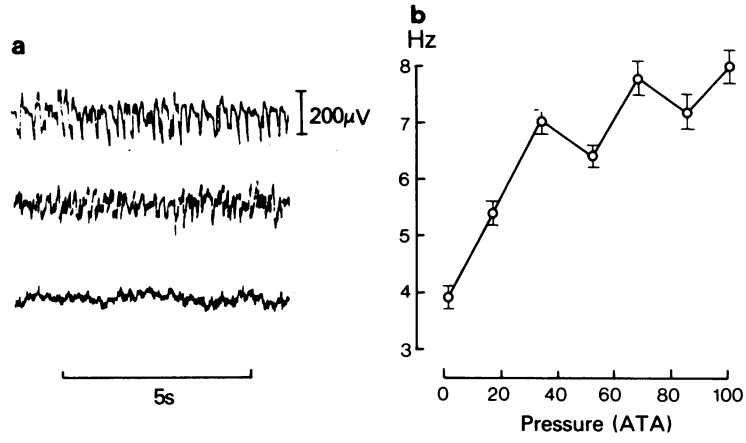


**Figure 4** This shows (a) the spontaneous electrocortical activity at pressures of 1.4 ATA, 17 ATA and 100 ATA, from top to bottom respectively. The graph (b) shows the geometric mean frequency (± s.e. mean) versus pressure.

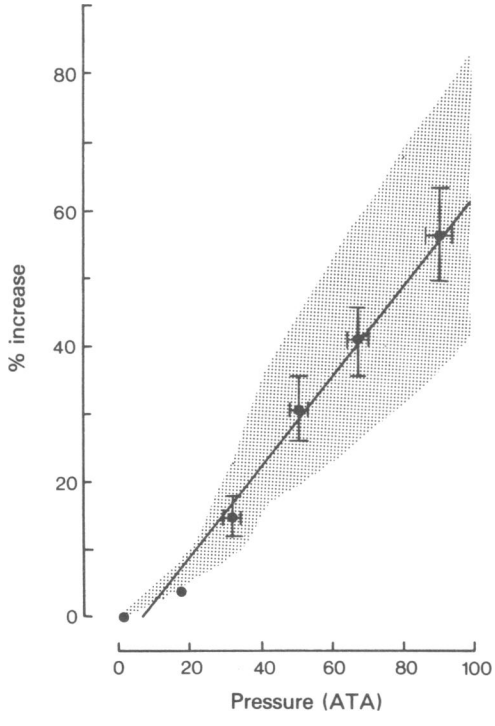


**Figure 5** The mean percentage increase in anaesthetic dose necessary to prevent an increase in response to a 10 V tail stimulus (ordinate scale) versus pressure (abscissa scale). Each point represents the mean of 6 experiments, vertical lines show s.e. mean. For this graph the mean increases at mean pressures were calculated by pooling the results into 20 ATA pressure groups. The shading represents the absolute scatter of the results and the line is the calculated regression line for the data ($y = 0.669x - 4.595$, coefficient of linear regression = 0.993; $P < 0.001$).

the relationship between pressure and required anaesthetic dose could best be described as a linear monotonic function with a slope which varied between 0.014 to 0.020% increase in dose, for each atmosphere increase in pressure (mean 0.17% increase per ATA ± 0.001).

## Discussion

The cerebral evoked response to electrical stimulation at the wrist, in the anaesthetized rat, can be considered as the sum of the electrical responses of a varied population of cortical elements. For the initial components of the cortical response these elements can be tentatively identified. For example, the amplitude of the first positive wave of the cortical response has been shown to be proportional to the discharge of thalamo-cortical relay cells and hence can be considered as a rough estimate of the size of the thalamo-cortical afferent volley. Since this part of the cortical response occurs before any cortical cellular activity can be detected with conventional recording techniques, it represents either the activity in incoming nerve fibres or the post-synaptic activity of dendrites coursing through, or cell somata located mainly in cortical layer IV. The amplitude of the first negative wave, on the other hand, is proportional to the discharge of cortical cells within the latency span of this wave (Angel, 1977). Thus a possible interpretation of the effects of increasing the anaesthetic depth with ethyl carbamate is that (a) it slows the transmission of the sensory volley from the periphery to the cerebral cortex (as evidenced by the increase in latency); (b) it decreases the size, or dispersion, of the thalamo-cortical volley and (c) it decreases the effectiveness of this volley in firing cortical cells (these latter two

points shown by the decrease in amplitudes of both the initial positive and negative waves of the cortical responses); with the apparent decrease in cortical cellular discharge (initial negative wave) decreasing at a steeper rate, with increasing anaesthetic depth, than the decrease in thalamocortical volley (initial positive wave: see Figure 1b). Under the conditions reported in this paper the effect of increasing the ambient pressure shows the exact reverse, that is, it restores the ability of the afferent volley to gain access to the cerebral cortex, both in time and in magnitude, and apparently restores the initial cortical cellular discharge to this corticopetal input.

The change in amplitude of the initial negative wave of the cortical response at a pressure of 100 ATA, i.e. a mean increase in size of 4.77 times, can be regarded as representing a shift in anaesthetic depth from 2.3 g/kg to 1.32 g/kg (from 19.5% to 93% in Figure 1b). Assuming a linear function relating the size of the initial negative wave of the cortical response to pressure, then this represents an 'apparent' removal of 9.80 mg of urethane for each atmosphere increase in pressure. A similar figure can be obtained from Figure 5 relating the increase in anaesthetic dose necessary to maintain the observed response to a stimulus at a constant level. With a mean increase in required dose of 65%, and again assuming a linear relation, this gives an apparent removal of 8.45 mg urethane for each atmosphere pressure increased. That these results represent an 'apparent' removal rather than an absolute (metabolic) removal, can be seen from Figures 2b and 3. A further indication of the apparent decrease in anaesthetic depth with increasing pressure is seen in the changes which occur in the spontaneous electrocortical activity. This activity changes from a high voltage: low frequency pattern to one of low voltage: high frequency as pressure is increased (see Figure 4a). That the effect of pressure cannot be explained by postulating an increase in activity of the enzyme system for anaesthetic degradation resulting in an increased metabolic removal of the anaesthetic, is indicated by the two experiments in which the pressure was decreased from 100 ATA to 68 ATA. In both experiments the latency and size of the initial negative waves at 68 ATA were not statistically significantly different if the responses were compared with increasing or decreasing pressure. If metabolic removal of the anaesthetic were increased by increasing pressure then a decrease in pressure should not result in an apparent increase in anaesthetic depth to the same level.

The importance of determining whether a particular effect of an anaesthetic is pressure reversible is two fold. Firstly, the initial observation that narcotized newts regain their normal motility at pressure (Johnson & Flagler, 1950; Lever *et al.*, 1971) and the subsequent demonstration that high pressure reverses the effects of anaesthetic chemicals, measured behaviourally, in mammals (Halsey, Wardley-Smith & Green, 1978) shows that the effect of the anaesthetic agent on the central nervous motor system is reversed and implies some form of sensory restoration. Taking the term anaesthesia in its literal connotation, i.e. a loss of sensation, the experiments reported in the present work show: (i) that anaesthesia induced with ethyl carbamate impedes transmission of sensory information in the tactile system to the cerebral cortex; (ii) that the cortical response to any information reaching it is decreased during anaesthesia and, if one equates cortical responsiveness with aesthetic interpretation, a decrease in sensory experience and (iii) that tactile sensation lost during anaesthesia at normobaric pressures is reversed on increasing the ambient pressure. Thus, high ambient pressures not only restore the motor responsiveness of the central nervous system, they also appear to restore its sensory responsiveness as well. Secondly, it might be concluded that the effect of ethyl carbamate on the somatosensory pathway, by virtue of its pressure reversibility, is a better system for studying the actions of anaesthetics on nervous tissue than the peripheral ganglia in which the effects of anaesthetics cannot be reversed by pressure (Kendig, Trudell & Cohen, 1975).

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## References

ANGEL, A. (1977). Processing of Sensory Information. *Progr. Neurobiol.*, **9**, 1–122.

ANGEL, A., BERRIDGE, D.A. & UNWIN, J. (1973). The effect of anaesthetic agents on primary cortical evoked responses. *Br. J. Anaesth.*, **45**, 824–836.

ANGEL, A. & UNWIN, J. (1969). The effect of anaesthesia on somatosensory cortex of the rat. *J. Physiol.*, **205**, 25P.

HALSEY, M.J., EGER, E.I., KENT, D.W. & WARNE, P.J. (1975). High pressure studies of anaesthesia. *Progr. Anaesthesiol.*, **1**, 353–361.

HALSEY, M.J., WARDLEY-SMITH, N. & GREEN, C.J. (1978). Pressure reversal of general anaesthesia—a multi-site expansion hypothesis. *Br. J. Anaesth.*, **50**, 1091–1097.

JOHNSON, F.H. & FLAGLER, E.A. (1950). Hydrostatic pressure reversal of narcosis in tadpoles. *Science*, **112**, 91–92.

KENDIG, J.J., TRUDELL, J.R. & COHEN, E.N. (1975). Effects of pressure and anaesthetics on conduction and synaptic transmission. *J. Pharmac. exp. Ther.*, **195**, 216–224.

LEVER, M.J., MILLER, K.W., PATON, W.D.M. & SMITH, E.B.

(1971). Pressure reversal of anaesthesia. *Nature*, **231**, 368–371.

MILLER, K.W. (1975). Pressure reversal of anaesthesia and the critical volume hypothesis. *Progr. Anaesthesiol.*, **1**, 341–351.

MILLER, K.W., PATON, W.D.M., SMITH, R.A. & SMITH, E.B. (1973). The pressure reversal of anaesthesia and the critical volume hypothesis. *Mol. Pharmac.*, **9**, 131–143.

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