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# Isomers of Long-Chain Alkane Derivatives and Nervous Impulse Blockage

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**Summary.** The potency to block nervous impulse of members of normal aliphatic homologous series of primary and secondary isomers of functional derivatives of alkanes was tested in bundles of a few axons from sciatic nerves of the toad *Bufo marinus*. For the primary substituted functional derivates of pentane, the relative potency series was I > H ≈ Br > Cl > $COOCH_3$ > F > $CH_2OH$ > $COCH_3$ > OH ≈ $NH_2$ > COOH. For the homologous series of primary alkanols, and from saturated Ringer's solutions, the time required to reversibly reduce the amplitude of the action potential to one-half its initial value were determined. The cut-off effect was detected at the level of dodecan-1-ol, while for the primary bromoalkanes it was bromooctane. However, solutions of the secondary isomer of the inactive primary homologues, such as tridecan-5-ol and tridecan-7-ol or 2-bromononane, were able to block nervous impulse conduction reversibly. From the concentration required for an equipotent effect it was calculated that the standard free energy for adsorption of primary alkanols was −705 cal mol⁻ $CH_2$. Similarly, for primary bromoalkanes a value of −733 cal $mol^{-1}$ $CH_2$ was obtained. The concentration required for an equipotent effect for primary isomer (either of alkanols or bromoalkanes) is lower than those obtained for the secondary isomers. Therefore, the latter are less potent than the former. Among secondary isomers the potency decreases as the functional group is moved away from the terminal carbon. The differential effect of structural isomers of long-chain alkane derivates around the point of cut-off cannot be explained in terms of differences in chemical properties, concentration in aqueous and membrane phases, or mean molecular volume. It is concluded that a volume related to that of the hydrophobic region of the agent and not its mean molecular volume should be responsible for an expansion of the target region.

**Key Words** alcohols · isomerism · nervous block · anaesthesia · cut-off effect · bromoalkanes

## Introduction

Volume expansion hypotheses of general anaesthesia revolve around the notion that the phenomenon occurs when a region of the cell membrane, which somehow modulates the excitability function, is caused to enlarge beyond a critical volume by the adsorption of an inert substance (Mullins, 1954; Miller et al., 1973; Janoff & Miller, 1983). This region has been thought to be nonpolar and its location and nature, either lipid or protein, is the subject of much research (Franks & Lieb, 1978, 1981, 1982; Janoff, et al., 1981). It appears to be a phase less hydrophobic than hexadecane, resembling more *n*-octanol. The effect caused by the adsorption of the inert chemical agent has been argued to occur either locally, at the level of the lipoprotein complex responsible for the gated flow of ions or, alternatively and less specifically, along the whole cell membrane (Seeman, 1972; Haydon et al., 1977*a,b*).

One of the intriguing puzzles in this context is the cut-off effect. This effect, better found in homologous series of hydrocarbon derivatives, manifests itself as the inability of higher members of a series to act as anaesthetic agents. Thus, in alkanols, a classical example, a systematic increase in potency is observed from methanol up to dodecanol while saturated solutions of tridecanol are inactive for exposure periods comparable to the survival time of experimental preparations (Meyer & Hemmi, 1935; Seeman et al., 1971). The simplest explanation for this effect has relied on the assumption, mainly through steric considerations, that the molecular volume of the inactive homologues is such that the few molecules absorbed into the target region can not produce the critical expansion. Recently, a phenomenological explanation for the cut-off has been advanced. Pringle et al. (1981) maintained that the maximum achievable concentration of tridecan-1-ol in the membrane phase is just below the 20 to 40 mM required level, whereas for tetradecan-1-ol it is one order of magnitude smaller. Although for the lower members of the homologous series, the observed reduction in solubility with chain-length extension is almost compensated by a proportional increase in the partition coefficient, in such a way that their product, the concentration in

the membrane phase, is nearly constant; for the higher homologues it has been argued that this is not so (Janoff et al., 1981). This later point is based in the sudden fall detected for the partition coefficient of the homologues higher than dodecanol (Salle, 1978).

A test of the validity of the cut-off effect could be done with the aid of structural isomers. Since they have very similar chemical and physical properties and more specifically, an almost identical mean molecular volume, they should have nearly equal potencies in blocking nerve impulse, i.e. the cut-off point should depend upon the number of aliphatic carbons in the chain, but not on the isomeric arrangement. In order to explore this possibility, axons from sciatic nerves were exposed to Ringer's solution containing structural isomers of members of homologous series of alkane derivatives in which one hydrogen was substituted by a functional group. The selection of the nature of the functional group derivative, was based on an analysis of the relative potency observed for various functional groups. Alkanols (backed up by bromoalkanes) were the obvious choice owing to their significant solubility in Ringer's and the easy manipulation of their isomeric arrangement. As an indicator of anesthetic action either the time or the concentration required for an equipotent effect on the extracellularly recorded compound action potential was chosen. The experimental results show that as a result of structural isomerism, the point of cut-off shifts to higher homologues in secondary isomers.

## Materials and Methods

### Materials

Alcohols were either purchased commercially from a variety of sources or, when necessary, synthetized. The catalytic reduction of the parent ketone over Li-Al hydryde or a Grignard based on aldehydes and bromoalkanes, followed by distillation, were the synthesis methods employed. 1-fluoropentane was synthethized by an exchange reaction at 145°C for several hours of bromopentane with finely ground and dried KF in anhydrous ethylenegly-col (Vogel, 1959). The methyl ester of caproic acid was produced by the methanolysis of the hexanoyl chloride. The main test for purity was gas-liquid-chromatography (GLC). In some samples, however, nuclear magnetic resonance and/or mass spectra were obtained to verify the nature of the synthethized compound. Great care was taken to use properly desiccated solvent. Chemical reagents were always of the highest purity available. Bromoalkanes, iodopentane, methylketone-pentane and alkanes in general were purchased commercially and purified by distillation if proven to be less than 99%. The water was single distilled in an all-glass apparatus. The amphibian Ringer's contained (in mM): 102 NaCl, 2.1 $MgCl_2$, 2.7 KCl, 1.9 $CaCl_2$, 2.0 $NaHCO_3$ and 0.36 $NaH_2PO_4$. Ringer's solution was adjusted to pH 7.4 and osmolarity of 210 mOsm. Solutions of a given concentration were either prepared directly or by dilution from saturated stock solution.

### Sciatic Nerve Measurements

The experiments were carried out in sciatic nerves from *Bufo marinus*. Young specimens, of good size and weight (*ca.* 130 g) of this local tropical species, were collected nearby (Camatagua reservoir, Aragua), kept indoors in a wet tank and used within 2 or 3 weeks of their capture. The freshly excised nerves were desheathed and a bundle of a few axons from the distal end of the peroneal branch was carefully dissected up to the proximal end near the spine. Excess nerve material from both ends was cut out before positioning the nerve in the experimental chamber. A length of the dissected nerve of about 4 cm was tied up at both extremes and mounted in a slot (0.5 cm/side) milled on a Perspex® bar. The bundle was made to rest by gravity on five equally spaced Pt wires. The slot was perfused from the bottom, via Teflon® and glass lines, with Ringer's solution delivered at a rate of 3 to 5 ml/min from a constant pressure reservoir. Flow was regulated with a Teflon needle valve. A suction line on top of the slot removed the wasted solution. Great care was exercised to avoid the presence of any grease near the Ringer's solutions. Given the dead volume of the slot and the rate of flow, the solution in the experimental chamber was exchanged 3 or 4 times per minute. Control experiments with tetrodotoxin containing Ringer's or Na-free Ringer's showed that within 90 sec of application of either of those Ringer's solutions, the compound action potential vanished. A variety of precautions were taken to preclude the evaporation of the more volatile agents from the experimental chamber.

The nerve was stimulated throughout the experiment at given intervals with pulses of constant amplitude and duration and of supra-maximal intensity. The elicited compound action potential (CAP) was recorded on a storage oscilloscope after being differentially amplified by two amplifiers in cascade. Once the signals of steady and stable amplitude were recorded, usually within a period of about 0.5 hr, the experiment was begun by switching to a Ringer's solution containing the test molecule. Under the conditions outlined above, a dissected nerve was usually able to maintain elicited compound action potentials, with amplitude equal to that observed at the onset of the experiment, for as long as 7200 min (3.2 days). In some cases, especially in those nerves treated with saturated Ringer's solutions of very long-chain molecules (such as tetradecanol and pentadecanol) some 8000 min elapsed without any clear sign of deterioration. The temperature in all experiments was kept at 18°C (±1°C).

### Distillation Purity and Solubility Determinations

Compounds were subjected to fractional distillation in an all-glass apparatus under reduced pressure. The temperature and the pressure in the system were adjusted such that the former parameter was kept constant and within the range 60 to 100°C (depending on the boiling point of the compound), while the latter was adjusted to a value below 10 mm Hg. This combination of conditions ensured a minimum of thermal breakdown. Vigreaux type columns of 30 to 40 cm in length were used. Normally the first two fractions were discarded. Pure fractions were kept in dark glass bottles.

**Table.** Density, mean molecular volume, solubiity, partition coefficient and nerve half block parameters for structural isomers of alkanols

| Isomer | Density $\left(\frac{20°C}{4°C}\right)$ (g/ml) | Mean molecular volume (ml/mol) | Ringer's solubility ± SEM (mM) | Partition coefficient | Nerve half block parameters | |
|---|---|---|---|---|---|---|
| | | | | | Concentration (mM) | Time ± SEM (min) |
| Propan-1-ol | 0.80350[a] | 74.81 | — | 1.78[f] | 190.0 | — |
| Propan-2-ol | 0.78350[a] | 76.71 | — | 1.12[f] | 400.0 | — |
| Butan-1-ol | 0.80980 | 91.53 | — | — | 60.0 | — |
| Pentan-1-ol | 0.81507 | 108.14 | 177.0 ± 0.7 | 2.1[e]; 36[f] | 19.0 | — |
| Pentan-3-ol | 0.82032 | 107.45 | — | 1.6[e]; 16[f] | 51.0 | — |
| Hexan-1-ol | 0.81871 | 124.81 | 47.0 ± 0.2 | 50[d] | 5.5 | — |
| Hexan-3-ol | 0.81958 | 124.67 | — | — | 9.0 | — |
| Heptan-1-ol | 0.82561 | 140.74 | 13.5 ± 0.12 | 170[d] | 1.8 | 1.5 ± 0.02 |
| Heptan-4-ol | 0.82435 | 140.96 | — | — | 4.9 | — |
| Octan-1-ol | 0.82703 | 157.46 | 3.4 ± 0.10 | 378[d] | 0.5 | 3.7 ± 0.05 |
| Octan-3-ol | 0.82505 | 157.84 | 8.8 ± 0.16 | 162[d] | 1.9 | 2.4 ± 0.08 |
| Nonan-1-ol | 0.82790 | 174.24 | 1.1 ± 0.06 | 1400[d] | 0.14 | 17.0 ± 0.73 |
| Nonan-2-ol | 0.82704 | 174.43 | — | — | 0.40 | — |
| Nonan-3-ol | 0.82625 | 174.59 | — | — | 0.45 | 7.2 ± 0.44 |
| Nonan-5-ol | 0.82267 | 175.35 | 2.0 ± 0.08 | — | 0.55 | 2.6 ± 0.11 |
| Decan-1-ol | 0.83025 | 190.65 | 0.254 ± 0.008 | 4500[d] | 0.05 | 69 ± 1.5 |
| Decan-5-ol | 0.82465 | 191.94 | 0.520 ± 0.02 | — | 0.10 | 11 ± 0.35 |
| Undecan-1-ol | 0.83168 | 207.18 | 0.063 ± 0.009 | 12000[d] | — | 321 ± 7 |
| Undecan-3-ol | 0.82863 | 207.95 | — | — | — | 40 ± 3 |
| Undecan-5-ol | 0.82730 | 208.28 | 0.155 ± 0.002 | — | — | 42 ± 5 |
| Dodecan-1-ol | 0.83204[b] | 223.93 | 0.010 ± 0.001 | 40000[d] | — | 2200 ± 160 |
| Dodecan-3-ol | 0.82679[b] | 225.35 | — | — | — | 235 ± 25 |
| Dodecan-5-ol | 0.82468[b] | 225.93 | 0.036 ± 0.002 | — | — | 208 ± 19 |
| Tridecan-1-ol | 0.82479[c] | 242.93 | 0.0025 ± 0.0001 | — | — | no effect |
| Tridecan-5-ol | 0.81971[c] | 244.44 | 0.0089 ± 0.0003 | — | — | 718 ± 87 |
| Tridecan-7-ol | solid | — | 0.0028 ± 0.0002 | — | — | 1400 |
| Tetradecan-1-ol | solid | — | 0.00049 ± 0.00001 | — | — | inactive |
| Tetradecan-5-ol | solid | — | — | — | — | inactive |
| Tetradecan-8-ol | solid | — | — | — | — | inactive |

[a] Weast, 1983.
[b] $\frac{25°C}{4°C}$
[c] $\frac{35°C}{4°C}$
[d] Data from Jain et al. (1978); lecithin bilayer/$H_2O$.
[e] Data from Leo et al. (1971); oil/$H_2O$.
[f] Hansch (*personal communication*); octanol/$H_2O$.

Purity was determined by gas-liquid chromatography (GLC) using a VARIAN 1440 unit equipped with a flame ionization detector, stainless steel columns ($\frac{1}{8}''$ OD) and a brass injector port. The output of the electrometer was fed into a Hewlett-Packard Integrator model 3390A. GLC columns (6 feet long) were packed with chromosorb W (80 to 100 mesh) as support and either SE-30 (3%) or FFAP (5 to 10%) as stationary phase. Most of the time both columns were joined to improve separation and reproducibility. Flow ratios of $N_2/H_2$/air were 1 : 1 : 8, respectively. Column temperature was usually under program control from 50 to 200°C at 5 to 8°C $min^{-1}$. The injection port was kept between 170 and 190°C and the detector port at 250°C. Samples of 2 to 5 $\mu l$ were injected through Teflon-lined septa. Great care was taken to purge clean the column before each run.

Purity of the compounds was determined using the area under the curve criteria. The overall sensitivity of the set-up was such that 0.1% impurities could be easily detected. Solubilities were determined using the "internal standard" approach. For this purpose an inert substance with a low boiling point was selected (*n*-butanol usually, otherwise ethanol). Solutions of known concentration of the standard and the test molecule were prepared and a response factor for the instrument obtained by comparing the areas under the curves obtained for the respective peaks. To the unknown solution, known concentration of the internal standard was added and from the response factor, the concentration of the unknown solution was calculated.

For those substances, whose concentration was too small to be determined directly by GLC, extraction from a large volume of Ringer's was employed. A small volume (5 to 20 ml) either of hexane or dichloromethane were equilibrated with 2 to 6 liters of Ringer's solution previously filtered through a Millipore filter type GS with 0.22 $\mu m$ pore size. After separation, the hexane samples were concentrated in a Kuderna-Danish type of evaporative-concentrator fitted with a Snyder type of column.

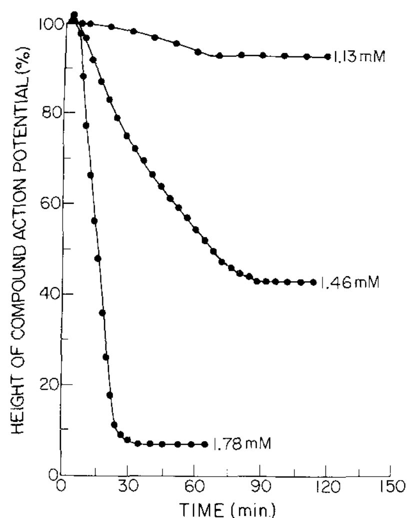


**Fig. 1.** Time course of the height of the compound action potential (on a percent basis) is plotted as a function of three concentrations of bromobutane in Ringer's solution (three different experiments). The interpolated concentration which induced a steady reduction of the compound action potential to half of its initial value was 1.4 mM ($C_{50}$)

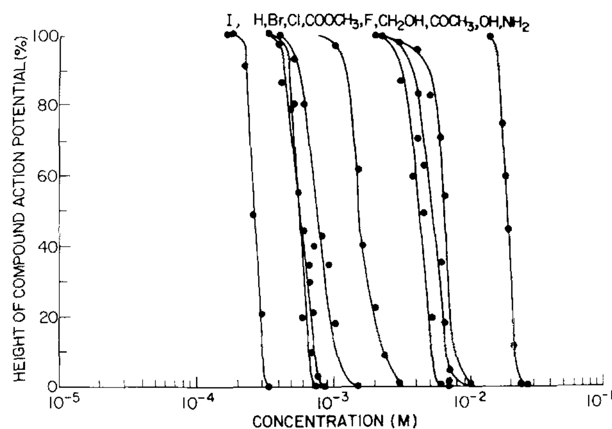


**Fig. 2.** Dose-response curves for the primary functional derivative isomers of pentane. The height of the compound action potential (on a percent basis) is plotted as a function of the concentration (molar) in Ringer's solution of the various molecules tested. The functional group indicated on top of each curve was substituted in carbon 1. The results for pentan-1-ol and 1-pentylamine are indistinguishable from each other. Only nine curves are shown

From the concentrated solution, samples were taken to be analyzed by the internal standard routine in GLC.

### Mean Molecular Volume

This parameter was determined experimentally as the reciprocal of the density of the dried pure liquid. The density measurement was based on an oscillation principle as employed in a precision density meter (Anton Paar model DMA 60-602), a generous loan from Dr. L. Mateu of IVIC, Caracas. The experimental cell was equilibrated at 20 ± 0.01°C (unless otherwise stated). Densities were determined as the average of five or more measurements. The measured values were corrected for the local air density and expressed in the more usual form, i.e. densities referred to water at 4°C. Since density data were reproducible to $1 \times 10^{-5}$ g and the instrument sensitivity was $1 \times 10^{-6}$ g cm$^{-3}$ no error is included in the Table, where the values for the density and the calculated mean molecular volume of the pure liquids are listed. The reported densities are within 0.1% of the values currently listed in the Handbook of Chemistry and Physics (Weast, 1983), except for *n*-hexanol and *n*-heptanol: their densities are 0.5% higher than the values reported previously.

## Results

### Potency of Functional Isomers

Dose-response curves permit the comparison of the relative potencies of a series of related molecules. Figure 1 shows, as a function of time, typical dose-response experiments in our preparation. It can be observed that millimolar concentration of 1-bromobutane in Ringer's causes nerve block conduction. For a given value of concentration there is reduction in the height of the compound action potential until a steady level is reached. The correlation of the concentration of the agent with the induced steady level of supression of the compound action potential permits the construction of dose-response curves. These can be characterized by an equipotent parameter such as the concentration required for a 50% block.

The relative potency of various functional groups was tested using molecules which shared a common hydrophobic residue; an aliphatic five carbon saturated chain with a functional group inserted into a primary carbon. The results obtained for the dose-response curves are shown in Fig. 2. For the functional derivatives the range of concentration required to induce an equipotent effect spanned four orders of magnitude, as judged by the concentration required by the most and least potent agents, iodopentane and valeric acid. The relative potency series found was: $I > H \approx Br > Cl > COOCH_3 > F > CH_2OH > COCH_3 > OH \approx NH_2 \gg COOH$. The curve for valeric acid is not drawn, since, for the salt form, even at concentrations bigger than 0.05 M, there was only a minor partial block.

For the halogen family it should be noticed that the potency series follows their crystal ionic radii and degree of hydrophobicity. For the carboxylic acid related group it is clear as well that the more hydrophobic the substitute, the higher the potency

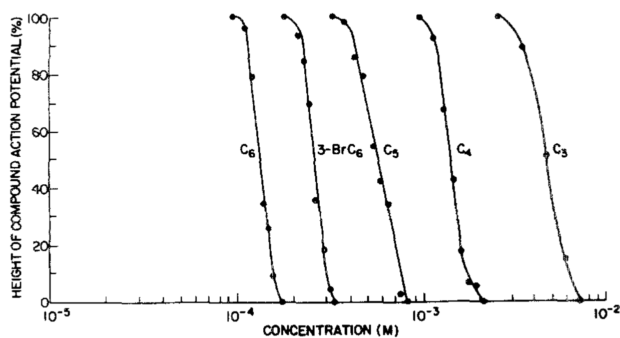


**Fig. 3.** Dose-response curves for various bromoalkanes. The height of the compound action potential (on a percent basis) is plotted as a function of the concentration (moles/liter) in Ringer's solution. With the exception of the curve labeled 3 BrC which corresponds to the secondary isomers substituted in carbon 3, the others correspond to primary bromoalkanes. The codes associated with each curve represent the number of carbon atoms in each homologue

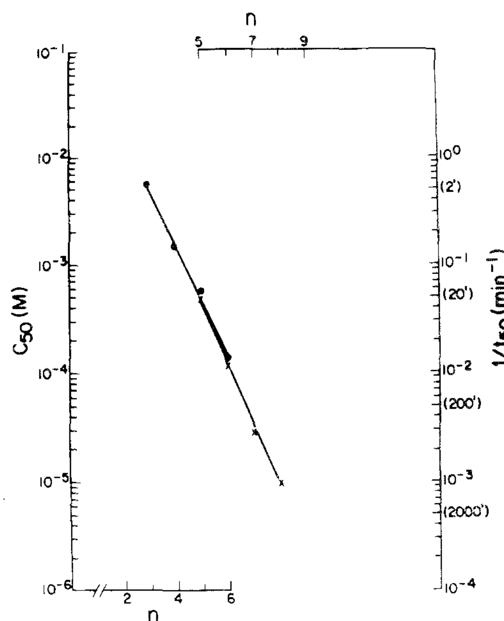


**Fig. 4.** Concentration of bromoalkanes in Ringer's (logarithmic scale) ($C_{50}$, circles), or the reciprocal of the time required (logarithmic scale) ($1/t_{50}$, crosses) by a saturated Ringer's solution to reversibly block the extracellularly recorded compound action potential to half of its initial value, are plotted as a function of the number of carbons in the chain. The experimental points (mean ± SEM of at least $n = 5$) were joined by a least-squares fitted regression line. The corrected slope of the lines represent the standard free energy of transfer per methylene group as stated in the text

observed. A similar trend was observed for very hydrophobic functional group residues, such as methyl and phenyl. However, their extreme high potency precluded the acquisition of dose-response curves. These were difficult to obtain, mainly because it took too long to reach a 50% reduction in action potential amplitude from a nonsaturated Ringer's. Moreover, give the vapor pressure of hexane and phenyl pentane, the maintenance of a given concentration level in solution over a long period of time is a very difficult experimental condition to meet. As a rule, it takes about twenty times longer for a solution containing the half-response concentration (as compared to the saturated Ringer's) to induce an equipotent effect. Nevertheless, from the time required from a saturated solution of hexane or phenylpentane to induce an equipotent effect, and which were about 206 and 385 min, respectively, and from a comparison with time required by iodopentane (62 min), it can be argued that the potency series should start with $C_6H_5 > CH_3$ followed by I.

## Concentration Required for an Equipotent Effect

Away from the point of cut-off it is feasible to look for the concentration in Ringer's required by various members of a homologous series to induce an equipotent effect. Figure 3 summarizes the dose-response curves for the primary bromoalkanes in the range of 3 to 6 carbons in the aliphatic chain (plus the secondary isomer 3-bromohexane). From the values of the concentration required to reduce the amplitude of the compound action potential to half of its initial value, and using the argument developed by Haydon and Urban (1983), a value of −733 cal $mol^{-1}$ can be calculated for the standard free energy of adsorption per methylene residue of this homologous series. The linear relationship between the log of the concentration and the number of methylene residues in the chain is graphically shown in Fig. 4.

From the concentration required to induce an equipotent level of impulse conduction block of homologues of the primary alkanol series (from propanol to decanol), a value for the free energy of adsorption of −705 cal $mol^{-1}$ $CH_2$ can be computed. For the secondary isomers, with the hydroxyl group inserted in the middle of the odd-number of carbons chain, a value of −657 cal $mol^{-1}$ $CH_2$ was found from propan-2-ol to nonan-5-ol. The relationship between the concentration required for half block and the number of carbons in the chain for this homologous series of secondary isomers is shown in Fig. 5. These standard free energies of transfer for methylene groups of alkanols are similar among themselves and comparable to others reported in the literature (Haydon & Urban, 1983). The values for the Ringer's concentration required to induce an equipotent effect for the alkanols are listed in the

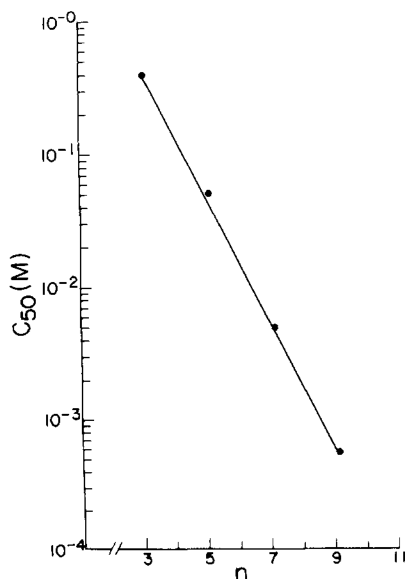


**Fig. 5.** Concentration of secondary isomers of alkanols in Ringer's (logarithmic scale) required to induce an equipotent effect is plotted as a function of the number of carbons in the chain. The hydroxyl group was substituted in the middle of the odd-number of carbons chain. Points were joined by the least-squares fitted regression line

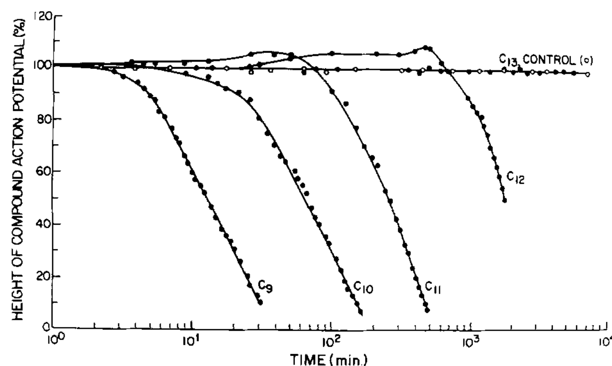


**Fig. 6.** Time course (logarithmic scale) of the height of the compound action potential (on a percent basis) is plotted as a function of various members of the normal primary aliphatic homologue series of alcohols saturated in Ringer's solution (●). Each code represents the homologue containing the indicated number of carbon atoms. The control curve is for Ringer's solution (○). For each homologue a number of experiments (2 to 8) were carried out and only a typical result is plotted for each alcohol

Table. Finally, for the iodoalkanes, in the range from 1-iodopropane to 1-iodopentane, a free energy of adsorption per $CH_2$ of $-857$ cal $mol^{-1}$ was estimated.

## Time Required for an Equipotent Effect

In order to study the behavior of agents and nerves around the point of cut-off, inevitably a kinetic type of experiment has to be done in which a time parameter for an equipotent block is sought, while referring the Ringer's solution to the saturated state. Under these conditions, the reciprocal of the time required to reach a given degree of nervous conduction block can be considered to represent a rate constant for the effect. Figure 6 shows the time dependency for the supression of the compound action potential for Ringer's solution saturated with members of the normal primary aliphatic homologous series of alkanols. In the curved labeled control experiment, it is observed that the amplitude of the action potential remained stable (and equal to its initial value) for a period of several thousand min. In a similar lapse and for tridecan-1-ol, no reduction in this magnitude was observable. However, dodecan-1-ol, induced in about 2200 min a reduction of the compound action potential to half of its initial value. These data lead to the conclusion, in agreement with other authors and experimental preparations (Richards et al., 1978; Pringle et al., 1981) that dodecan-1-ol, is the last active member of this homologous series. It should be mentioned that an extrapolation of the linear dependence of the log of the time for the equipotent effect on the number of carbons in the chain, predicts that tridecan-1-ol should have been active well within the survival time of control experiments.

Figure 7 shows similar data but for nerves exposed to Ringer's solution saturated with members of the normal secondary (substituted in carbon 5) homologous series of aliphatic alcohols. It is observed that tridecan-5-ol is capable of reducing the action potential height to one-half its initial value with a time constant of 718 min, while dodecan-5-ol induced an equipotent effect in about 208 min. However, the effect, if any, of tetradecan-5-ol was not distinguishable from the control experiment. In this figure, it is explicitly shown that the effects described above are reversible. Indeed, if tridecan-5-ol is removed from the Ringer's at minute 700 (when the action potential was 60% blocked) the nerve almost totally recovers (97%) by minute 2000.

For Ringer's solution saturated with members of the normal secondary homologous series of aliphatic alcohols, in which the hydroxyl group is located in the middle of an odd-number carbon chain, it was found that tridecan-7-ol reduced the height of the compound action potential in about 1400 min, while pentadecan-8-ol failed to produce an equipotent effect within the time lapse established for control experiments. These results are shown in Fig. 8. It should be noticed (*see* Table) that both, nonan-5-ol and undecan-6-ol, induced an equipotent effect more rapidly than the parent primary isomers.

For the bromoalkanes it has been determined

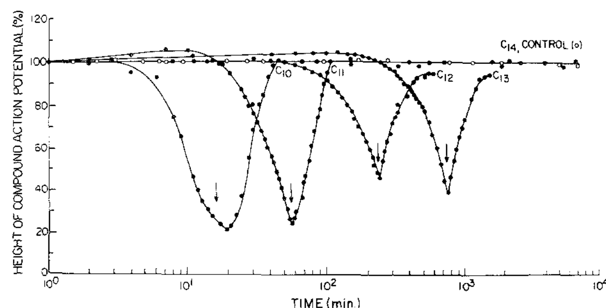


**Fig. 7.** Time course (logarithmic scale) of the height of the compound action potential (on a percent basis) is plotted as a function of various members of the normal secondary (substituted at carbon 5) homologous series of aliphatic alcohols. Each code represents the homologue containing the indicated number of carbon atoms. The control curve is for Ringer's solution (O), while the other curves are for Ringer's solution saturated with the test molecule (●). Arrows mark the time when the Ringer's solution containing the alcohol was switched to Ringer's in order to test the reversibility of the phenomenon

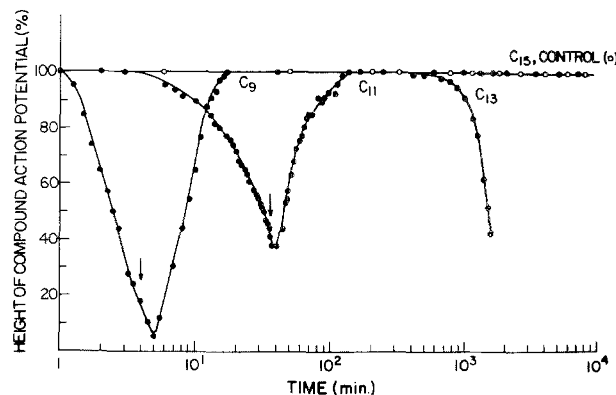


**Fig. 8.** Time course (logarithmic scale) of the height of the compound action potential (on a percent basis) is plotted as a function of various members of the odd-number of carbons secondary aliphatic homologous series of alcohols substituted in the medial carbon. Each code represents the number of carbons in the chain. The control curve is for Ringer's solution (O), while the other curves are for Ringer's solution saturated with the test molecule (●). Arrows mark the time when Ringer's solution containing the test molecule was switched to Ringer's in order to test the reversibility of the phenomenon

that Ringer's solution saturated with 1-bromononane showed no activity. The last active member of the normal primary aliphatic homologous series of bromoalkanes was determined to be 1-bromooctane which reduced to half the initial amplitude of the compound action potential in 1020 ± 56 min. The secondary structural isomer, 2-bromononane, however, was able to induce an equipotent effect in 3346 ± 127 min. The time dependency for the impulse conduction block for members of the homologous series of primary bromoalkanes is shown in Fig. 9.

A correlation of the log of the reciprocal of the time required for an equipotent effect (or the rate constant) on the number of methylene groups in the hydrocarbon chain should be linear. In this case, the slope, should yield the free energy of adsorption per $CH_2$ if the rate-limiting process were to be the adsorption of the inert agent into the target region. From the data in the Table, and for the primary alcohols, in the range of heptanol to dodecanol, a value of $-815$ cal $mol^{-1}$ is calculated for this free energy. For the primary bromoalkane homologous series this point is graphically illustrated in Fig. 4. In the right-hand side of this figure it is shown that if the log of the rate constant is plotted versus the number of carbons in the chain, the experimental points clearly fall on a straight line of slope $-712$ cal $mol^{-1}$ $CH_2$.

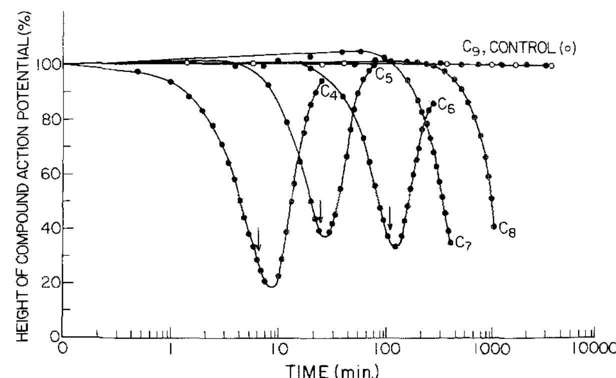


**Fig. 9.** Time course (logarithmic scale) of the height of the compound action potential (on a percent basis) is plotted as a function of various members of the normal primary aliphatic homologous series of bromoalkanes. Each code represents the homologue containing the indicated number of carbon atoms. The control curve is for Ringer's solution (O), while the other curves are for Ringer's solution saturated with the test molecule (●). Arrows mark the time when the Ringer's solution containing the test molecule was switched to Ringer's in order to test the reversibility of the phenomenon

## Potency of Structural Isomers

If one looks at the concentration required for the alkanols to induce a 50% reduction in the amplitude of the compound action potential (Table), it is observed that in all the cases studied, pentanol, heptanol, octanol and nonanol, the primary isomers are more potent than the secondary, since a smaller concentration of the primary isomer is required to induce an equipotent effect. Among the secondary isomers, those having the hydroxyl group nearer to the terminal carbon of the chain tend to be the most

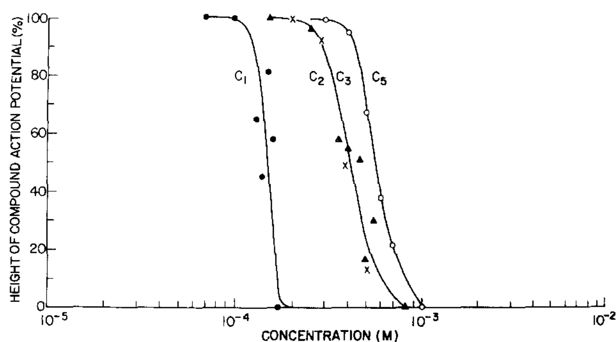


**Fig. 10.** Dose-response curves for nonanol structural isomers. The height of the compound action potential (on a percent basis) is plotted as a function of the concentration (molar) in Ringer's solution of the primary isomer (C1, ●) and the secondary isomers substituted in carbon 2 (C2, ×), carbon 3 (C3, ▲) and carbon 5 (C5, ○)

potent. This point is specifically illustrated in Fig. 10 where dose-response curves of the structural isomers for nonanol are shown. It should be noticed that the biggest part of the effect appears to occur in moving the hydroxyl from carbon 1 to carbon 2. For an equipotent condition, several-fold more molecules of nonan-2-ol are required than those of the primary isomer, while only 20% more molecules are required by nonan-5-ol when compared to nonan-3-ol. This observation is not a reflection of a particular property of the alkanols but more generally, is a constant feature of alkane derivatives. For bromoalkanes, while 260 μM of 3 bromohexane are required to reduce by half the compound action potential height, only 135 μM of the primary isomers of bromohexane are required to induce an equipotent effect. This point is graphically shown in Fig. 3.

From the time required to block the action potential from a Ringer's solution saturated with nonanol isomers, it can be concluded that secondary isomers act faster than the primary ones. Moreover, from the values of the time it takes to reach an equipotent effect among the secondary isomers, those having the functional group further away from the terminal carbon seem to be the fastest acting. If this kinetic data is analyzed as rate constants, that is, as the inverse of the time required for an equipotent effect, it turns out that, as in the concentration case, secondary isomers are less potent.

## Discussion

### Concentration of Isomers in the Membrane

The measurements summarized in Figs. 6–8 demonstrate that structural isomers of *n*-tridecanol have differential effects in blocking the conduction of the nervous impulse. If this fact is complemented with the similar observation for the isomers of bromononane, it would seem fair to conclude that structural isomers of functional derivatives of long-chain alkane molecules around the point of cut-off have a differential anesthetic potency: secondary isomers are active but the primary one is not.

An explanation for this differential effect of structural isomers around cut-off should be looked for in terms of concentration or of volume changes at the membrane phase and not in terms of the nature of the experiment. This last point, nevertheless, deserves some comment since the conclusion of a differential effect for isomers involved a kinetic measurement. It would appear at first sight that the conclusion reflects more the process leading to the interaction at the site of action than the interaction at the site itself. This, however, does not seem to be the case. As was shown, the standard free energies of adsorption per methylene group derived either from the kinetic approach or from the thermodynamically more rigorous instance, that based on the concentration required for an equipotent effect, were very similar for all isomeric homologous series tested, alkanols and bromoalkanes. Moreover, although it could be thought that the somewhat higher solubility of secondary over primary isomers (*see below*) might explain why it takes secondary isomers less time to reach an equipotent condition when compared with primary isomers, this argument does not seem to be conclusive since they showed a differential effect in blocking nerve conduction, even though the solubility of tridecan-7-ol was not very different from that of the primary isomer. Finally, it should be recalled that from the linear extrapolation of the time dependency for an equipotent effect on the number of methylene groups, tridecan-1-ol should have reduced the action potential amplitude to one-half the initial value well within 6000 to 7000 min, a period during which control nerves did not show signs of deterioration. This last point is even more striking for the bromoalkane series: saturated solutions of 1-bromononane should have acted well before 4000 to 5000 min and it did not, while the secondary isomer 2-bromononane did.

The concentration of a molecule in the membrane phase is given by its concentration in the Ringer's times its partition coefficient in that phase. Solubility and partition coefficient data relevant to this discussion are briefly summarized in the Table. Secondary isomers seem to be more soluble in Ringer's than primary isomers by a factor of 2 to 3. For a very long-chain molecule, and for those having the functional group very far away from the terminal

carbon, however, this factor is more likely, to be about 1 as shown with tridecanol. Although partition coefficient data for higher homologues in a membrane-like phase are not available, there are values for the isomers of lower alkanol homologues such as pentanol and octanol (*see* Table). The data indicate that the partition coefficients of secondary isomers are smaller than the value recorded for the primary isomer by a factor of about 2, the exact values apparently depending on the position of the hydroxyl group along the hydrocarbon chain (Hansch & Anderson, 1967; Leo et al., 1971; Jain et al., 1978; Jan & Wray, 1978). This finding seems to be also true for the octanol/$H_2O$ system. The partition coefficient of the primary isomer of propanol is larger than the value for the secondary isomer, or for other functional derivatives. The partition coefficient of butylamine in octanol is 9.3 while that of butyl-2-amine is 5.5 (Hansch, *personal communication. See also* Table). At a given chain length, thus, the gain produced in Ringer's solubility by displacing the hydroxyl group from a terminal carbon to any other would appear to be almost fully compensated by the loss in partition coefficient. It would seem fair then to conclude that the concentration of primary and secondary isomers in an excitable membrane is very similar, especially for those homologues near the point of cut-off.

The other point of interest is the mean molecular volume. From data shown in the Table, it can be concluded that in the pure state the change in this parameter due to isomerism is very small and of the order of 0.3 to 0.7%. Nevertheless, in view of the recent data of Mori et al. (1984), the possibility that structural isomers might have very different mean molecular volumes when dissolved in a phase needs to be evaluated. This effect, however, needs to be somewhat large and in the order of a few percents. It should be recalled that between sequential homologues of alkanols the change in mean molecular volume is about 9%. Any volume expansion caused by the adsorption of primary and secondary isomer would appear, thus, to be very similar since it is composed of the product of two terms which are not affected by isomerism, the concentration in the membrane phase and their mean molecular volume.

## Hydrophobic Volume Hypothesis

The question then is, how to reconcile the fact that isomeric molecules with very similar concentration and mean molecular volume in the membrane phase show such a marked difference in blocking nerve impulses. The supposedly weaker isomers—the secondary—are active whereas the more potent isomers—the primary—are not. This paradox could be resolved by assuming that the crucial parameter in the phenomenon is not the mean molecular volume of the anesthetic molecule but rather an apparent hydrophobic volume.

Since the hydroxyl group renders polar some of its neighboring methylene residues, if it is attached to a terminal carbon the number of affected methylene residues should be smaller than in a secondary isomer, where it will render polar a similar number of methylene residues but from the two hydrophobic chains attached to the secondary carbon. Thus, the volume of the hydrophobic region of a secondary isomer should be different and *smaller* than that of the corresponding primary isomer. This thesis is consistent with the observed potency of structural isomers: since secondary isomers show a smaller hydrophobic volume than the primary isomer, a few more molecules of the former will be required to induce an equipotent volume expansion effect. Around the point of cut-off, the close steric requirements could lead to a differential effect.

The effective hydrophobic volume for interaction (that to be accounted for in any expansion theory) could, in principle, be calculated from the mean molecular volume of the normal aliphatic alkanes by comparing the activities of homologues at a point of equipotent effect.

As long as the block of conduction in a peripheral nerve is supposed to reflect the phenomenon of general anesthesia, then the experiments reported herein confirm somewhat an expansion hypothesis inasmuch as it is found that there is a limiting finite volume fraction of the agent which, if incorporated into the cell membrane, perturbs its function. Indeed, among the most potent isomers tested, either saturated aliphatic alcohol or unsaturated aliphatic alcohol, there has been not one of 16 carbons in the chain which has been shown to have anesthetic activity in whole animals (Pringle & Miller, 1978) or to block conduction in isolated nerve preparations.

The effect described for structural isomers should be further investigated in iodo-substituted alkanes, basically because they are the most hydrophobic of all the functional derivatives. This is shown in Fig. 3 and in the free energy of adsorption data, and it is just a reflection of the major size of the iodine ion and its large electron density. For these compounds, the effect of structural isomerism might be more striking and even quantifiable with the assistance of more direct approaches such as the dynamic X-ray diffraction (Padron et al., 1980). Finally, the results presented herein clearly call for caution when analyzing the interaction of anesthetic agents with membranes inasmuch as the perturba-

tion introduced by the hydrophobic nature of the probe should be explicitly taken into account.

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