tion is less restrictive than that in the interior, and structural regularities (helixes, sheets, and bends) each prefer certain residues. This introduces indeterminacy since residue replacement rules are probably not identical in all subclasses. It is also conceivable that the link between amino acid properties and exchange rate varies somewhat with time and type of organism. In any case, the formula is an improvement, not a final solution. Adding other parameters helps correlations somewhat. The present parameters are the best set of three, but are not only ones that could appear in the formula.

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9. Properties must be weighted for combining. If the values in Table 1 for the three properties are used in the formula without weighting ($\alpha = \beta = \gamma = 1$), correlation against *RSF* will give nearly the same result as when difference is determined by v alone. This is because expressing v in Å^3 inflates its importance in the correlation relative to c and p. Inverse mean weighting is a scale change in the distance space to accommodate to the arbitrary units of each property. Inverse mean weighting (legend to Table 1) gives $\alpha^{\frac{1}{2}} : \beta^{\frac{1}{2}} : \gamma = 68.7 : 16 : 1$ and $R = -.72$ for the D : log *RSF* regression. When the formula is optimized by computer to maximize the correlation between D and log *RSF*, the square roots of the weighting factors = 54 : 17.6 : 1 and $R = -.74$. Thus, the inverse mean scale change gives nearly optimum weights. Incidentally, no linear combination of c, p, and v correlates better with *RSF* than this distance formula. Glycine, which is undefined by the hetero element/carbon ratio (see text), has an arbitrarily assigned c (the mean of the 14 nonring side chains). Proline, whose delta C joins to alpha N, is counted as having an atom of N in its side chain. Polarity is averaged: $p = (PR + PA)/2$, where *PR* is the "polar requirement" of C. R. Woese [*Naturwissenschaften* **60**, 447 (1973)] and *PA* (adjusted to same scale as $PR) = 13.66 - 14.85\ R_f$, where R_f is the amino acid mobility of A. A. Aboderin [*Int. J. Biochem.* **2**, 537 (1971)]. Side chain molecular volume is the residue volume (Å^3) of D. E. Goldsack and R. C. Chalifoux [*J. Theor. Biol.* **39**, 645 (1973)] minus the constant peptide volume (Gly residue — Gly side chain volume, the latter estimated as 3 Å^3). Proline's side chain sweeps out relatively less volume because it is pinned to alpha N; we therefore estimate the effective v_{Pro} as one-half its formal value. Ignoring the three refinements (taking into account the side chain volume of Gly in v determinations and the influence of Pro's side chain particularly on c and v) weakens the correlations slightly.
10. Amino acid residue abbreviations used in this report are: Arg, arginine; Leu, leucine; Pro, proline; Thr, threonine; Ala, alanine; Val, valine; Gly, glycine; Ile, isoleucine; Phe, phenylalanine; Tyr, tyrosine; Cys, cysteine; His histidine; Gln, glutamine; Asn, asparagine; Lys, lysine; Asp, aspartic acid; Glu, glutamic acid; Met, methionine; and Trp, tryptophan.
11. Further evidence is found by separating the 190 amino acid pairs into three classes according to codon relatedness and correlating D with *RSF* in each class. The Spearman rank coefficient (—.70) for correlation of D with *RSF* among the 101 pairs in the class with one common base is not greatly lower than that (—.82) among the 75 pairs having two common bases; however, that (—.43) among the 14 pairs with no common bases does help confirm a tendency for the code's structure to facilitate mutations to similar amino acids.
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Reverse Transcriptase in Normal Rhesus Monkey Placenta

Abstract. *Particles with the morphology of type C virus have been identified from primate placentas by electron microscopy. A reverse transcriptase (RNA-dependent DNA polymerase) was isolated and purified from microsomal pellets of two fresh placentas of rhesus monkeys in the early stages of gestation. This enzyme was biochemically similar yet immunologically distinct from the reverse transcriptases of known tumorigenic type C RNA viruses isolated from primates, but was immunologically related to a reverse transcriptase isolated from a type C virus obtained from normal baboon placenta. These particles may represent endogenous viruses and may function in the transfer of genetic information during embryogenesis.*

Several investigators, using electron microscopy, have reported that particles with the appearance of type C viruses bud from placental syncytial trophoblasts of the rhesus monkey (*1*), and of other primates (*2, 3*). Despite the presence of structures morphologically similar to oncogenic viruses, no mention has been made of any disease state in any of the maternal sources of the placentas (*1–3*).

The function of these type C particles is still unknown. Nor is it known whether they are biochemically similar to oncogenic type C viruses, whether they represent congenital transplacental infection by a tumor virus, or whether they are endogenous viruses that may be nononcogenic to the host.

We have confirmed the presence of type C viral particles in rhesus monkey placenta by electron microscopy; we now report the presence of a viral reverse transcriptase (RNA-dependent DNA polymerase) in two fresh placentas obtained from rhesus monkeys in early gestation. This enzyme is biochemically similar to but immunologically distinct from reverse transcriptases of known primate type C RNA tumorigenic viruses (woolly monkey sarcoma and gibbon ape lymphosarcoma viruses); nevertheless, it is immunologically related to a reverse transcriptase isolated from a virus propagated by cocultivation of normal baboon placental extract with heterologous cells.

Two rhesus monkey placentas (3.0 and 4.2 g) were obtained from normal mothers by cesarian section at days 33 and 36 of gestation, respectively (normal gestation is 165 days). In separate experiments, these tissues were homogenized in isotonic buffer, the nuclei and mitochondria were removed by differential centrifugation, and the resulting supernatants were centrifuged through a 25 percent sucrose "cushion" to obtain a microsomal pellet. The pellets were solubilized in high salt and detergent, and the nucleic acids were removed by passage of the extract over a fibrous diethylaminoethyl (DEAE)-cellulose column (*4*).

The placental extracts were then passed over a microgranular DEAE-cellulose column. Under certain conditions (*5*), viral reverse transcriptase and DNA polymerase II ($s_{20,w} = 3.4S$) elute from microgranular DEAE-cellulose columns at low salt concentrations (below 0.075*M* KCl) and may thus be separated from DNA polymerase I ($s_{20,w} = 6S$ to $8S$) and DNA-polymerase III (R-DNA polymerase) which elute at a higher ionic strength (between 0.10*M* and 0.35*M* KCl) (*6*). By virtue of their removal at this step, cellular DNA polymerases I and III are not sources of confusion in the sub-

sequent isolation of viral reverse transcriptase. Therefore, the microgranular DEAE-cellulose column was washed with 0.075*M* KCl and that pool was further purified by phosphocellulose chromatography. [See (*5*) for the nomenclature of the cellular DNA polymerases.]

Figure 1 shows the phosphocellulose chromatographic appearances of extracts of microsomal pellets, prepared as described above, from the following sources: (i) phytohemagglutinin (PHA) stimulated normal human blood lymphocytes; (ii) normal rat kidney cells infected with a known type C RNA tumor virus (murine sarcoma virus); (iii) the rhesus placenta. The same batch of phosphocellulose resin was used in all experiments. Reverse transcriptase elutes at 0.26*M* KCl in the tumor virus infected cells (Fig. 1B), but is absent or not detectable by this method in normal, uninfected cells (Fig. 1A). This enzyme transcribes the synthetic RNA-DNA hybrid $(A)_n \cdot (dT)_{12-18}$ and more significantly, fails to utilize the synthetic DNA · DNA template, $(dA)_n \cdot (dT)_{12-18}$, well-known properties of purified RNA viral reverse transcriptases (*7*). The rhesus placental extract (Fig. 1C) contains a small but distinct peak (2500 count/min) of $(A)_n \cdot (dT)_{12-18}$-transcribing activity, eluting at 0.26*M* KCl, the exact elution point of the known reverse transcriptase (Fig. 1B). Cellular DNA polymerase II is present in all three cases, eluting at approximately the same salt strength (0.58*M* KCl) and preferring $(dA)_n \cdot (dT)_{12-18}$ as template-primer. In comparison to the abundant DNA polymerase II in the placental extract, there is only a minute amount of the reverse transcriptase. Furthermore, since $(A)_n \cdot (dT)_{12-18}$ may be utilized as a template-primer by DNA polymerase II (*8*) as well as reverse transcriptase, the enzyme eluting at 0.26*M* KCl comprises only 19 percent of the total $(A)_n$-transcribing activity in the material applied to the phosphocellulose column.

The placental phosphocellulose activity eluting at 0.26*M* KCl was pooled and concentrated. This enzyme showed preference for $(C)_n \cdot (dG)_{12-18}$ and $(A)_n \cdot (dT)_{12-18}$ as compared to $(dA)_n \cdot (dT)_{12-18}$ as template-primers (Table 1). In addition, enzyme activity was greater with Mn^{2+} as divalent cation than with Mg^{2+}. These findings are the same as those of the known primate type C viral reverse transcriptases (*9*) and are, in fact, characteristics so far unique for all mammalian type C viral reverse transcriptases (*7, 10*). The absence of activity in the presence of $(dT)_{12-18}$ or $(dG)_{12-18}$ alone rules out the possibility of contamination with terminal deoxyribonucleotidyl transferase.

The pooled, concentrated preparation from phosphocellulose chromatography was layered on a glycerol gradient (5 to 20 percent) and subjected to velocity sedimentation. The reverse transcriptases from placenta and from woolly monkey sarcoma virus cosediment (data not shown), each having a molecular weight of approximately 62,000 as determined by three external protein markers (bovine serum albumin, ovalbumin, and human immunoglobulin G) (*11*).

Thus, the placental reverse transcriptase has chromatographic properties, template and cation preferences, and a sedimentation coefficient similar to those of mammalian type C viral reverse transcriptase. The placental enzyme was distinguished, however, from the reverse transcriptases of known tumor-associated primate type C viruses by use of antibody prepared in rats against the reverse transcriptase of woolly monkey sarcoma virus (*12*). This anti-

Table 1. Reverse transcriptase from rhesus monkey placenta: effect of template-primer and divalent cations on enzyme activity. Assay mixtures contained 10 μl of enzyme and were initiated by adding 40 μl of a mixture which gave a final concentration of 0.05*M* tris · HCl, *p*H 7.5; 0.001*M* dithiothreitol; either 0.01*M* $MgCl_2$ or 0.0008*M* $MnCl_2$; 80 μM each of unlabeled deoxynucleoside triphosphate [dATP for assays containing $(A)_n \cdot (dT)_{12-18}$ and $(dA)_n \cdot (dT)_{12-18}$, dCTP for assays containing $(C)_n \cdot (dG)_{12-18}$, and none for assays containing $(dT)_{12-18}$ and $(dG)_{12-18}$]; 14.5 μM [^{3}H]TTP (28,000 count min^{-1} pmole^{-1}) or 93 μM [^{3}H]dGTP (8800 count min^{-1} pmole^{-1}); and template-primer (31 μg/ml). All reactions were performed at 37°C for 30 minutes. Acid-insoluble precipitates were collected on nitrocellulose filters, and the radioactivity was counted in a liquid scintillation counter. Enzyme activity is expressed as picomoles of ^{3}H-labeled deoxynucleoside monophosphate incorporated per milliliter of reaction mixture.

Template-primer	Tritium labeled substrate	Divalent cation	Enzyme activity
$(A)_n \cdot (dT)_{12-18}$	TTP	Mn^{2+}	3.140
$(A)_n \cdot (dT)_{12-18}$	TTP	Mg^{2+}	< 0.500
$(C)_n \cdot (dG)_{12-18}$	dGTP	Mn^{2+}	4.980
$(C)_n \cdot (dG)_{12-18}$	dGTP	Mg^{2+}	2.600
$(dA)_n \cdot (dT)_{12-18}$	TTP	Mn^{2+} or Mg^{2+}	< 0.500
Primer alone [$(dT)_{12-18}$ or $(dG)_{12-18}$]	TTP or dGTP	Mn^{2+} or Mg^{2+}	< 0.500

Table 2. Effect of antibody (IgG) to primate viral reverse transcriptases on the enzyme activity of the reverse transcriptase. Variable amounts of IgG were mixed with 10 μl of enzyme. Each sample was brought to a volume of 20 μl by the addition of buffer (0.1*M* tris · HCl, *p*H 8.0) or a sufficient amount of nonimmune (control) IgG to bring the total IgG input to 100 μg (except in the reactions containing IgG to M7 polymerase where the total IgG input was 20 μg) and the mixture was kept at 0°C for 10 minutes. Reverse transcriptase assay mixtures were processed as described in Table 1, except that the incubation period was 120 minutes at 30°C. The template-primer was $(A)_n \cdot (dT)_{12-18}$ and the divalent cation was Mn^{2+}, except in the reactions containing the Mason-Pfizer viral enzyme, where Mg^{2+} was substituted (*9*). Antibody to woolly monkey sarcoma virus reverse transcriptase was prepared in a rat while antibodies to Mason-Pfizer and M7 virus reverse transcriptase were prepared in rabbits. Control IgG was purified from serums of unimmunized rats and rabbits, respectively. IgG against woolly monkey and Mason-Pfizer virus reverse transcriptase was isolated from serums by Sephadex G-200 chromatography (*20*), concentrated by precipitation in 50 percent ammonium sulfate, and dialyzed against 0.1*M* tris · HCl. IgG against M7 virus reverse transcriptase was purified as described (*21*).

Source of reverse transcriptase	Inhibition (25 and 50 percent) of enzyme activity by immune IgG to reverse transcriptase of (micrograms of IgG):						
	Control	Woolly monkey virus		Mason-Pfizer virus		Baboon placental virus	
	25%	25%	50%	25%	50%	25%	50%
Woolly monkey virus	> 100	10	45	> 100	> 100	*	*
Mason-Pfizer virus	> 100	> 100	> 100	39	74		
Rhesus monkey placenta	> 100	> 100	> 100	> 100	> 100	9	20
Baboon placental (M7) virus	> 20	†	†			3	9

* Data of Todaro and Sherr (*17*) demonstrated lack of inhibition of woolly monkey sarcoma virus reverse transcriptase by immune IgG prepared against M7 reverse transcriptase. † Experimental data of Sherr *et al.* (*15*) showed lack of inhibition of M7 virus reverse transcriptase by immune IgG prepared against woolly monkey sarcoma virus reverse transcriptase.

body, like that prepared in rabbits by others (*13*), inhibits the reverse transcriptases of the woolly monkey sarcoma and gibbon ape lymphosarcoma viruses but does not inhibit cellular DNA polymerases I (*12, 13*), II (*12, 13*), or III (*14*). The placental enzyme was not inhibited by this antibody at concentrations sufficient to inhibit the homologous enzyme by at least 50 percent (Table 2).

The placental reverse transcriptase was also distinguished from that of the Mason-Pfizer virus, a primate RNA virus that differs morphologically from type C viruses and has a unique reverse transcriptase which, unlike that from the placenta, favors Mg^{2+} over Mn^{2+} when transcribing synthetic template-primers (*9*). Moreover, the Mason-Pfizer reverse transcriptase has been reported to have a sedimentation coefficient (*9*) higher than that of the placental and all other known mammalian type C virus reverse transcriptases. Antibodies prepared in rabbits against Mason-Pfizer reverse transcriptase did not inhibit the placental enzyme (Table 2).

While this report was in preparation, we became aware that Dr. G. Todaro's laboratory had isolated infectious type C virus (M7) from baboon placental tissue as well as from other baboon cells by cocultivation with a canine thymus cell line (*16*). This baboon virus contained a reverse transcriptase biochemically like that of mammalian type C viruses; this enzyme was not inhibited either by antibodies to the reverse transcriptase from the woolly monkey sarcoma virus or feline leukemia virus but partially cross-reacted with antibody to the reverse transcriptase of the endogenous feline virus RD114 (*15*). In addition, the baboon virus complementary DNA (product of an endogenous DNA polymerase reaction) to a large degree hybridized back to its own RNA and slightly hybridized to the RNA of RD114 virus, but it did not hybridize at all to the RNA's of woolly monkey sarcoma, gibbon ape lymphosarcoma, or feline leukemia viruses (*16*). Antibody prepared in rabbits to M7 reverse transcriptase does not cross-react with woolly monkey sarcoma virus reverse transcriptase (*17*), but it significantly inhibits the rhesus monkey placental reverse transcriptase (Table 2).

Our results and those on baboon placental virus obtained by cocultivation (*15, 16*) suggest that primate endogenous virus particles are morphologically and biochemically similar to those of oncogenic RNA viral particles, but that the reverse transcriptase and genomic information contained in these units differ substantially from those inserted into primate RNA viruses that are known to be tumor-causing or tumor-associated. Because the placental particles were obtained from fresh normal tissue and have not yet been shown to be related to disease, we believe they represent endogenous type C viruses. The finding of these structures in two different primate species and the demonstration of the interrelatedness of their polymerases suggest that these particles may function in a normal physiological process such as information transfer for cytodifferentiation and is consistent with the possibility that some tumorigenic type C RNA viruses may represent a genetically mutated or a genomically recombined form of naturally occurring endogenous viruses (*18*).

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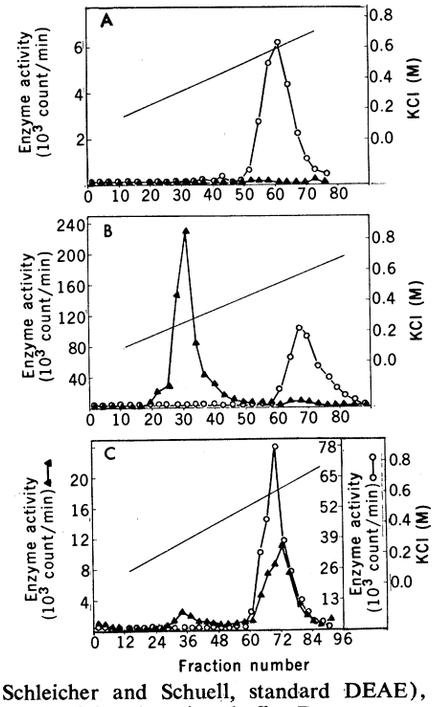

Fig. 1. Comparison of phosphocellulose chromatography elution profiles of DNA polymerase activities that had been eluted from DEAE cellulose columns by washing with 0.075*M* KCl. Tissue culture cells were disrupted manually in a tight fitting glass Dounce homogenizer, and placental tissue was disrupted with a motor-driven Teflon pestle in buffer containing 0.05*M* tris·HCl, *p*H 7.5; 0.02*M* dithiothreitol; 0.0005*M* EDTA; 0.005*M* $MgCl_2$; and 0.25*M* sucrose. Nuclei were removed by centrifugation at 900*g* for 10 minutes, mitochondria were removed by centrifugation at 10,000*g* for 15 minutes, and microsomal pellets were obtained by centrifuging the remaining supernatant through a sucrose cushion (25 percent by weight) at 171,000*g* for 120 minutes. The microsomal pellets were solubilized in 1.0*M* NaCl and 0.5 percent Triton X-100, particulate debris was removed by a 171,000*g* spin for 1 hour, and the extracts were dialyzed against a buffer containing 0.05*M* tris·HCl, *p*H 7.5; 0.001*M* dithiothreitol; 0.0001*M* EDTA; and 20 percent ethylene glycol (buffer D). The preparations were passed over fibrous DEAE-cellulose columns (column volume: 1.5 ml of wet packed resin per gram of starting tissue; Schleicher and Schuell, standard DEAE), washed off with buffer D containing 0.35*M* KCl, dialyzed against buffer D to remove salt, layered onto microgranular DEAE-cellulose columns (column volume: 1.5 ml of wet packed resin per gram of starting tissue; Whatman DE-52), washed off with buffer D containing 0.075*M* KCl, dialyzed against buffer containing 0.05*M* KCl, and then chromatographed on phosphocellulose columns (column volume: 0.75 ml of wet packed resin per gram of starting tissue; Whatman, P-11). Salt gradients ranged from 0.10*M* KCl to 0.75*M* KCl in buffer D; 0.02 percent Triton X-100 and bovine serum albumin (0.1 mg/ml) were added to the elution buffers. Assays were performed on every third fraction as described in the legend to Table 1. (A) Phytohemagglutinin (PHA) stimulated normal human blood lymphocytes (5.5×10^6 cells) were prepared as described (*19*). (B) Normal rat kidney cells (7.5×10^6 cells) infected with the Kirsten strain of murine sarcoma virus (MSV) were grown in Eagle's minimum essential medium containing 5 percent heat-inactivated fetal calf serum and 1 percent penicillin and streptomycin. (C) The placenta (33-day specimen; 3.0 g) was obtained from a rhesus monkey maintained for the Virus Cancer Program of the National Cancer Institute by Bionetics Laboratories, Kensington, Maryland. The phosphocellulose columns were assayed for DNA polymerase activity with $(dA)_n \cdot (dT)_{12-18}$ (○—○) and $(A)_n \cdot (dT)_{12-18}$ (▲—▲). Both assays were with Mn^{2+}.

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tris·HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.
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Inert Gas Narcosis, the High Pressure Neurological Syndrome, and the Critical Volume Hypothesis

Abstract. *The hypothesis that general anesthesia or pressure-induced convulsions occur when a hydrophobic region is expanded or compressed, respectively, by critical amounts is consistent with recent data obtained with mice. Calculations show that anesthesia occurs at an expansion of 1.1 percent and convulsions at a compression of 0.85 percent, the latter site of action being more compressible.*

The replacement of nitrogen by helium as the inert gas diluent in deep-diving breathing mixtures has removed the constraint of nitrogen narcosis (*1*), and simulated depths of 600 m (2000 feet, 61 atm) have been reached recently in France. However, a new barrier to deeper diving is the high pressure neurological syndrome, a hyperexcitability which first manifests itself in man at about 20 atm as a coarse tremor of the limbs (*2*). At higher pressures (60 to 100 atm) convulsions occur in experimental animals, including primates, and manned diving programs have consequently adopted cautious compression schedules (for example, 7½ days in the 600-m dive). Addition of narcotic gases to the breathing mixture has an ameliorating effect in animals (*3*).

Pressure reversal of anesthesia is another example of an effect of pressure on the central nervous system, and has led to the formulation of the critical volume hypothesis (*4*). This states that anesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical volume by the absorption of an inert substance. An applied pressure opposes this expansion and reverses anesthesia. In this report, it is proposed that the hypothesis may be extended to include the high pressure neurological syndrome by assuming that convulsions occur when some hydrophobic region has been compressed beyond a certain critical amount by the application of pressure. Absorption of an inert gas will compensate for such compression and raise the convulsion threshold pressure. This extension of the critical volume hypothesis has the attraction of offering a unified description for the interaction between pressure and narcotic gases in the central nervous system with respect to hyperexcitability and anesthesia. It could also provide a theoretical foundation for the use of inert gas mixtures in deep diving. The few studies that have been made of the effect of anesthetics and pressure on simple membranes suggest that the hydrophobic region is membranous in nature (*5*, *6*).

The fractional expansion, E, that occurs when a gas at a partial pressure, P_a, dissolves in a bulk solvent is given by

$$E = V_2 x_2 P_a / \overline{V}_m \quad (1)$$

where $\overline{V}_2$ is the partial molar volume of the gas in the solvent of molar volume V_m, and x_2 is the mole fraction solubility of the gas in that solvent when its partial pressure is 1 atm. In addition, physical compression of the liquid occurs according to its compressibility, β, and the total pressure, P_T (fractional compression $= \beta P_T$). In fact, for the less soluble gases, helium and neon, the compression term is larger than the expansion term and net compression results; hence they are not anesthetics. For the more soluble anesthetic or narcotic gases, such as N_2, Ar, and N_2O, net expansion occurs (*4*). Equation 1 must be corrected for gas imperfections and for the slight dependence of solubility on total pressure. The nature of these corrections has been given in a previous paper (*4*), in which the critical volume hypothesis accounted for pressure reversal of anesthesia data for newts. Here, the treatment will be applied to mammals.

Quantitative data for the pressure reversal of anesthesia in mice are available for three gas mixtures—He : N_2O, Ne : N_2O, and H_2 : N_2O (*7*)—while comparable data for the elevation of convulsion threshold are available for He : N_2, He : N_2O, and He : H_2 (*3*). The study of the high pressure neurological syndrome is complicated by the apparent dependence of the convulsion threshold on the strain of mice used and, to some extent, on the compression rate employed. While these variations deserve more detailed investigation, they are not large, and the data used in this study are internally consistent, having been obtained in one laboratory by a standardized procedure.

The expansion caused by dissolution of the inert gases (Eq. 1) was calculated for the experimental isonarcotic and isoconvulsion end points. This is shown in Fig. 1 as a function of pressure for the model solvent benzene. Such a plot should yield a linear relation where the slope gives the compressibility of the site of action and the intercept gives the critical volume