*Biochemical Pharmacology*, Vol. 34, No. 16, pp. 2839–2846, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00


# EFFECTS OF ANESTHETICS AND DICHLORODIFLUOROMETHANE ON THE ACTIVITIES OF GLYCERALDEHYDE-PHOSPHATE DEHYDROGENASE AND PECTIN METHYLESTERASE

DAVID M. LAVERTY and OWEN FENNEMA*
Department of Food Science, University of Wisconsin-Madison, Madison, WI 53706, U.S.A.

(*Received* 3 *September* 1984; *accepted* 2 *January* 1985)

**Abstract**—Solutions of glucose-3-phosphate dehydrogenase (GPD) and pectin methylesterase (PME) were exposed to various anesthetics and dichlorodifluoromethane (F-12) to determine the abilities of these chemicals to inhibit enzyme activity. An aqeuous solution of PME was exposed to saturation levels of the test chemicals for 30 min at 21°. All test chemicals were inhibitory (measured after release of the test chemical) with propane being most inhibitory followed in order by F-12, cyclopropane, Ethrane ($F_2HCOF_2CCHClF$) and halothane ($CF_3CHClBr$). GPD was exposed to various concentrations of F-12 and halothane for various times at 0° and 33°. Halothane at 33° and a saturation concentration reduced the initial reaction velocity of GPD to zero after a 10-min exposure period. F-12 was somewhat less inhibitory than halothane, but inhibition in all instances was irreversible. Halothane was found to affect the circular dichroism and optical rotary dispersion spectra of GPD, with the magnitude of the changes generally increasing with treatment time. The observed changes were believed to arise from side-chain transitions of GPD.

Short-chain halogenated hydrocarbons (SCHs) and anesthetics are known to associate with proteins in an aqueous environment [1], often resulting in conformational changes [2, 3] and in inhibition of enzymes and of some metabolic activities [4–13]. In simple, non-cellular systems, the effect of these chemicals on proteins is direct and probably involves charge–charge interactions or hydrophobic association [5, 9]. In cellular systems, these chemicals are believed to have profound effects on membranes, but it is not clear whether membrane proteins are directly affected or whether the chemicals affect the proteins indirectly by altering the structure of membrane lipids [4, 14]. In any case, anesthetic gases generally affect soluble enzymes less than membrane-bound enzymes [4]. Studies of the effects of anesthetic chemicals and similar compounds on proteins and enzymes are important not only to gain a greater understanding of anesthesia, but also to assess whether these chemicals are potentially useful as a means of inhibiting enzyme activity in foods.

The purpose of this study was to investigate the effects of several anesthetics and dichlorodifluoromethane on the activities of pectin methylesterase (PME) and glyceraldehyde-3-phosphate dehydrogenase (GPD). Pectin methylesterase was selected because it is important in fruit ripening and food processing, and glyceraldehyde-3-phosphate dehydrogenase was selected because it is an important metabolic enzyme that has been studied intensively.

* Author to whom all correspondence should be addressed.

## MATERIALS AND METHODS

*Test chemicals.* Test chemicals included dichlorodifluoromethane (F-12) ($CCl_2F_2$, E.I. du Pont de Nemours & Co., Wilmington, DE, food grade), propane ($CH_3CH_2CH_3$, Matheson Gas Products, Inc., 99.0% pure), cyclopropane ($C_3H_6$, Liquid Carbonic Co., U.S.P. grade), halothane ($CF_3CHClBr$, Fluothane, Ayerst Laboratories, anesthetic grade) and Ethrane ($F_2HCOF_2CCHClF$, Ohio Medical Products, research grade 99.9% purity). These chemicals were selected because: (a) their effects on enzymes are potentially important and little studied, (b) all except F-12 and propane are general anesthetics and are regarded as nontoxic and moderately inert at concentrations used clinically, (c) F-12 is approved by the Food and Drug Administration (U.S.) as an immersion freezant for foods, and (d) vapor pressures of the chemicals at room temperature are within reason for practical applications.

To compare the relative effects of the various test chemicals used in this study it was considered appropriate to express concentrations in terms of fractional saturation values, $C/C_s$, where $C$ is the weight of chemical dissolved in the liquid medium and $C_s$ is the solubility of the gas in the liquid medium at the same temperature and pressure. The fractional saturation value is an approximation of chemical potential [15] and is considered to be an appropriate means of expressing concentration in studies of this kind [16, 17]. To estimate the fraction of saturation achieved, it was necessary to obtain values for the solubility of test chemicals in the liquid phase and the amounts of test chemicals that existed in the vapor space under the conditions used. The solu-

bilities of both test chemicals in water were determined experimentally. In the case of F-12, the experimental solubility value differed less than 2% from the value reported by du Pont, Inc., so du Pont values were used at the two temperatures. For halothane, no reliable solubility values were found so total reliance was placed on experimental determination of solubility at various temperatures using a gas chromatographic procedure. The solubilities were $0.325 \pm 0.045\%$ (w/v) (95% C.L.) at 5°, $0.276 \pm 0.036\%$ (w/v) at 26° and $0.230 \pm 0.030\%$ (w/v) at 36°. The desired values at 0° and 33° were obtained graphically. The amount of test chemical occupying the vapor space was determined based on published vapor pressure values.

The procedure used to calculate fractional saturation values involved an assumption that the solubilities of test chemicals in water were the same as those in the test solutions. This assumption is considered appropriate since the error involved is likely to be small. For example, solubilities of oxygen and nitrogen in water and plasma differ only 2.5% (*ca.* 0.3 M in dissolved particles) [18]. The solutions employed in this study had concentrations ranging from about 0.2 to 0.4 M, so errors of a similar magnitude should have occurred.

To determine the amount of test chemical present, an aerosol can (200 × 202, double-epoxy lined, stainless steel cap) containing all reaction constituents was weighed, the test chemical added, the cap secured and the can reweighed.

*Enzyme.* Pectin methylesterase (PME) (EC 3.1.1.11) catalyzes demethylation of pectin resulting in release of methanol and a decline in pH. A unit of PME is defined here as that amount of enzyme that will cause a decline in pH from 7.30 to 7.19 in 10 min under specified conditions. Test samples contained 25 ml of 0.5% (w/v) pectin (Grade II, Sigma) in 0.1 M NaCl, and 0.48 μg PME enzyme. The pH was adjusted to $7.30 \pm 0.01$, and this moment was regarded as "zero" time. The extent of enzyme reaction was determined by measuring the decline in pH [19]. PME activities of treated and control samples were determined from a standard curve of change in pH versus reaction time.

The activity of glyceraldehyde-3-phosphate dehydrogenase (GPD) (EC 1.2.1.9) was determined by the method of Velick [20] which involves measurement of the increase in NADH at 340 nm, with arsenate (disodium arsenate·$7H_2O$, reagent grade) substituted for inorganic phosphate (the normal physiological reactant).

The basic 5.1-ml sample consisted of 1.44 ml of Tris buffer (0.5 M, pH 7.4, 0.25 ionic strength), 0.04 ml of 0.02 M $NAD^+$ (beta-DPN, $NAD^+$, Sigma Grade III, yeast source, Sigma Chemical Co., St. Louis, MO), 0.12 ml of 0.1 M L-cysteine (free base, Sigma) and 0.10 ml of 0.0044 M GPD (yeast source, 3× crystallized, Sigma). This solution was agitated for 10 min to allow GPD to associate with $NAD^+$ and cysteine, thereby favoring maximum activity. At the conclusion of the treatment period, a 1.7-ml aliquot was removed from the aerosol container and placed in a cuvette containing 1.0 ml of 0.0044 M glyceralde-3-phosphoric acid and 0.3 ml of 0.17 M disodium arsenate·$7H_2O$ (reagent grade). The

Table 1. Amounts of test chemicals required to achieve various fractions of saturation*

| Test chemical | Molecular weight | Temp. (°) | Amount of test chemical for various fractions of saturation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.077s† | | 0.155s† | | 0.31s† | | 0.62s† | | 1.25s† | |
| | | | Amt. added (g±0.01) | Est. amt. dissolved (g/ml) | Amt. added (g±0.01) | Est. amt. dissolved (g/ml) | Amt. added (g±0.01) | Est. amt. dissolved (g/ml) | Amt. added (g±0.01) | Est. amt. dissolved (g/ml) | Amt. added (g±0.01) | Est. amt. dissolved (g/ml) |
| Dichlorodi-fluoromethane | 121 | 0 | 0.12 | 0.0002 | 0.23 | 0.0004 | 0.46 | 0.0008 | 0.93 | 0.002 | 1.9 | 0.003 |
| | | 33 | 0.27 | 0.0001 | 0.54 | 0.0003 | 1.1 | 0.0006 | 2.2 | 0.001 | 4.1 | 0.002 |
| Halothane | 197.5 | 0 | 0.01 | 0.0003 | 0.02 | 0.0006 | 0.04 | 0.001 | 0.08 | 0.002 | 0.16 | 0.005 |
| | | 33 | 0.03 | 0.0002 | 0.06 | 0.0005 | 0.12 | 0.001 | 0.25 | 0.002 | 0.50 | 0.004 |

* Closed 97-ml aerosol cans, each containing 5.1 ml of aqueous solution, were employed.

† The degree of saturation equals $C/C_s$, where $C$ is the weight of chemical dissolved in the liquid medium and $C_s$ is the solubility of the gas in the liquid medium at the same temperature. The amount dissolved is taken to be the amount of test chemical that dissolves in water at the temperature and pressure used.

absorbance $340_{nm}$ increase was monitored for 1 min, and the reaction rate was determined. First-order reaction kinetics with respect to GPD concentrations prevailed during this period.

The initial reaction velocity of a treated sample was usually compared to that of an untreated sample and the result reported as "percent activity remaining" after treatment. When GPD activity was reported in absolute units, a molar extinction coefficient of $6.22 \times 10^6$ for reduced nicotinamide adenine dinucleotide (NADH) was used to convert Δ absorbance/min to moles NADH. In this instance, a unit of GPD activity at 33° was that amount of enzyme that oxidized 1.0 μmole of $NAD^+$ to NADH per min at pH 7.4.

*General procedure.* The enzyme solution was added to the aerosol can, and the desired test chemical was added in liquid form. Those chemicals with relatively high vapor pressures were added through the valve of the attached aerosol cap by means of a high pressure buret [16], whereas those with relatively low vapor pressures were added by means of a syringe prior to attaching the aerosol cap.

In the studies involving PME, the test chemicals were added at a fractional saturation value of 1.25, with the vapors in the headspace being accounted for in this calculation. At 21°, 0.27 g halothane, 0.90 g cyclopropane, 2.1 g F-12, 1.1 g propane, or 0.4 g Ethrane were required to achieve 1.25 saturation(s). PME samples were allowed to react for 30 min at 21°, while being agitated on a platform shaker for 28 min at 120 cpm.

In the studies involving GPD, the test chemicals were used at several concentrations and at temperatures of 0° and 33°. The weight of test chemicals needed to achieve various fractions of saturation are indicated in Table 1. GPD samples were agitated on a platform shaker for various times at 180 cpm. At the conclusion of the test period, the pressure in the container was released over a 10-sec interval, the sample temperature readjusted to the original value, and the sample removed and analyzed.

*Circular dichroism (CD) and optical rotary dispersion (ORD) measurements.* CD and ORD measurements were performed with a Jasco spectropolarimeter, model J-20 (Japan Spectroscopic Co., Ltd., Tokyo, Japan). A path length of 0.5 was used, and the enzyme concentration was 4 mg/100 ml or less. The enzyme concentration was calculated from absorbance measurements at 280 nm, using the extinction coefficient reported by Krebs *et al.* [21]. Ellipticities and specific rotations were corrected for path length and protein concentration [22]. Mean residual rotations based on 233 nm troughs in the ORD spectra were calculated using a mean residual weight of 109 for GPD [23].

## RESULTS AND DISCUSSION

The effects of halothane, F-12, Ethrane, cyclopropane or propane at a concentration of 1.25s on the ability of PME to catalyze demethylation of pectin in aqueous solution at 21° were investigated. Four replicates were used for each treatment along with suitable controls. From the results in Table 2 it is evident that all test chemicals significantly reduced the activity of PME, with propane being the most inhibitory and halothane the least. The effectiveness of propane would suggest that hydrophobic associations are involved in the inhibition.

The authors are unaware of any other study that has dealt with the effects of anesthetics or short-chain halogenated hydrocarbons on the activity of PME. However, some of the test chemicals in Table 2, or similar chemicals, have been shown to exhibit inhibitory effects against enzymes other than PME [4, 5, 24–26].

GPD in aqueous solution was exposed to halothane or F-12 at a concentration of 1.25s. Results in Fig. 1, expressed in terms of initial reaction velocity, show that both halothane and F-12 inhibited GPD activity and that the extent of inhibition increased greatly with treatment time. At 33°, samples exposed to halothane for 10 min or to F-12 for 20 min exhibited no significant GPD activity. At 0°, GPD in the presence of halothane became virtually inactive after 30 min, whereas F-12 at 0° failed to completely inactivate GPD during a 100-min exposure period.

The effects of various concentrations (1.25, 0.62, 0.31, 0.155, 0.077s) of halothane and F-12 on GPD activity were examined at 33° and 0°. Four treatment times were selected for each combination of chemical concentration and temperature. From the results

Table 2. Activity of pectin methylesterase as influenced by various chemicals administered at saturation values of 1.25

| Test chemical* | Units of activity† (±95% C.L.) Control‡ | Treated‡ | $\frac{\text{Activity treated}}{\text{Activity control}} \times 100$ |
|---|---|---|---|
| Propane | 0.48 ± 0.05 | 0.18 ± 0.03 | 38 |
| Dichlorodifluoromethane | 0.66 ± 0.05 | 0.30 ± 0.07 | 45 |
| Cyclopropane | 0.57 ± 0.08 | 0.30 ± 0.05 | 57 |
| Ethrane | 0.61 ± 0.09 | 0.41 ± 0.04 | 67 |
| Halothane | 0.58 ± 0.05 | 0.42 ± 0.04 | 72 |

* All samples were allowed to react for 30 min at 21°, with agitation imposed for 28 min at 120 cpm.

† A unit of enzyme is defined as that amount of enzyme which will cause a decline in pH from 7.30 to 7.19 in 10 min under the conditions specified in the text.

‡ Means of four replicates.

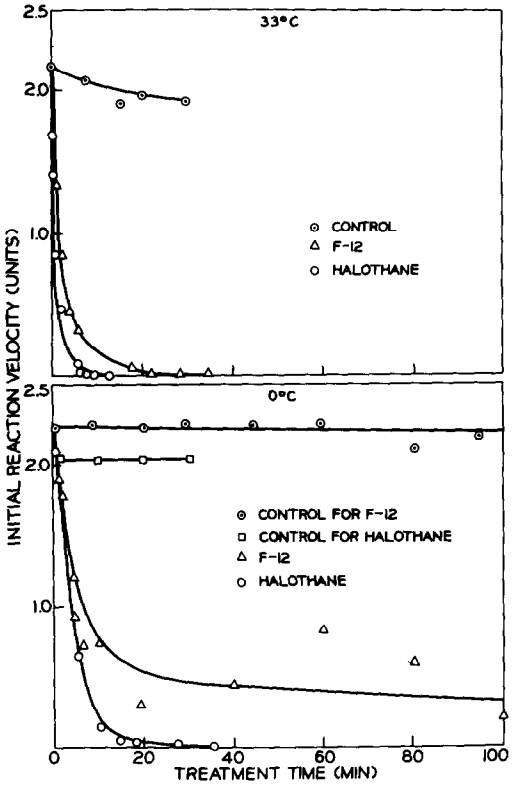


Fig. 1. Effects of halothane and dichlorodifluoromethane on the activity of glyceraldehyde-3-phosphate dehydrogenase (chemical concentration, 1.25s; agitation, 180 cycles/min; treatment temperatures, 0° and 33°). One unit of enzyme reduces 1 μmole of $NAD^+$ per min under conditions of the experiment. Each point on the graph represents a single observation.

presented in Fig. 2 it is evident that chemical concentration, treatment time and treatment temperature all had significant effects on the extent of GPD inactivation. When halothane was applied at low concentrations (0.077 and 0.155s) at either 0° or 33°, only a slight loss of GPD activity occurred even after the longest treatment times (Fig. 2, a and b). As the concentration of halothane was increased, GPD was more rapidly inactivated at both temperatures, with a concentration of 1.25s at 33° reducing GPD activity to 7% of the control value after 3.5 min. At the higher concentrations (0.31, 0.62, 1.25s) and at any given treatment time, halothane was always more inhibitory at 33° than at 0°.

Results with F-12 (Fig. 2, c and d) were similar to those with halothane except that F-12 was less inhibitory than halothane at any given condition. The comparative inhibitory effects of halothane and F-12 are in accord with that reported by Lund *et al.* [25] in their study involving invertase, and with that of Laverty and Fennema [27] in their study involving malate dehydrogenase, NADH dehydrogenase and GPD from *Eschericha coli*. It is interesting, however, that PME was inhibited to a greater extent by F-12 than halothane. This result was unexpected and cannot be explained from the information available.

The energy of activation ($E_a$) for inactivation of GPD was calculated using data from Fig. 2. In the temperature range 0° to 33°, the calculated $E_a$ value for inactivation of GPD by halothane (concentration, 1.25s) was 3400 cal/mole and the calculated $E_a$ value for inactivation of GPD by F-12 (concentration, 1.25s) was 2200 cal/mole. These $E_a$ values are quite low compared to values obtained when enzymes are denatured by heat. $E_a$ values for heat inactivation of lipase, amylase, pepsin, trypsin and invertase are in the range of 40,000–60,000 cal/mole [28]. The calculated $E_a$ values for inactivation of GPD are also low when compared to an $E_a$ of 12,500 cal/mole for a typical chemical reaction in which the reaction rate doubles with a 10° rise in temperature.

The activation energy for diffusion of non-electrolytes at low concentrations in liquid solutions is about 600 cal/mole [29]. Thus, the calculated $E_a$ values for GPD inactivation compare more closely to the $E_a$ for a diffusion process than to those for either a typical chemical reaction or heat denaturation of enzymes. This suggests that the rate at which GPD is inactivated by halothane and F-12 may be closely related to the rate at which the hydrocarbon derivative diffuses to the enzyme in solution. If this is so, an increased rate of GPD inactivation should occur when the vigor of agitation is increased. This relationship is observed in Fig. 3, at agitation rates in excess of 60 cpm.

Although it is clear that halothane and F-12 can temporarily inactivate GPD, it was considered desirable to determine whether this activation is permanent. Agitated solutions of GPD at 0° and 33° were treated for various times with halothane or F-12, each at a concentration of 1.25s. At the conclusion of the treatment the chemical was released, control and treated samples were sparged with moist nitrogen gas for 30 min, and the samples were then held quiescently in a constant temperature bath at 33°. Enzyme activity was determined at 0, 0.5, 1, 2 and 4 hr after removal of the test chemical. None of the treated samples exhibited increased GPD activity during a 4-hr period following release of the test chemicals (data not shown).

Additional samples containing GPD were treated with halothane for a length of time sufficient to cause total inactivation of GPD. Halothane was sparged from the samples, as described, and they were then allowed to stand at room temperature. None of the samples exhibited GPD activity during a 22-hr period following release of halothane. Control samples retained 76% of their original activity after the 22-hr holding period.

These results indicate that unbound halothane or F-12 can be removed almost completely from treated samples without causing any increase in the activity of GPD. Thus, it can be concluded that halothane and F-12, as employed in these studies, inhibit GPD irreversibly.

This finding agrees with that of Warmbier *et al.* [24] wherein they found that liquid F-12 inhibits *o*-diphenoloxidase irreversibly. However, other investigators have treated microsomal tissues with low concentrations of halothane or diethylether and found inhibition of ATPase and glucuronyltransferase to be reversible [30, 31]. Treatment time, con-

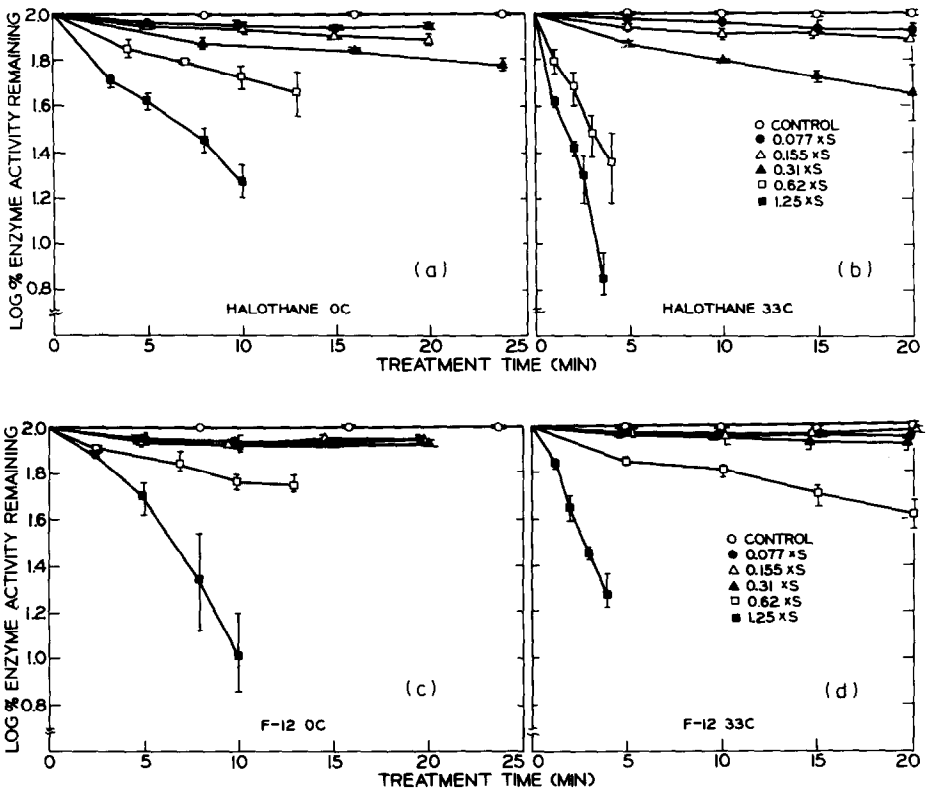


Fig. 2. Effects of various concentrations of halothane (a and b) and dichlorodifluoromethane (c and d) on the activity of glyceraldehyde-3-phosphate dehydrogenase at 0° and 33° (agitation, 180 cycles/min; pH 7.4). Each variability symbol represents a range of three observations.

centration of the test chemical, and other factors may influence whether inhibition is reversible.

An additional experiment was conducted to determine if the presence of various solutes influences the ability of halothane to inactivate GPD. Halothane at a concentration of 1.25s was applied to samples containing GPD, buffer and one of various solutes. Samples were maintained at 33° and agitated for 4 min at 180 cpm. Halothane was then released and GPD activity was determined. Results in Fig. 4 indicate that the presence of 5% (w/w) dextrose or 1% (w/w) soluble starch (A.C.S., Allied Chemical) had no significant effect on the ability of halothane to inhibit GPD. When the substrate, 0.0016 M glyceraldehyde-3-phosphoric acid (G-3-P), was present during treatment with halothane, lower activities were observed for both control and treated samples. However, inhibition was such that the relative differences in activities of control and treated samples were similar to those obtained when no G-3-P was present during treatment. The lower GPD activities in samples containing G-3-P are presumably attributable to instability of G-3-P in the buffer, resulting in a lower substrate concentration at the moment activity was being determined. For samples containing G-3-P prior to treatment, the enzyme reaction was initiated by adding arsenate.

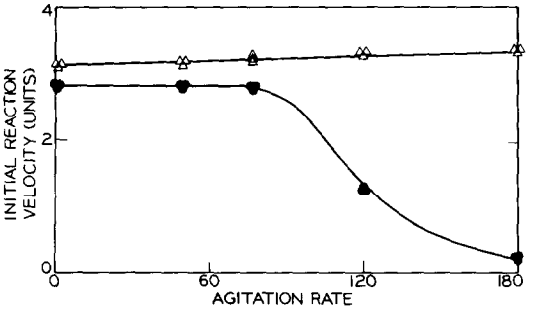


Fig. 3. Initial reaction velocity of glyceraldehyde-3-phosphate dehydrogenase as influenced by various agitation rates during exposure to halothane (1.25s) for 10 min at 27°. Key: (△——△) control (no agitation), and (●——●) treated.

When 5% (w/w) bovine serum albumin was present in the samples, inactivation of GPD by halothane was very slight, indicating that albumin had a protective effect. The ineffectiveness of halothane when albumin was present may have resulted from hydrophobic association of halothane with albumin thus lessening contact of halothane with the enzyme. Laasberg and Hedley-White [3] showed that addition of serum albumin to pure water greatly increases the solubility of halothane, indicating a significant association between albumin and halothane.

The ability of halothane to inhibit GPD activity, and reports that various anesthetic gases (including halothane) alter protein structure [2, 14], stimulated

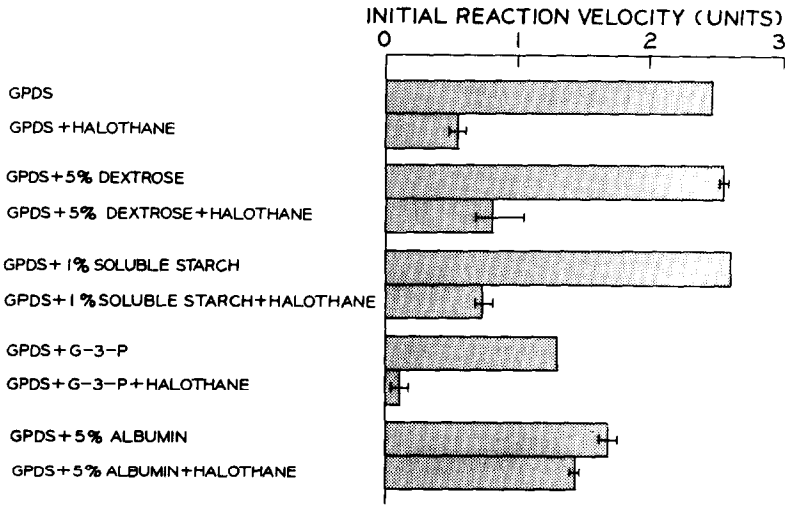


Fig. 4. Effects of various solutes on the ability of halothane to inhibit glyceraldehyde-3-phosphate dehydrogenase (halothane concentration, 1.25s; temperature, 33°; treatment time, 4 min; agitation, 180 cycles/min). Each variability symbol represents the range of three observations; for data without variability symbols, variability was too small to indicate. One unit of GPD activity is the amount of enzyme that reduces 1 μmole of $NAD^+$ per min under conditions of the experiment. Solutes used: 5% (w/w) dextrose, 1% (w/w) soluble starch, 0.0016 M glyceraldehyde-3-phosphoric acid (G-3-P), and 5% (w/w) bovine serum albumin. GPDS is GPD enzyme in Tris buffer (pH 7.4; ionic strength 0.25).

a study of the effect that halothane has on the structure of GPD. Optical rotatory dispersion (ORD) and circular dichroism (CD) were chosen as techniques to observe possible changes in protein structure.

Halothane at a concentration of 0.31s was applied to aqueous solutions of GPD in 1 nM EDTA (pH 7.4) for 2, 6, 10 or 14 min. This concentration of halothane was selected because it resulted in reasonably slow but significant inactivation of GPD, thus making structural changes in the enzyme relatively easy to follow. Following treatment, only samples retaining more than 50% of their original activity were analyzed since greater inactivation resulted in turbidity that interfered with CD and ORD measurements.

Figures 5 and 6 are the CD and ORD dispersion spectra, respectively, of untreated and halothane-treated GPD. These curves represent duplicate observations at each treatment time. The negative ellipticity band observed in the 215 nm region of the CD spectrum for untreated GPD (yeast origin) agrees with reported observations on rabbit-muscle GPD; however, the bands in the 250–280 nm range have a more positive ellipticity than those for rabbit-muscle GPD [32–34]. Some possible explanations for this discrepancy are differences in the origin of GPD, pH and/or purity of the enzymes used.

When halothane was applied to aqueous solutions containing GPD (Fig. 5), the CD bands diminished in the 250–300 nm range and the large band at 215 nm was virtually unaffected. Changes in bands at 250–300 nm became more prominent as the treatment time was increased. Changes in ellipticity bands in this area of the u.v. range are usually attributed to side-chain transitions of aromatic amino acids in asymmetric environments [35]. The lack of change in the far u.v. range indicates that little change occurred in the peptide backbone structure of GPD when it was exposed to halothane [35].

The negative rotation at 233 nm in the ORD spectrum of untreated GPD is characteristic of this enzyme (Fig. 6). The magnitude of this rotation, expressed as mean residual rotation, was −3900 and this value falls within the range of −3300 to −4120 that has been reported for yeast and muscle GPD

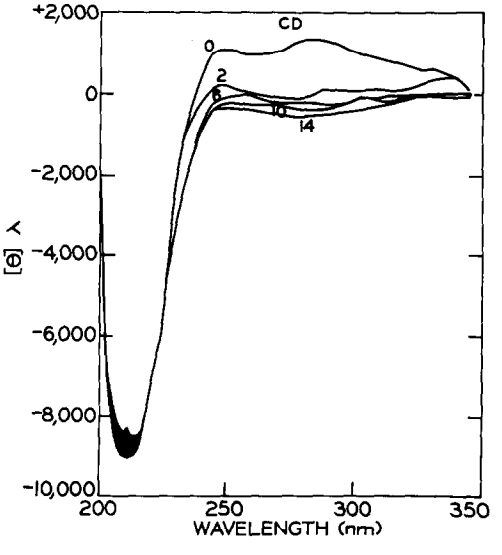


Fig. 5. Effect of halothane on the circular dichroic (CD) spectra of glyceraldhyde-3-phosphate dehydrogenase (halothane concentration, 0.31s; GPD concentration, 3 mg/100 ml; 1 mM EDTA present in solution; pH 7.4; samples agitated at 180 cycles/min during treatment; numerals on curves indicate treatment time in min).

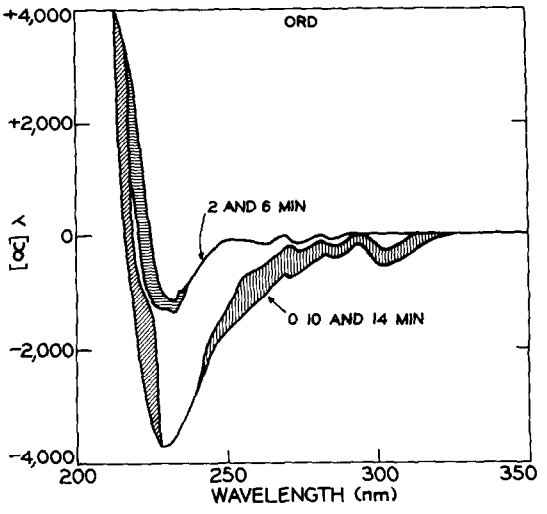


Fig. 6. Effect of halothane on the optical rotatory dispersion (ORD) spectra of glyceraldehyde-3-phosphate dehydrogenase. See the legend of Fig. 5 for experimental details.

[23, 32, 36–38]. The amount of rotation at 233 nm first decreased and then increased back to the original value as treatment time was increased. Attaching structural meaning to these transitions at 233 nm is questionable when, as is apparently true in this instance, extensive interference exists between side-chain and aromatic amino acids [38]. The above experiment was repeated (data not shown), and essentially the same results were obtained.

These results are in good agreement with those of Balasubramanian and Wetlaufer [2]. They observed alteration of the structure of β-lactoglobulin when various anesthetics (one of which was halothane) were applied. Their results suggested rearrangement of side chains of the protein rather than alteration of the backbone structure. Schoenborn [39] applied cyclopropane to myoglobin, and drew similar conclusions based on analysis by X-ray diffraction.

Laasberg and Hedley-White [3] applied halothane to a hemoglobin solution and observed a less negative rotation at 233 nm in the ORD spectrum than that which occurred with untreated hemoglobin. After comparing this observation with results obtained when alpha- and beta-hemoglobins were treated with halothane, they suggested that halothane binding produces a change in the beta-structure of the protein molecule. Inoue and Timasheff [40] exposed solutions of β-lactoglobulin to increasing concentrations of 2-chloroethanol. The CD and ORD curves indicated that the α-helix content of β-lactoglobulin increased with exposure to increasing concentrations of 2-chloroethanol.

Thus, it is abundantly clear that the anesthetic gases and the short-chain halogenated hydrocarbon tested here, when used at or near saturation levels, have strong inhibitory effects on PME and GPD. These effects are irreversible and involve structural changes, at least in GPD.

*Acknowledgements*—Research was supported by The College of Agricultural and Life Sciences, University of Wisconsin, Madison. The assistance of Professors J. H. Perrin and P. A. Hart, School of Pharmacy, University of Wisconsin-Madison, in conducting and interpreting the experiments on CD and ORD is gratefully acknowledged.

## REFERENCES

1. A. Steward, P. R. Allott, A. L. Cowles and W. W. Mapleson, *Br. J. Anaesth.* **45**, 282 (1973).
2. D. Balasubramanian and D. B. Wetlaufer, *Proc. natn. Acad. Sci. U.S.A.* **55**, 762 (1966).
3. L. H. Laasberg and J. Hedley-White, *Fedn Proc.* **30**, 603 (1971).
4. C. D. Richards, in *International Review of Biochemistry; Biochemistry of Cells Walls and Membranes II* (Ed. J. C. Metalfe), Vol. 19, p. 157. University Park Press, Baltimore (1978).
5. M. A. Singer, *Biochem. Pharmac.* **29**, 2651 (1980).
6. H. Rottenberg, *Proc. natn. Acad. Sci. U.S.A.* **80**, 3313 (1983).
7. P. J. Snodgrass and M. M. Piras, *Biochemistry* **5**, 1140 (1966).
8. R. N. Miller and F. E. Hunter, Jr., *Molec. Pharmac.* **6**, 67 (1970).
9. A. M. Casanovas, M. F. Malmary Nebot, Ph. Courrière and J. Oustrin, *Biochem. Pharmac.* **32**, 2715 (1983).
10. P. Seeman, *Pharmac. Rev.* **24**, 583 (1972).
11. R. A. Harris, J. Munroe, B. Farmer, K. C. Kim and P. Jenkins, *Archs Biochem. Biophys.* **142**, 435 (1971).
12. B. A. Britt, W. Kalow and L. Endrenyi, *Biochem. Pharmac.* **21**, 1159 (1972).
13. J. F. Biebuyck, J. F. P. Lund and H. A. Krebs, *Biochem. J.* **128**, 711 (1972).
14. P. Gróf and J. Belágyi, *Biochim. biophys. Acta* **734**, 319 (1983).
15. J. Ferguson, *Proc. R. Soc. B* **127**, 387 (1939).
16. B. A. Prior, O. Fennema and E. H. Marth, *Appl. Microbiol.* **20**, 139 (1970).
17. B. A. Prior, O. Fennema and J. Pate, *Appl. Microbiol.* **29**, 685 (1975).
18. E. S. West, *Textbook of Biophysical Chemistry*, 3rd Edn, p. 72. Macmillan, New York (1963).
19. L. P. Somogyi and R. J. Romani, *Analyt. Biochem.* **8**, 498 (1964).
20. S. F. Velick, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), p. 401. Academic Press, New York (1955).
21. E. G. Krebs, G. W. Rafter and J. M. Junge, *J. biol. Chem.* **200**, 479 (1953).
22. J. H. Perrin and P. A. Hart, *J. pharm. Sci.* **59**, 431 (1970).
23. I. Listowsky, C. S. Furfine, J. J. Betheil and S. Englard, *J. biol. Chem.* **240**, 4253 (1965).
24. H. C. Warmbier, O. Fennema and E. H. Marth, *J. Fd Sci.* **37**, 702 (1972).
25. D. B. Lund, O. Fennema and W. D. Powrie, *Archs Biochem. Biophys.* **129**, 181 (1969).
26. H. Oujesky and R. Fuerst, *Devs ind. Microbiol.* **15**, 405 (1974).
27. D. M. Laverty and O. Fennema, *Microbios*, in press.
28. J. R. Whitaker, *Principles of Enzymology for the Food Sciences*, p. 345. Marcel Dekker, New York (1972).
29. R. H. Perry, C. H. Chilton and S. D. Kirkpatrick (Eds.), *Chemical Engineers Handbook*, p. 14.21. McGraw-Hill, New York (1963).
30. I. Ueda and W. Mietani, *Biochem. Pharmac.* **16**, 1370 (1967).
31. B. R. Brown, *Anesthesiology* **37**, 483 (1972).
32. J. J. M. DeVijlder and B. J. M. Harmsen, *Biochim. biophys. Acta* **178**, 434 (1969).
33. D. W. Darnall and T. D. Barela, *Biochim. biophys. Acta* **236**, 593 (1971).

34. W. Boers, C. Oosthuizen and E. C. Slater, *Biochim. biophys. Acta* **250**, 35 (1971).
35. S. Beychok, in *Poly-Alpha-Amino Acids* (Ed. G. D. Fasman), p. 293. Marcel Dekker, New York (1967).
36. M. T. A. Behme and E. H. Cordes, *J. biol. Chem.* **242**, 5500 (1967).
37. Y. Shibata and M. J. Kronman, *Archs Biochem. Biophys.* **118**, 410 (1967).
38. R. Jaenicke and W. B. Gratzer, *Eur. J. Biochem.* **10**, 158 (1969).
39. B. P. Schoenborn, *Fedn Proc.* **27**(3), 888 (1968).
40. H. Inoue and S. N. Timasheff, *J. Am. chem. Soc.* **90**, 1890 (1968).