EXHALATION KINETICS OF SOME VOLATILE ORGANIC COMPOUNDS

by

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DEDICATION

This thesis is dedicated to my parents Mr. and Mrs. Bhalchandra P. Kulkarni whose encouragement and assistance made my desire for higher education fruitful.

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INTRODUCTION

The analysis of expired air as a method for the investigation of the metabolism of administered compounds and their excretion kinetics has attracted the attention of scientists for many years. The first systematic effort in this direction was reported by Cushney[1] in 1911. Compounds including ethanol, methanol and chloroform were intravenously administered to experimental animals. Analysis of their blood and expired air at regular intervals suggested that the exhalation of the volatile compounds from the lungs is purely a physical process involving the partition of the exhaled compound between lung air and blood. He also observed that while substances such as chloroform and ethylene chloride were entirely eliminated through the expired air, only a small fraction of substances such as ethanol and ethyl acetate was exhaled, the rest apparently being metabolized in the body.

Need for suitable medico-legal tests for intoxication gave further impetus to the analysis of expired air. Harger and co-workers[2] developed a chemical test for the determination of the concentration of ethanol in blood by the analysis of expired air. Various other workers have suggested different chemical tests for determination of the concentration of ethanol and acetone in expired air, notable among them being the methods by Liljestrand and Linde[3] and by Haggard and co-workers.[4] All these methods

of analysis had severe drawbacks;

1. The sensitivity was low.
2. The reproducibility was relatively poor.
3. There was a certain amount of non-specificity involved due to the fact that these methods are generally based on oxidation-reduction reactions and any substance that can reduce the reagent thus gives a positive response.

The search for new and sensitive methods of analysis of volatile substances logically leads one to gas-liquid chromatography. The development of gas chromatography has significantly increased the capability of analysis of volatile compounds in trace quantities mainly with respect to sensitivity but also with respect to accuracy to a certain extent. This makes the analysis of human expired air a significant tool for the investigation of metabolism of volatile substances or substances which when administered are metabolized to volatile substances. Thus the information obtained from the exhalation kinetics of these substances should be utilizable in the investigation of the kinetics of their absorption and/or elimination.

The analysis of expired air for the determination of blood concentrations of administered substances which are relatively volatile offers several obvious advantages over the direct analysis of blood or urine samples;

1. Samples can be taken more frequently.

2. The time required for the collection and the analysis of the samples is much shorter.

3. The analysis is easier since there is less interference by other substances as compared to blood or urine samples. In particular steps such as distillation and protein precipitation are avoided.

4. Collection of the blood samples from human subject has to be done under the supervision of a physician or similar qualified person. Collection of expired air samples does not require such supervision.

The work described in this report was intended to be an investigation into the development of a highly sensitive method for the analysis of expired air. This method as described in Section I of this report, makes it possible to carry out investigations of the exhalation kinetics of volatile organic compounds administered to human subjects in very small doses. The quickness and the convenience of this method, coupled with its high sensitivity and specificity, offer obvious advantages over the usual methods of analyses of blood or urine samples.

The investigations reported in Section II deal with the concentration of volatile organic compounds in normal human expired air and the studies of exhalation kinetics of ethanol, isopropanol, acetone and paraldehyde following oral administration. The interpretation of the results of these investigations is made in the light of the information concerning the metabolism of these compounds reported by previous workers.

It might be pointed out that the studies reported herein represent only a beginning step into the field with the aim of discovering where some of the important problems lie with regard to investigation of the kinetics of elimination of relatively volatile drugs (or drugs giving rise to exhalation of volatile organic compounds) through the investigation of their exhalation kinetics.

SECTION I

PHYSIOLOGICAL BACKGROUND

Expired air is a mixture of alveolar air and extra-alveolar air, (the latter is also known as dead-space air). Figure I shows the lung volume at different stages in respiration.

The air exhaled each time during the normal course of respiration is called 'tidal air' and constitutes only about one-seventh of the mean lung volume. The initial portion of the exhaled air contains the extra-alveolar or dead-space air. It is not in equilibrium with pulmonary blood and its composition is different from that of the alveolar air. Thus the composition of expired air depends on:

(a) The relative proportions of alveolar and dead-space air.

(b) The composition of the alveolar air.

(c) The extent of intermixing between the alveolar and the dead space air by the process of diffusion and by the mechanics of respiration.

Thus the composition of different expired air samples from the same subject under same initial condition may vary significantly unless the conditions of collection of the expired air samples are rigorously specified and controlled. In fact the main cause of poor results obtained by earlier workers in the analysis of expired air might well be attributed to these variations.

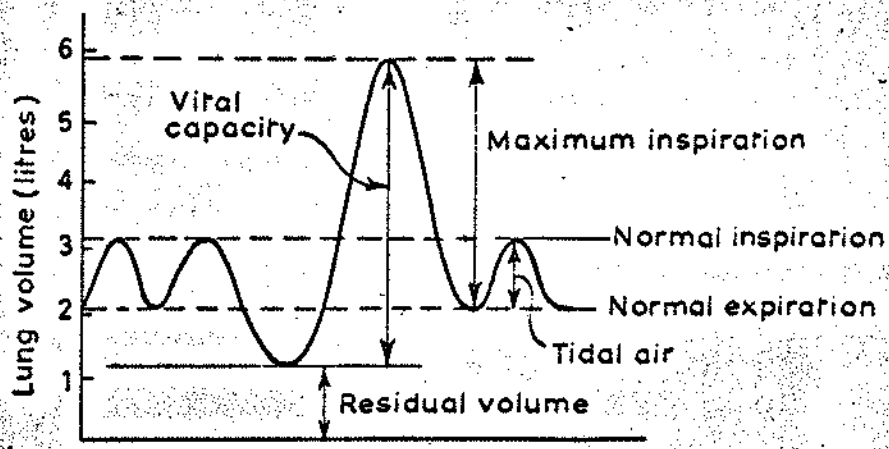

Fig. 1 Lung volume at different stages of respiration

*Lung volumes reported by Guyton.[9]

Table I lists the values of some respiratory characteristics in average human subjects. It is clear from the extremely large ratio of surface area of the respiratory membrane to the volume of the pulmonary blood and to the volume of the alveolar air, that the alveolar air should be very rapidly equilibrated with pulmonary blood.

Harger, Lamb and Hulpieu[5] first tried to correlate the concentration of ethanol in expired air to the concentration of ethanol in alveolar air by measuring the ratio of ethanol content of expired air to carbon dioxide content of expired air. Their assumption was that the coefficients of diffusion of CO_2 and of ethanol through the alveolar membrane separating the alveolar blood from alveolar air are not different. However it was pointed out later that the diffusion coefficient of a volatile compound diffusing out through the alveolar membrane is dependent on the solubility of the substance in the membrane. Since the solubility of CO_2 in aqueous fluid in the alveolar membrane is very small, its diffusion coefficient is much smaller than the diffusion coefficient of ethanol and was shown to be only about 1/40th as large as the latter. Hence, the method of correlating the concentration of an exhaled substance in expired air to its concentration in alveolar air on the basis of CO_2 content of the expired air is quite questionable.

Table I

SOME RESPIRATORY CHARACTERISTICS OF AVERAGE ADULT HUMAN SUBJECTS[9]

Volume of pulmonary blood	60 to 100 mls.
Total surface area of respiratory membrane	50 to 70 sq. meters
Mean lung volume	2550 mls.
Tidal volume	500 mls.
Normal inspiration volume	2800 mls.
Maximum inspiratory capacity	5800 mls.
Residual lung volume (volume at maximum expiration)	1200 mls.
Vital capacity (volume of air exhaled between the point of maximum inspiration to the point of maximum expiration)	4600 mls.
Normal dead space air volume	150 mls.
Dead space air volume at maximum inspiration	225 mls.

*Values reported by Guyton.[9]

Harger and co-workers in their later investigations[5,6] found that the composition of rebreathed air is the same as that of alveolar air. They also verified the partition ratio for alcohol between blood and alveolar air by a series of in vivo determinations of ethanol content of blood and of expired air from human subjects to whom ethanol was previously administered. However, when the concentration of the substance being estimated in the expired air is very low, the rebreathed air samples may give erroneous results due to the adsorption of the substance on the walls of the flexible container used for rebreathing. For the same reason various other mechanical devices suggested by different workers for obtaining alveolar air samples are unsatisfactory. Hence the use of equilibrated vital capacity air, as described below, was preferred to rebreathed air.

An additional source of error in the analysis of expired air comes from the difficulty of obtaining reproducible quantities of gaseous samples. The variation is further aggravated by the fact that normal expired air is at a lower temperature than the body temperature and may vary by as much as 2° C.[5]

In the method described in this work, an attempt is made to overcome these difficulties and at the same time increase the sensitivity of the method using the assumption that the alveolar air is saturated with respect to water vapor at the body temperature.

Christie and Loomis[7] have reported that the partial pressure of water vapor in normal alveolar air is 2mm.Hg. lower than the saturation vapor pressure of water at the body temperature. But since the collection of the samples in the method to be described involves holding the breath for 12 seconds, it can be safely assumed that the alveolar air in these samples is saturated with respect to water vapor at the body temperature.

The air used for the collection of expired air samples in this method is vital capacity air (air between the point of maximum inspiration and the point of maximum expiration) following the holding of the breath for 12 seconds after full inspiration. The use of this procedure entails the following advantages:

1. Vital capacity of an individual is remarkably reproducible. Hence the error due to the variations in the proportions of alveolar and dead space air is reduced.

2. The maximum dead space air volume is 225 mls. compared to 5800 mls. of the maximum inspiratory capacity in normal human subjects. Thus the ratio of dead space air to the alveolar air is substantially lowered (amounting to less than 4%).

3. Holding the breath for 12 seconds after full inspiration increases the probability of the saturation of respired air with respect to water vapor at the body temperature and also reduces the error caused by fluctuations of temperature of expired air.

4. The large volume of vital capacity air makes it possible to devise a condensation scheme by the use of which aqueous liquid samples are available for analysis. The analysis of liquid samples is much simpler in comparison to analysis of gas samples. Secondly, the use of a condensation scheme gives the samples in a higher concentration than the air and thus produces an increase in sensitivity as will be shown in the calculations to follow.

SAMPLE COLLECTION TECHNIQUE

Figure 2 shows a diagramatic sketch of the sample collection set-up. The subject steadily inhales air to the point of maximum inspiration and holds the breath at this point for 12 seconds. At the end of this period he blows the air slowly and steadily through a siliconized Pyrex glass cell cooled by immersion in liquid nitrogen. The other end of the cell is connected to a spirometer* with which the flow rate is roughly maintained at about 400 mls. per second. The spirometer also provides a low but relatively constant resistive pressure during the exhalation and serves as a check from time to time on the vital capacity of the subject.

During the flash condensation of the expired air, all the volatile compounds present in the expired air along with water vapor condense in the cell. The cell is then taken out, quickly stoppered and the condensate is carefully melted allowing CO_2 to escape cautiously. The resulting aqueous droplets are collected into a single drop by rolling them over the siliconized inner surface of the cell by gentle rotation of the cell. The projection at the lower end of the cell where this aqueous solution

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* "Precision" wet test meter, Precision Scientific Co., Chicago, Ill.

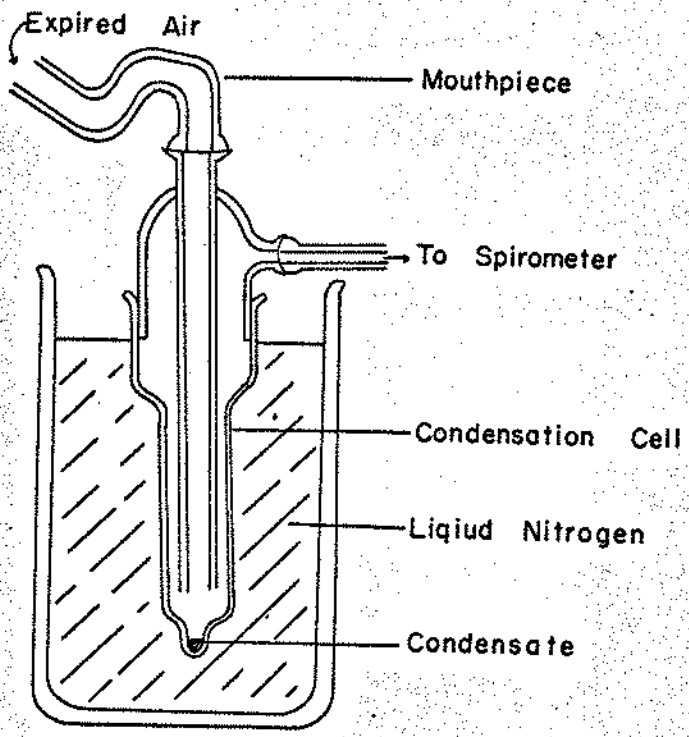

Fig. 2 Assembly used for low-temperature condensation of equilibrated vital capacity air.

collects makes it easier to withdraw samples of this aqueous solution with the help of a gas chromatographic microsyringe.**

Five Ml. of this aqueous solution was subjected to gas chromatographic analysis. For each experiment the same amount of a standard solution containing a known concentration of the substances being estimated was also injected immediately after the samples.

**Hamilton 701-NCH syringes were used for all studies, Hamilton Company, Whittier, California.

OUTLINE OF CALCULATIONS

Since the expired air collected by the procedure described is to be considered saturated with respect to water vapor at 37° C, the composition of the aqueous condensate is dependent only on the composition of the expired air and is independent of the actual volume of the expired air from which the condensate is obtained. (The volume only determines the quantity of the condensate obtained.)

Assuming ideality, the volume of 1 mole of water vapor at 37° C and 1 atmos. pressure = $\frac{22.415 \times 310.2}{273.2}$ L. = 25.48 L. Saturated water vapor pressure at 37° C is 47.1 mm.Hg.

∴ Partial pressure of water vapor in the expired air sample = (47.1/760) atm.

∴ 1 mole or 18 mls. of water are contained in $\frac{25.48 \times 760}{47.1}$ L. of expired air.

∴ 1 L. of condensate = $\frac{1000 \times 25.48 \times 760}{18 \times 47.1}$ L. of expired air.

∴ Concentration of a substance in expired air = 4.39×10^{-5} X the concentration of the substance in the condensate.

(At pressures significantly different from 1 atm. appropriate changes in the calculation are necessary.)

ANALYTICAL TECHNIQUE

(A) Outline of Gas-Chromatographic Analysis of the Condensate:

Gas-chromatography of dilute aqueous solutions is difficult due to the broadening effect of the large excess of water on the peaks of the solutes. Furthermore many stationary phases such as esters are susceptible to hydrolysis and this may result in a very unsteady baseline particularly if higher temperatures must be used.

The compounds estimated in the following studies include ethanol, methanol, acetone, acetaldehyde, isopropanol and paraldehyde. Most of the stationary phases recommended for gas-liquid chromatography of alcohols such as glycerol, diglycerol, carbowaxes and SE-30 can separate these compounds effectively from their mixtures, but were found unsatisfactory for separations from aqueous solutions of these compounds. Furthermore for very dilute solutions the adsorption of the compounds on solid support presents a problem in itself.

For the gaschromatographic analysis of expired air, the selection of the column material must be based on the following considerations:

1. The solid support used must not significantly adsorb the compounds being estimated.

2. Separation between acetone, methanol, and ethanol should be complete although this is very difficult to achieve.

3. The tailing should be small and the ratio of peak height to peak area should be as high as possible.

4. The efficiency of the column should not be affected by prolonged exposure to the water contained in the samples.

5. The retention time should not exceed 4 to 5 minutes to permit frequent sampling and rapid results.

6. Either the peak height or the peak area of the compound to be estimated must be proportional to the total amount of the compound injected into the chromatograph. The former case is preferred for its convenience.

As far as the detector is concerned, flame ionization detector is best suited for the analysis, because it is insensitive to water, carbon dioxide and inert gases but very sensitive to combustible organic compounds. Furthermore it has the advantage that the detector response is directly proportional to the concentration of the substance being detected in the carrier gas[8] over large concentration ranges.

The chromatograph used in these studies was an Aerograph Hy-Fi model 600 equipped with an Aerograph Hydrogen Generator and a gold plated flame ionization detector-head.*

The columns used were made of 1/4 inch stainless steel coils with Swagelock fittings.

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*Wilkens Instrument and Research Co., Walnut Creek, California.

(B) Selection of the Gas-chromatographic Column and the Operating Conditions:

Solid Support:

Solid supports such as Celite,* crushed firebrick, Chromosorb G* and Gaschrom** were found to be unsatisfactory due to severe adsorption and consequent tailing of the peaks. Deactivation of Gaschrom by treatment with a 1 percent solution of dichlorodimethyl-silane in xylene substantially reduced the tailing and for initial experiments this material was used as solid support. Anakrom ABS, 70-80 mesh,*** was found to cause much less tailing even without the treatment with the siliconizing agent. Furthermore columns prepared with it appeared to pack better. For these reasons this material was used as a solid support for all subsequent work.

Stationary Phases:

A large number of substances were examined as potentially suitable stationary phases for the required gaschromatographic separation of acetaldehyde, acetone, methanol, ethanol, isopropanol and paraldehyde from dilute aqueous solutions. These included the substances

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*Johns Manville and Company, New York, New York.

**F & M Scientific Corporation, New Castle, Delaware.

***Analabs, Inc., Hamden, Connecticut.

recommended for separation of alcohols in gas-chromatographic literature (PEG 1500, PEG 6000, glycerol, diglycerol, SE-30, glyceryl monostearate, didecylphthalate). In addition many substances such as Arochlor (a mixture of chlorinated polyphenyls*), Craig polyester (adipic acid ethyleneglycol co-polymer), mineral oil, hexadecane, stearic acid, Ortho phthalamide, cetyl alcohol and its homologues and substituted amides of azeleic acid and sebacic acid were also tested. These examinations led to the following observations:

1. Highly polar compounds such as glycerine, diglycerol and carbowaxes retain polar compounds much longer but the alcohol peaks are broad and overlap with one another. Thus the quantitative estimation of ethanol, methanol and isopropanol in presence of one another is not possible.

2. Non-polar stationary phases such as mineral oil and hexadecane retain acetone longer than methanol and ethanol. However all peaks are highly unsymmetrical with pronounced tailing. In most cases there is an overlap of methanol and acetone peaks. Furthermore for aqueous solutions these columns gave a very unsteady baseline with a reverse peak following injection. This reverse

*Monsanto Chemical Company, Saint Louis, Missouri.

peak was attributed to very small retention volume of water on the column and its effect on the hydrogen flame.

Although these columns were not found suitable for quantitative analyses, 10% mineral oil on Fluoropak,* and 10% stearic acid on Anakrom ABS were used for confirmation of the identification of the compound detected by means of their retention volumes as shall be described later.

3. Stationary phases containing ester groupings such as didecylphthalate, were found to be affected by hydrolysis causing very unsteady baselines even at low column temperatures.

4. Long chain aliphatic alcohols such as cetyl alcohol and behenyl alcohol as well as substituted amides of long chain aliphatic dicarboxylic acids such as N,N,N',N',tetramethyl or N,N,N',N',tetraethylazeleamides or sebacamides were found most suitable for the required separation.**

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*The Fluorocarbon Company, Anaheim, California.

**Separation of ethanol, methanol and acetone. (The author wishes to thank C. P. Hall and Co., Chicago, Illinois, for free samples of some of these compounds, the remainder were synthesized as part of this work.)

The tetraalkyl diamides are extremely stable to hydrolysis and columns prepared using them can be used at temperatures up to 120° C. without difficulty. They give good separation between acetaldehyde, acetone and methanol, however, there is some overlap of alcohol peaks. Long chain alcohols, on the other hand, gave good separation of alcohols but there was slight overlap of acetone and methanol. Taking advantage of the additivity of the retention volumes due to fractional lengths of the column, various proportions of the two were successfully used in a mixed packing.

(A column with a mixed packing can be considered to be made up of numerous individual column segments in series. The retention volume of a substance in such a column is the total sum of the retention volumes in all the segments in the series.)

The separation of acetaldehyde, acetone, methanol, ethanol and paraldehyde obtained using one of the mixture columns is shown in the chromatograph (Fig. 3).

Procedure for the preparation of N,N,N',N',tetraethyl-sebaccimide:

Diethylamine (twice the theoretically required amount) was dissolved in ether and cooled in ice-bath. Sebacyl chloride was carefully added dropwise with constant stirring and cooling. After the addition was complete the mixture was extracted twice with 5% hydrochloric acid to

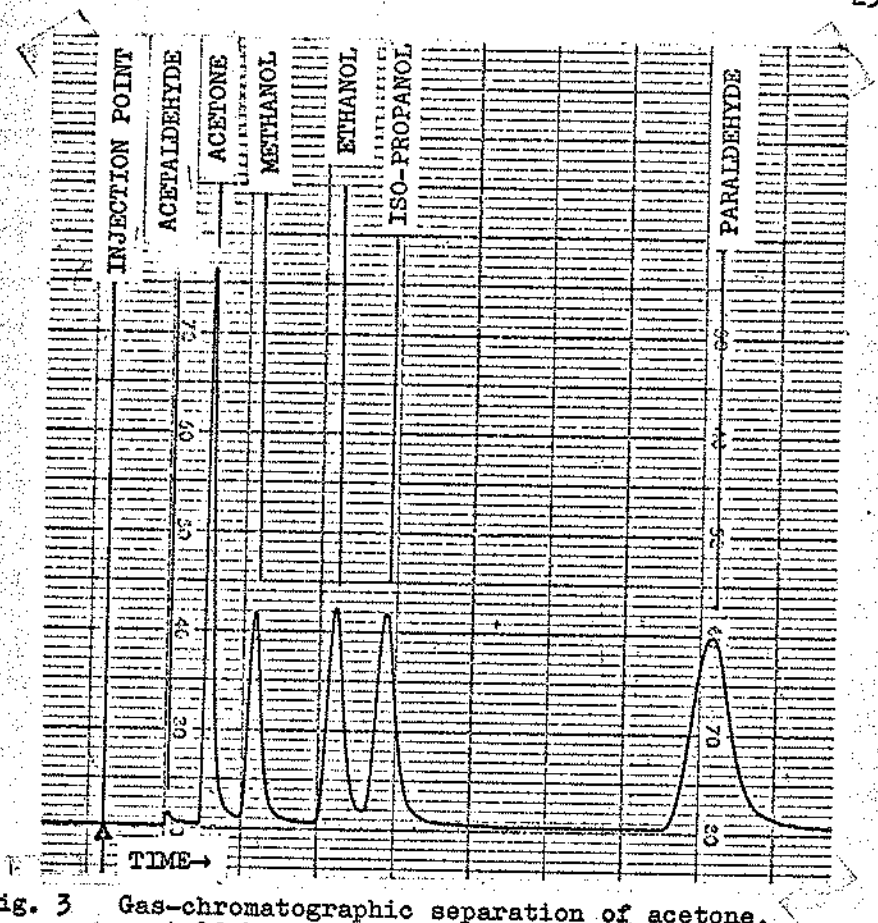


Fig. 3 Gas-chromatographic separation of acetone, acetaldehyde, methanol, ethanol, isopropanol and paraldehyde from dilute aqueous solution.

Column: 8' 6", 1/8" diameter, containing 10% behenyl alcohol and 5% N,N,N',N',tetraethylsebacamide on Anakrom ABS at 70° C.

Carrier gas: N_2, 20 mls./min. Hydrogen flow: 30 mls./min.

Injected sample: 5 microliters of aqueous solution containing acetone (0.1% v/v), ethanol (0.1% v/v), methanol (0.1% v/v), isopropanol (0.1% v/v) and slight trace of acetaldehyde.

remove the excess of the amine, then twice with 5% sodium hydroxide to remove any sebacic acid or sebacyl chloride and finally twice with distilled water. The ethereal solution was then filtered through anhydrous sodium sulfate and evaporated to give the substituted amide. This was further purified by column chromatography using reagent grade basic alumina and an eluent consisting of 15% chloroform in dry petroleum ether. After the complete removal of the solvent under vacuum the substituted amide was left behind as a pale yellow viscous liquid which was very difficult to crystallize and was used as such.

In the preparation of N,N,N',N',tetraisopropyl-sebacamide, the same procedure was used, using di-iso-propylamine in place of diethylamine. The residue of the substituted amine was obtained in the form of a solid which was recrystallized from ether by cooling in Dry-Ice-acetone bath. The resulting white crystals had a melting point of 74° C.

The amount of the stationary phase on the support material:

With the stationary phases and solid supports selected, a coating of 10% to 15% w/w of the stationary phase on the support was found suitable. If the proportion of the stationary phase is made lower, the tailing is increased while the retention volumes are decreased. On the other hand, if the proportion of the stationary phase is

higher, the peaks are broader and although the individual retention volumes are increased, the overlap of adjacent peaks is also increased.

The coating of the support with the stationary phase was done by flash evaporation. The stationary phase was dissolved in just enough chloroform to form a uniform thin slurry on the addition of the required amount of solid support. The slurry was stirred occasionally for a period of 15 minutes. Chloroform in the slurry was then evaporated rapidly on a steam bath with gentle but efficient and continuous stirring to prevent uneven coating. When the evaporation was apparently complete, the heating on steam bath was continued with frequent stirring till the smell of chloroform was undetectable. The coated support was then transferred to an oven at about 120° C. where it remained overnight.

The columns were packed by gently applying vacuum at one end of a column plugged with glass wool while introducing the coated support at the other end in very small portions with the help of a small paper funnel. For obtaining uniform packing the column was thoroughly tapped after each addition. Passing a stream of nitrogen through the columns at a rate of about 20 mls./min. at 120° C. for approximately 12 hours before use was found sufficient to produce steady baseline at the sensitivities required.

In the case of mixed columns the coating was carried out separately for each stationary phase before packing the required proportions of the coated support in the same column. The stationary phase percentages reported represent the percentages based on total column contents.

The column length for most of the experiments was 5 feet. Increasing it further was observed to have no special advantages. This can be partially explained on the basis of the following considerations: (1) Beyond an optimum length under one set of operating conditions, the number of theoretical plates per unit length of the column decreases rapidly. (2) For longer columns uniform packing becomes considerably difficult. For longer columns a higher carrier gas pressure is required in order to obtain the desired flow rate. Thus for very long columns the actual flow rate of the carrier gas in the initial portion of the column can be much smaller than its flow rate at the end of the column.

Operating parameters

Carrier gas flow rate: A rate of 15 to 30 mls. per minute was found suitable. The hydrogen flow rate was kept approximately the same as the carrier gas flow rate.

Temperature: As far as the temperature is concerned, lower temperatures give better separation, especially between ethanol and

isopropanol. However, with lowered temperature, the sharpness of the peaks decreases and the tailing increases since the adsorption on the solid support is greater at lower temperature. For substituted amide-heavy alcohol columns the optimum temperature was found to be between 65° and 75° and temperatures from 55° to 85°[7] gave satisfactory separations of acetaldehyde, acetone, methanol, ethanol, isopropanol and paraldehyde; these temperatures were used for all studies.

(C) Identification of Volatile Compounds in Expired Air:

The identification of the compounds detected in expired air was accomplished by the comparison of the retention times of their peaks on the chromatograph with those obtained from aqueous solutions of known substances under identical conditions of operation. Further confirmation can be obtained using the values of retention times of the peaks on another column with a stationary phase of quite different (polar on non-polar). The principle behind this procedure is that if two different compounds have the same retention time on one column, the probability of their having identical retention times on the other column is negligible particularly when the number of peaks obtained is rather limited in the region investigated as it is in this case. Mineral oil (10% (w/w) on Fluoropak

and 10% w/w stearic acid on Anakrom ABS were used as secondary columns for this purpose. It should be pointed out that although the separation obtained on these columns is unsatisfactory for quantitative analysis they can very well be used for qualitative identification. The retention volumes obtained on three of such columns are shown in Table II.

(D) Quantitative Estimation of the Compounds Detected in Expired Air:

The quantitative estimation of a compound from its peak on the gas-chromatograph may be done in two ways:

1. Peak Height method, and
2. Peak Area method.

Whereas the former measures the maximum response of the detector, the latter measures the integrated response. The choice between the two methods depends on the following major factors, among others:

(1) The type of the detector used.

(2) The nature of the peak, particularly with respect to the half width and the extent of tailing.

(3) The stability of the baseline, and

(4) The ease and accuracy with which peak area can be determined.

For a flame ionization detector the response is proportional to the concentration of the substance being detected in the carrier gas. The peaks obtained with the

Table II

RETENTION VOLUMES OF ACETONE, METHANOL AND ETHANOL ON DIFFERENT GAS CHROMATOGRAPHIC COLUMNS

Column	Retention volume* (mls. of N_2 per inch of column length) Acetone	Methanol	Ethanol
1. 10% stearic acid on Anakrom ABS. 89° C.	0.019	0.015	0.022
2. 10% N,N,N',N', tetramethyl-azeleamide on Anakrom ABS. 75° C.	0.30	0.56	0.73
3. 10% behenyl-alcohol and 5% N,N,N',N', tetraethyl-sebacamide on Anakrom ABS. 70° C.	0.34	0.39	0.6

*Uncorrected for mobile phase volume of the column.

gas-chromatographic set up described before are relatively sharp with a small half-width. Thus the determination of peak areas of these peaks will involve large error even when an electromechanical integrator is used. Hence the peak height method was used. Peak-height concentration relationships were determined for ethanol, isopropanol, paraldehyde and acetone over wider concentration ranges than those anticipated in these experiments. The results indicated that the peak-height is proportional to the concentration (for the same injection volume). As an example Figure 4 indicates the linearity of the peak-height with concentration for ethanol while Table III gives the values of peak heights at different concentrations along with the extent of variation in one of the columns used. (It must be pointed out that the variations observed include the injection-volume errors in the Hamilton 701 NCH syringe.) The results indicate that for very dilute solutions the percentage error is increased, but an average error of $\pm 5\%$ may be assumed for the range of concentrations observed in these studies. The peak height variations for isopropanol and paraldehyde are slightly lower while those for acetone and methanol were slightly higher than for the solutions of ethanol at similar concentrations.

It is important to mention that all the variables such as injection temperature, carrier gas flow rate,

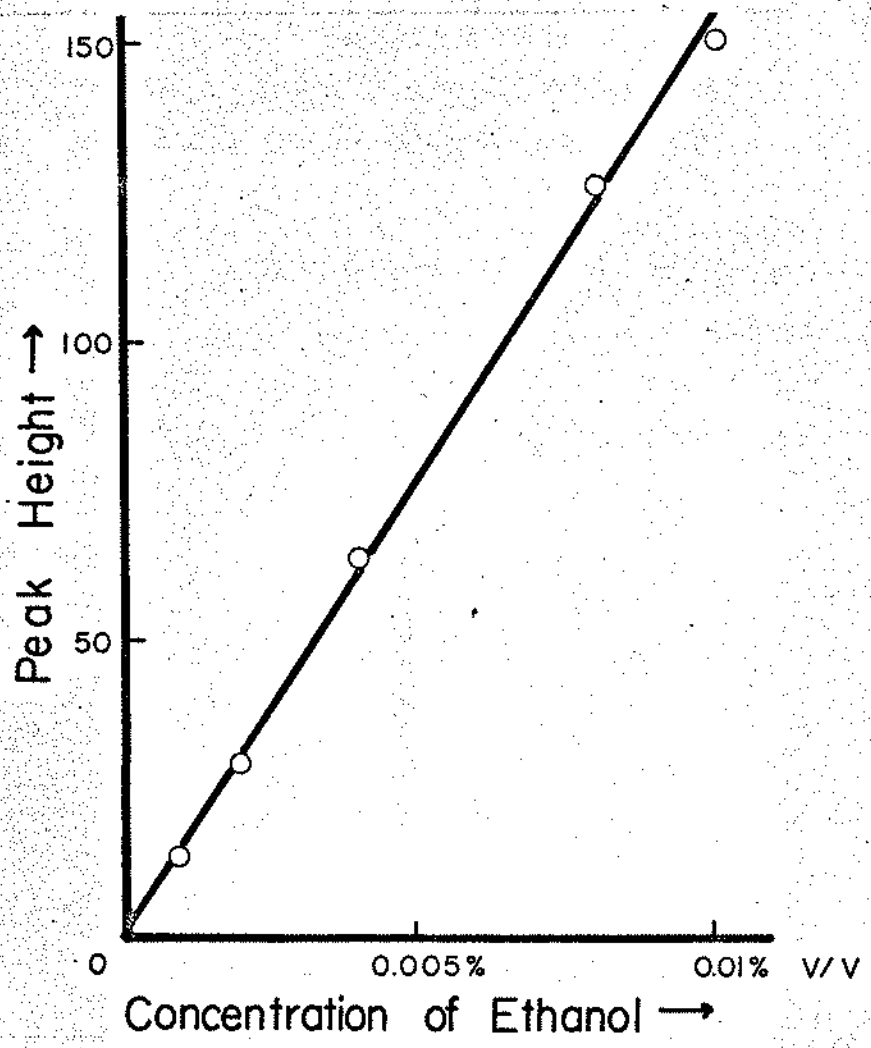


Fig. 4 Peak-height and concentration relationship.

Column: 5', 5% N,N,N',N',tetramethylazeleamide and 5% behenyl alcohol on Anakrom ABS.

Temp.: 85° C. H_2: 15 mls./min. N_2: 20 mls./min.

Injection volume: 5 microliters.

Table III

LINEARITY OF PEAK-HEIGHT AND CONCENTRATION

FOR ETHANOL AND ITS MAXIMUM VARIATION RANGE*

Concentration of ethanol	0.001%	0.002%	0.004%	0.008%	0.01%
Mean peak-height	14.5	29.6	64.5	127	151
Maximum variation range	±6%	±3.8%	±2.3%	±0.7%	±0.8%

*Column: 5', 5% N,N,N',N',tetramethylazeleamide and 5% cetyl alcohol on Anakrom ABS.

Temp.: 85° C.; N_2: 20 mls./min.; H_2: 15 mls./min.

Injection volume: 5 microliters.

hydrogen flow rate must be kept rigidly constant since the variations in any of these factors will cause much larger errors in the peak-height method than in the peak-area method.

During every experiment, standard dilute solutions of the substances being estimated were injected using the same injection volume setting as for the samples and under identical conditions. The peak-heights from these standard solutions were used as the standard peak-heights for calculation purposes.

EVALUATION OF THE CONDENSATION SCHEME

As it was our purpose to use quantitative condensation for breath collection, studies of the collection scheme were carried out. Their object was:

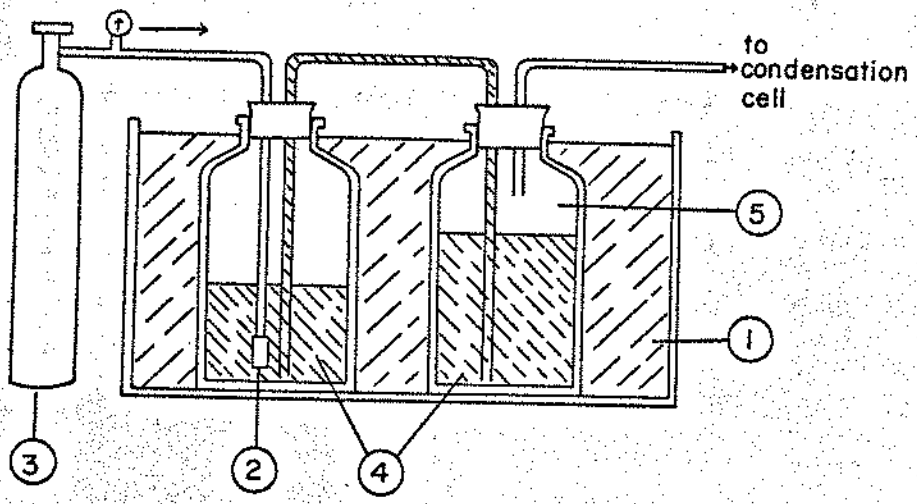
(1) To check the reproducibility of obtaining condensate from the same expired air sample, under conditions similar to those used for the collection of the samples.

(2) Comparison of the results obtained for the condensate from a known sample with the theoretically calculated value.

The known sample used in this case was air in equilibrium with a solution of ethanol of a known composition. Ethanol was selected because the value of its partition coefficient between air and water at 37° C. has been reported in the literature.[10]

The experimental set up used for this experiment is illustrated in Fig. 5.

Two 15-liter bottles were immersed in a constant temperature waterbath at 37° C. One of the bottles was almost completely filled with a 0.1% solution of ethanol while the other was empty. Air was bubbled into the filled bottle very slowly through a gas-dispersion tube located near the bottom and the solution allowed to flow into the other bottle under the pressure of the air. After



1 - Constant temperature bath

2 - Gas dispersion tube

3 - Compressed air cylinder fitted with regulator valve

4 - Dilute aqueous solution of ethanol at 37° C.

5 - Air in equilibrium with the ethanol solution

Fig. 5 The experimental set up for the evaluation of the condensation scheme used for the collection of expired air samples.

nearly three-fourths of the solution had flowed out into the other bottle, the air supply was stopped and the air already in the bottle allowed to come into equilibrium with the ethanol solution for a period of three days. At the end of this period a small portion of this solution was withdrawn and analyzed on the gas-chromatograph to determine the exact concentration of ethanol in it.

For the evaluation experiment the bottle containing the air was then connected to the condensation cell immersed in liquid nitrogen. By using compressed air and a regulator valve the liquid from the solution bottle was pumped into the air bottle at a predetermined rate displacing the air in equilibrium with ethanol solution through the condensation cell that was being held under conditions identical to those used for the collection of expired air samples. By previous experiment the pressure of the compressed air required for obtaining the desired flow rate of the air sample was determined. This rate was approximately the same as the collection rate used for the collection of the expired air samples. After 8 liters of the air had passed through the cell, the cell was disconnected, quickly stoppered, and the condensate analyzed in the manner described previously; the concentration of ethanol in it was calculated. Comparison of this value with the value obtained from the analysis of the solution by using the partition coefficient of

ethanol between water and air (Harger and co-workers)[10] indicated that the value obtained from the condensate was 73% ±3% of the value obtained by using the partition coefficient. The variation in this value was considered within the limits of experimental error in view of the:

1. Low accuracy of determination of the distribution coefficient of ethanol between air and water in very dilute solutions.
2. Large temperature coefficient of the distribution coefficient (12% per degree at 30 degrees C.),[10] and
3. The possibility of slight entrainment of very small droplets of the solution along with the air during the rapid transfer of solution from solution bottle to air bottle.

SECTION II

EXHALATION OF VOLATILE ORGANIC COMPOUNDS IN HUMAN EXPIRED AIR UNDER NORMAL CONDITIONS

The first significant attempt to detect volatile organic compounds present in human expired air by gas-chromatography was reported by Southwestern Research Institute[11] in 1960. The volatile compounds were collected by adsorption on cooled ascarite. Subsequently they were desorbed by warming and the vapor analyzed by gas-liquid chromatography on a Carbowax column so that compounds originally in concentrations as low as a few parts per billion in expired air could be detected. The compounds reported to have been detected by this procedure include acetone, acetaldehyde, methanol, ethanol, furan, methylfuran, isoprene, propionaldehyde, dimethyl sulfide, diethyl sulfide and four unidentified compounds. However, no quantitative data is reported.

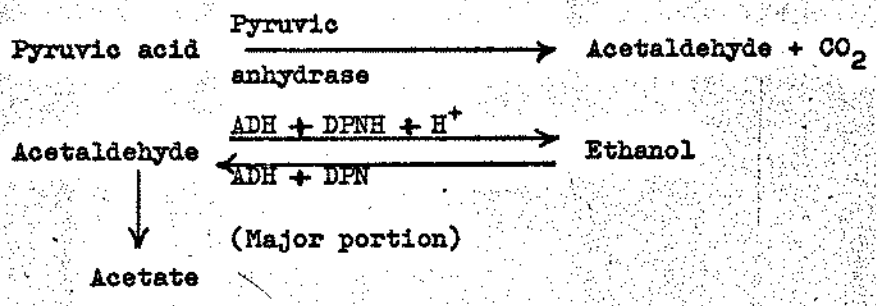
With the method reported in this thesis, the compounds such as ethanol, methanol, isopropanol and acetone can be detected in expired air in concentration as low as 0.001 micrograms per liter and the sensitivity could be raised by as much as ten times with better baseline control. The sensitivity for compounds with heavier molecular weights is somewhat lower. Analysis of expired air of several subjects revealed that acetone, methanol and ethanol are

present in human expired air at all times. Their concentrations in expired air vary not only from subject to subject but from time to time in the same subject as indicated in the sample data shown in Table IV and Fig. 6.

Ethanol in normal human expired air:

Ethanol content of expired air, varied from 0.01 μg./L. to 1.01 μg./L. and was in agreement with values of concentration of ethanol in blood in normal subjects reported by Lester[12] (using dist. coeff. value for ethanol between air in blood at body temperature as reported by Harger[10]). After meals or after ingestion of glucose the ethanol content of expired air increased severalfold attaining concentrations as high as 2.75μg./L.

The presence of ethanol in expired air can be explained on the basis of its endogenous production in the body from pyruvic acid by the following mechanism:



McManus, Contag and Olson[13] have confirmed this explanation with the use of radioactive pyruvid acid.

Table IV

CONCENTRATION OF ETHANOL AND METHANOL IN THE EXPIRED AIR OF SEVERAL HUMAN SUBJECTS UNDER NORMAL CONDITIONS

Subject Number	Concentration in Expired Air (μg./L.) Ethanol	Methanol
1	0.011	0.06
2	0.045	0.078
3	0.027	0.074
4	0.094	0.206
5	0.37	0.32
6	0.20	0.49
7	1.11	0.35
8	0.17	0.35
9	0.83	0.32
10* (a)	0.527	0.253
10* (b)	0.544	0.170

*These samples were taken at two different times from a subject after antibiotic treatment of the gastro-intestinal tract.

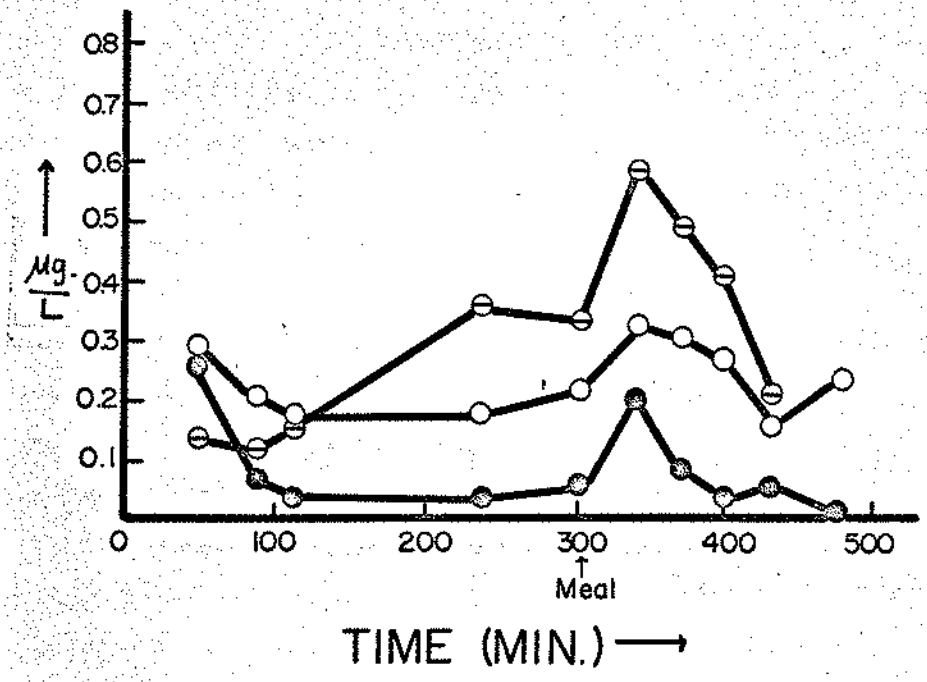


Fig. 6 Variation in the concentration of acetone (-○-), methanol (-⊖-) and ethanol (-●-) in the expired air of a representative human subject.

Methanol in normal human expired air:

The concentration of methanol in expired air was observed to be ranging from 0.06 µg./L. to 0.49 µg./L.

Western and Osburn[14] have suggested that all 'normal methanol' in humans results from dietary sources. Some support to this point of view may be found since some foods, particularly pectin containing fruits contain or produce methanol during growth or processing.[14,15] Tobacco also contains methanol.[16] However, both the investigations of Western and Osburn and the investigations reported herein show no direct relationship between methanol in expired air and smoking or diet. This contention is supported by observations of methanol concentration in expired air after intake of food and after long periods of fasting, such as those reported in Table V.

Observation with other subjects also showed that methanol content of expired air increased after meals reaching a maximum between 1 to 2 hours. According to Kendal and Ramanathan[17] complete absorption of a small orally ingested dose of methanol takes about 24 hours in man. In view of this the origin of methanol in expired air from dietary sources is possible.

On the other hand according to available information in the literature regarding the elimination of methanol at low concentrations,[44] its biological half-life in man appears to be between 6 to 7 hours. Thus if the origin of methanol

Table V

VARIATION IN THE CONCENTRATION OF METHANOL IN THE EXPIRED AIR OF A REPRESENTATIVE HUMAN SUBJECT AFTER FASTING AND AFTER INTAKE OF FOOD

Period of Fasting	Methanol Concentration (μg./L. in expired air)
3 Hrs.	0.54
15	0.18
16	0.45
17	0.35

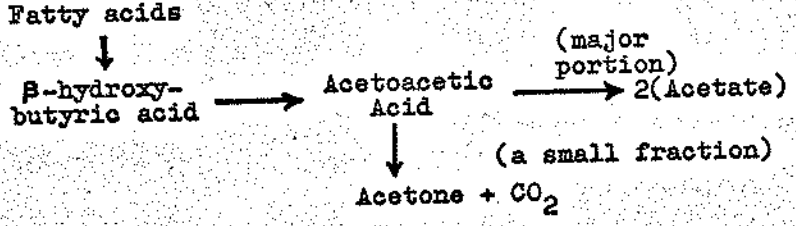
Time After Lunch	Methanol Concentration (μg./L. in expired air)
0.5 Hrs.	0.28
1	0.72
1.5	1.95
2	0.44
3	0.64

in expired air is attributed to dietary source alone, the methanol content of expired air after long periods of fasting must be extremely low. This seems quite unreasonable in view of the data presented. Thus if a subject shows a methanol content of 0.54 µg./L. after fasting for 3 hours, the methanol content after fasting for 17 hours should drop to about 0.12 µg./L. The actual value found however was 0.35 µg./L. (in subject A as indicated before).

No significant change in the methanol content of expired air was observed in case of subject B after rather extensive antibiotic treatment of the gastrointestinal tract. This suggests that the formation of methanol in the gastrointestinal tract as a result of bacterial action is very unlikely. Thus it seems more likely that methanol is produced as a result of some metabolic process. There seems to be, however, no current explanation based on a metabolic degradation process that would produce methanol.

Acetone in normal human expired air:

The presence of acetone can be attributed to decarboxylation of aceto-acetic acid.



In a recently reported work, Gordon and Goldberg[18] administered 1-C^{14} glucose, 6-C^{14} glucose, 1-C^{14} palmitate and 1-C^{14} β-hydroxybutyric acid intravenously to normal human subjects and measured $C^{14}O_2$ content of their expired air at regular intervals. It was found that after administration of 1-C^{14} palmitate $C^{14}O_2$ was detected in expired air in a few minutes and reached peak activity within 20 to 30 minutes. $C^{14}O_2$ content of expired air after administration of the same activity of 1-C^{14} glucose or 6-C^{14} glucose was much smaller and reached its peak value after approximately 80 minutes. The authors suggested from these results that metabolic degradation of fats goes on in the body at all times, although the amount of fat oxidized may be replenished by the formation of fats from carbohydrates. In presence of such constant and active fat metabolism the by products of fat metabolism could be expected to be formed and might well explain the presence of acetone as a normal constituent of expired air.

The concentration of acetone in expired air was found to vary from 0.08 to 3.5 μg./L. More than 90% of the samples showed acetone content between 0.16 and 0.23 μg./L. After vigorous muscular activity and after meals the acetone content of expired air increased. This is in general agreement with the qualitative observations of Forssner[19,20] regarding the increase in the elimination of acetone bodies in urine after muscular work and after intake of food.

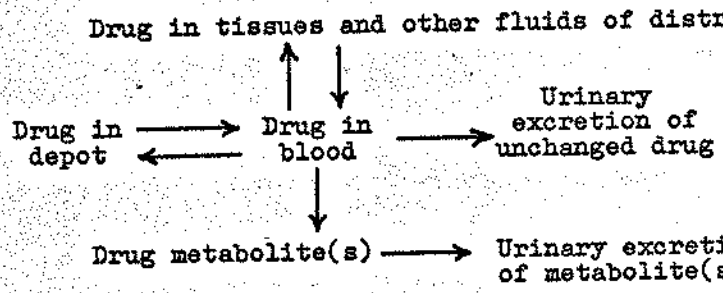
APPLICATION OF GAS CHROMATOGRAPHIC ANALYSIS OF EXPIRED AIR TO THE STUDY OF KINETICS OF ELIMINATION OF ADMINISTERED VOLATILE COMPOUNDS

The gas chromatographic method developed as a result of this research and described in Section I offers a significant tool for the investigation of metabolism of administered compounds which are either volatile or give rise to volatile products on degradation in the body. Using this technique the kinetics of elimination of ethanol, acetone, isopropanol and paraldehyde was studied. Paraldehyde is mainly eliminated as such in urine and expired air although a very small fraction may be oxidized in the body. In comparison to this ethanol is mainly oxidized in the body while a small fraction is eliminated as such in urine and expired air. Both isopropanol and acetone when administered show evidence of interconversion in the body. In both the cases the elimination takes place mainly in the form of acetone. Thus these administered compounds belong to three distinctly different categories. The results of the exhalation studies are considered in the light of the theoretical considerations of the elimination kinetics of orally administered compounds as elaborated in the next subsection.

A. Theoretical Consideration of Elimination Kinetics of Orally Administered Compounds

As described earlier if a compound administered is sufficiently volatile the information about its concentration in expired air can be used for understanding its elimination kinetics from the blood.

The first systematic attempt at mathematical interpretation of blood level data of administered compounds was made by Teorell[36] in 1937. He suggested the relatively simplified scheme for drug distribution in the body as shown:



The drug depots, blood, urine, etc. are considered as separate compartments, and the passage of the drug from one to another is considered to be regulated by one or more rate constants. In most cases these constants are shown to be first order rate constants. Even though this scheme is rather simple, it was found adequate enough to explain the kinetics of absorption, distribution, excretion and metabolism of many substances.

The next systematic attempt at mathematical interpretation of blood level data of orally administered compounds was a modification of Teorell, by Wagner and Nelson.[21] The method given by them consists of consideration of different kinetic models based on the assumption of various patterns of absorption, excretion and metabolism of the administered compound. By comparison of the blood level data with the blood level patterns deduced from different models, important information can be obtained about the kinetics of elimination and in certain cases also the kinetics of absorption of the administered compound.

The models considered by Wagner and Nelson[21] include,

(1) Zero order absorption followed by first order elimination.

(2) Zero order absorption by two simultaneous zero order absorption processes independent of each other followed by first order elimination.

(3) First order absorption followed by first order elimination.

(4) Absorption by two simultaneous independent first order absorption processes followed by first order elimination.

(5) Absorption by simultaneous first order and zero order absorption processes followed by first order elimination.

The main purpose behind these models was to explain the absorption and elimination kinetics of various dosage forms including sustained release preparations.

A modified approach based on the work of Wagner and Nelson can be used for the consideration of elimination kinetics of volatile compounds. The assumption of a constant "apparent volume of distribution" (V) is important for the consideration of these models. The concept of apparent volume of distribution was first introduced by Dominguez[22] in connection with kinetics of absorption of certain drugs. Although in certain cases it may be directly related to blood volume or total body fluid volume, it should be pointed out that it is strictly a mathematical constant relating the amount of the administered compound absorbed (A) and its concentration in blood (C) in absence of elimination or metabolic transformation. Thus in the absence of elimination or transformation $A = C \cdot V$.

When the volume of the orally administered dosage form is relatively small, the effective volume of the source of absorption process (V^1) is usually assumed to be a constant relating the concentration of the compound in the gastrointestinal fluid (G) at any time, and the amount of unabsorbed compound (U) at the same time, such that $U = V^1G$.

The first order elimination constant Ke similarly is the total sum of all independent first order elimination processes of excretion and metabolic degradation.

Model I - <u>First order absorption followed by first order elimination</u>

$$G \xrightarrow{k_a} C \xrightarrow{k_e}$$

When the concentration of the compound being absorbed is relatively low in the absorption source as well as in cases where the absorption is more or less diffusion controlled it is logical to assume that absorption takes place by first order process. In such a case,

$$\frac{-dG}{dt} = k_a G$$

$$G = G_o e^{-k_a t} = \frac{A_t}{V^1} e^{-k_a t} ,$$

$$\frac{dC}{dt} = \frac{V^1}{V}\left(-\frac{dG}{dt}\right) - k_e C = \frac{A_t k_a}{V} e^{-k_a t} - k_e C$$

All terms have their previous definitions, G_o is the initial gastro-intestinal concentration, and A_t is the total drug administered. Integration of this equation followed by substitution of the integration constant with the value obtained from the initial conditions ($t = o$, $C = o$) one obtains,

$$C = \frac{k_a A_t}{V^1(k_e - k_a)} \left(e^{-k_a t} - e^{-k_e t} \right)$$

Thus for relatively large values of t,

$\frac{d(\ln C)}{dt} = -k_a$ if k_a is smaller than k_e and

$\frac{d(\ln C)}{dt} = -k_e$ if k_e is smaller than k_a.

Thus the smaller of two rate constant becomes the rate determining constant.

Thus the apparent half life of disappearance of the substance observed in such a case is not necessarily $(0.693/k_e)$, as usually assumed, unless there is sufficient evidence that k_a is greater than k_e.

The semilogarithmic plot of C versus time in this model will show an apparent lag time the value of which will depend on the relative magnitudes of k_a and k_e, other factors being (unassumed) constant.

Model II - Zero order absorption followed by first order elimination

$$G \xrightarrow{k_a^o} C \xrightarrow{k_e}$$

When the dose of the administered compound is relarively large or if the absorption takes place through a transport mechanism that is able to be saturated at relatively low concentrations of the compound being absorbed, the absorption is likely to be a zero order process. If this is followed by a first order elimination process, then,

$$\frac{dC}{dt} = k_a^o - k_e C$$

where k_a^o is the zero-order rate constant for absorption and all other terms have their previous meanings. Integration of this differential equation followed by substitution of the value of the integration constant using initial conditions t = o, C = o, one obtains,

$$C = \frac{k_a^o}{k_e}\left(1 - e^{-k_e t}\right)$$

Thus C exponentially approaches a maximum steady state value of $\frac{k_a^o}{k_e}$. For any finite total amount (A_t) of the administered compound, however, the absorption is complete at $t = \frac{A_t V^1}{k_a^o V}$ and the corresponding value of the maximum blood level is,

$$C_{max.} = \frac{k_a^o}{k_e}\left(1 - e^{-\frac{k_e A_t V^1}{k_a^o V}}\right)$$

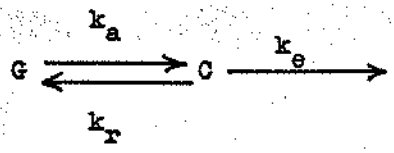
Beyond this point there is no further absorption

$$\frac{dC}{dt} = k_e C \quad \text{or} \quad \frac{d(\ln C)}{dt} = -k_e$$

Thus beyond $C_{max.}$ the decrease in C becomes first order with respect to time with the apparent half life of

$(0.693/k_e)$. In this case, in the semilogarithmic plot of C versus time, no convex portion can be expected as the concentration decreases from C_{max}.

Model III - <u>First order absorption with simultaneous recycle and first order elimination</u>



Many substances when orally administered are known to exhibit enterohepatic recycling. These substances are absorbed from the gastro-intestinal tract and on passage through the liver are partially re-excreted into the gastro-intestinal tract. In such case if k_r denotes the first order re-excretion constant then,

$$\frac{-dG}{dt} = k_a G - k_r C \quad \text{(i)} \quad \text{and} \quad \frac{dC}{dt} = \frac{V^1}{V}\left(\frac{-dG}{dt}\right) - k_e C$$

$$= \frac{k_a V^1 G}{V} - \left(k_e + k_r V^1/V\right) C \quad \text{(ii)}$$

Initially, when C is very small in comparison to G

$$\frac{-dG}{dt} = k_a G$$

$$G = G_o e^{-k_a t} = \frac{A_t}{V^1} e^{-k_a t}$$

$$\frac{dC}{dt} = \frac{k_a A_t}{V} e^{-k_a t} - (k_e + k_r V^1/V)C$$

Thus for initial portion the equation assumes the form similar to that for two consecutive first order processes as in model I.

If k_a and k_r are relatively much larger than k_e, a steady state is soon reached such that G/C is nearly equal to k_r/k_a and therefore substituting the value of G in the equation for $\frac{dC}{dt}$, one obtains,

$$\frac{dC}{dt} = -k_e C \qquad \text{or} \qquad \frac{d(\ln C)}{dt} = -k_e$$

and the value of the final apparent half life is $(0.693/k_e)$.

In the portion between these two extremes the relationship between C and time is complex and can be given by the simultaneous solution of the differential equations (i) and (ii), a difficult process mathematically, but the graphs for such a solution may easily be produced by analog computation.

B. Studies on Exhalation Kinetics of Paraldehyde

Although paraldehyde was widely used as a hypnotic for a long time, the information about its elimination kinetics is virtually absent in the literature. As mentioned earlier most of the paraldehyde administered is excreted unchanged although a small portion may be metabolized. In the studies reported herein, exhalation

kinetics of paraldehyde was studied after oral administration of a small dose.

Two milliliters of paraldehyde were administered to the fasting subject in hard gelatin capsules with as little water as possible to ensure that the absorption took place as rapidly as possible without oral contact. At regular intervals the expired air was analyzed according to procedure described previously. The experiment was repeated with several subjects.

Results:

Fig. 7 shows a typical semi-logarithmic plot of the concentration of paraldehyde versus the time after administration. It shows the following features,

1. The absorption of paraldehyde was very rapid as indicated by steep initial rise of the curve.
2. The curve soon assumed the form of a short "plateau" which lasted for a period of 2 to 3 hours.
3. The plateau was followed by a rapid fall of concentration of paraldehyde in expired air which continued until it finally became exponential.

Since the absorption appears to be very rapid in the initial period, it can be assumed that the absorption process is not the rate-determining step in the elimination kinetics of paraldehyde. Hence the apparent half life values obtained from the semilogarithmic plots are

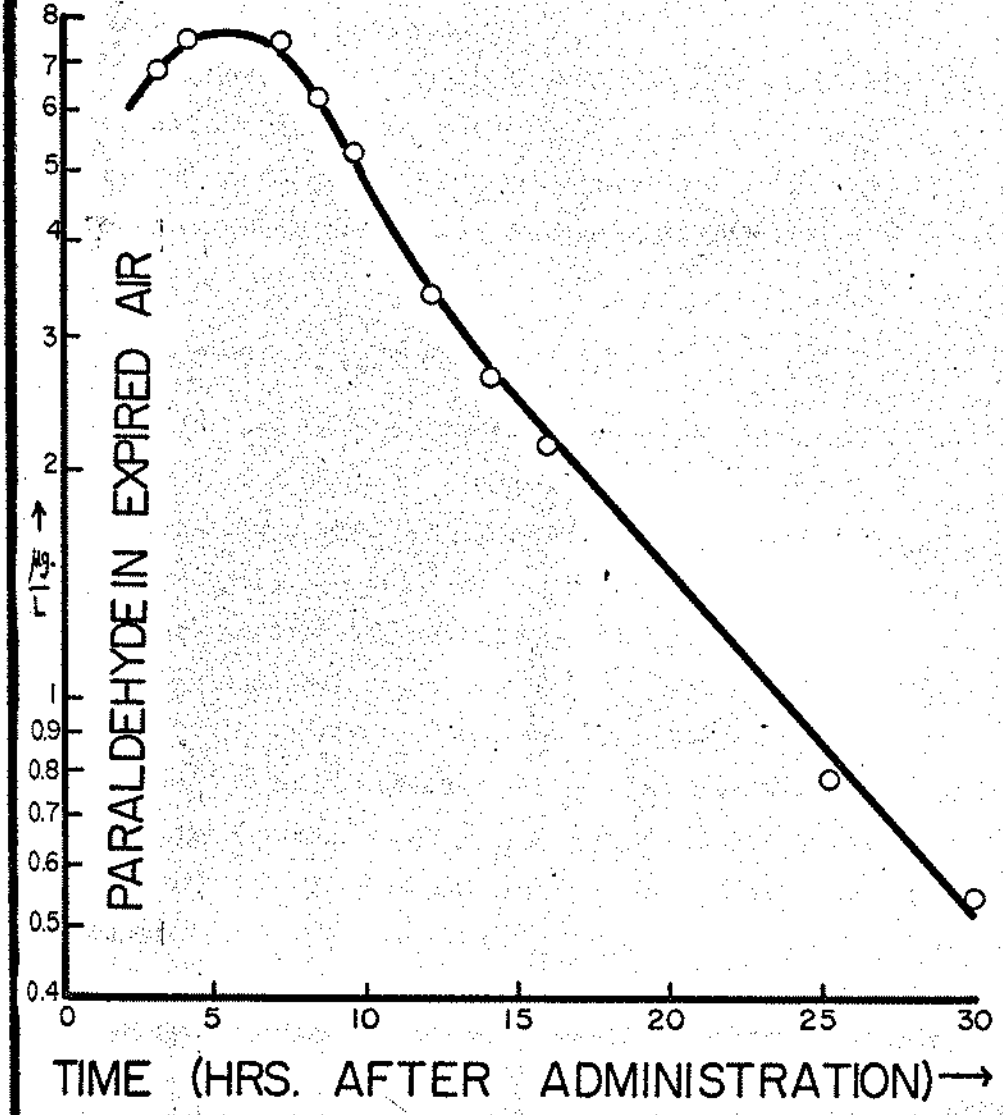


Fig. 7 Exhalation kinetics of paraldehyde following oral administration of 2 mls. of paraldehyde to a fasting subject.

assumed to be the values determined by the values of the elimination rate constants.

The values of apparent half life and the approximate values of time after administration after which the decrease in the paraldehyde concentration in expired air became exponential (apparent lag time) are listed in Table VI.

Discussion of the results:

The apparent half life of paraldehyde varied between 280 min. and 435 min. In general the variation of the apparent half life for different studies made with the same subject was found to be much less than that between different subjects, as might be expected.

It was observed that in all subjects the experiments in which the values of apparent lag-time were smaller, the half life of paraldehyde was also smaller, suggesting that experiments in which the absorption was faster, the apparent half life of paraldehyde was smaller. However in view of the various models considered earlier in this section this observation was attributed to coincidental variation. Wagner[23] in his article on the interpretation of semilogarithmic plots of blood level and urinary excretion data considers that the rate constant for the clearance of the drug in the body should be independent of the route of administration in a given species.

TABLE VI

EXHALATION KINETICS OF PARALDEHYDE FOLLOWING ORAL ADMINISTRATION OF 2 mls. OF PARALDEHYDE TO FASTING SUBJECTS

Subject	Expt. no.	Apparent lag-time	Apparent half life
A	1	11 hrs.	405 min.
A	3	13.5 hrs.	405 min.
B	1	3 hrs.	280 min.
B	2	9 hrs.	435 min.
C	1	11 hrs.	405 min.
C	2	less than 5 hrs.*	375 min.
D	1	less than 6 hrs.*	340 min.
D	2	less than 6 hrs.*	296 min.
D	3	6 hrs.	370 min.

* In these experiments the value of apparent lag time could not be correctly estimated from the data recorded.

Volenbruck[24] had observed in case of ethanol elimination curves at different doses and after fasting and after food intake, that despite large variations in absorption processes the elimination curves became equal in all cases after complete absorption. This indicates that the variation in the absorption rate of a compound cannot lead to a variation in the apparent half life of the compound even if the absorption is relatively slow.

Thus in the absence of any reasonable explanation the variation between the values of apparent half life and apparent lag time are assumed to be due to biological variation.

C. A Brief Survey of the Previous Methods of Study of Elimination Kinetics of Ethanol

The first significant attempt at mathematical interpretation of elimination kinetics of ethanol in experimental animals was made by Widmark.[25] After administration of alcohol to dogs, samples of their blood were analyzed[26] at regular intervals. Alcohol from the samples was distilled out and absorbed in 3N sulfuric acid. A known amount of potassium dichromate was added to oxidize ethanol quantitatively to acetic acid and the excess of oxidizing agent was estimated bromometrically. The results indicated that the elimination of ethanol was a zero order process. Various modifications in the

dichromate method of determination of alcohol level of blood were suggested by different workers. Notable among these were the methods suggested by Haggard and Greenberg[27] and by Harger[28] in 1934 (later revised and improved[5]). These methods used expired air samples for the first time instead of blood samples.

A much more sensitive method of determination of ethanol concentration in blood based on the reaction between ethanol and diphospho-pyridine nucleotide (DPN) in presence of crystalline alcohol dehydrogenase was developed by Bucher and Redetzki[29] and by Theorell and Bonnichsen[30] in 1951. The method is based on the determination of the initial velocity of the reaction,

$$\text{Ethanol} + \text{DPN} \rightleftharpoons \text{Acetaldehyde} + \text{DPNH} + \text{H}^+$$

in a buffered solution at known constant pH. The alcohol is first quantitatively distilled from the blood sample and the aqueous condensate is used for analysis. Semicarbazide is used to suppress the reverse reaction by reacting with the acetaldehyde formed.

$$\frac{-d(\text{DPN})}{dt} = k\,(\text{EtoH}) \times (\text{DPN})$$

The velocity of the reaction is determined by measuring the decrease in the absorbance of DPN at 340 mμ in a spectrophotometer.

Results obtained by Fritz and Marshall[31] indicate that the enzyme method is very accurate and can be used to determine alcohol concentrations in blood as low as 10 mg. % (which approximately corresponds to the ethanol level of 50 µg/L. in expired air). The analysis of dog blood showed that the accuracy of the method was within ±5% at an ethanol level of 30 mg.%.

The first gas-chromatographic method for determination of the concentration of ethanol in blood for medico-legal purposes was reported by Parker and co-workers[32] in 1962. This method employed direct injection of blood samples into a gas chromatograph using a column of 40% Castor wax on Chromosorb and flame ionization detector. The effective range of determination of blood alcohol concentration was reported to be from 23 mg.% to 180 mg.% with an accuracy of ±5%.

This column shows an overlap between acetone and ethanol peaks, however, and since acetone is always present in blood, this method is unsuitable except when the blood level of ethanol is very high. Furthermore since this method uses direct injection of blood samples the column has to be changed very often. Similarly special devices such as rolled stainless steel screens have to be used with varying success to protect the injection port from contamination and also must be replaced often.

A critical examination of the methods of determination of the concentration of ethanol in blood indicates that although many of them (particularly the enzyme method) may be extremely accurate at high concentrations of ethanol in the blood, none of them has a sensitivity comparable to the method based on the condensation of expired air reported in this work.

D. A Brief Survey of the Past Work on the Elimination Kinetics of Ethanol

Widmark[25] who made the first systematic investigation of elimination kinetics of ethanol, concluded that the elimination of ethanol from the body is a zero order process and thus obeys the following relationship,

$$A = Wr\ (C_t + Bt)$$

where A is the total amount of ethanol consumed, W is the body weight and C_t is the concentration of ethanol in blood at time t, r and B being two constants.

Widmark's conclusion was soon challenged by various workers. Haggard and Greenberg,[33] using the method of analysis of expired air by dichromate method, found the elimination of ethanol to be a first order process with an average elimination rate constant 0.18 per hr.

Lundquist and Wolthers[34] demonstrated in 1958 that the oxidation of ethanol by diphosphopyridine nucleotide

(DPN) in presence of alcohol dehydrogenase and simulated physiological conditions proceeds according to the familiar Michaelis-Menten kinetics. They also found using the more sensitive ADH-DPN method of analysis, that when ethanol is administered in small or moderate doses, about 2% of it is eliminated in expired air and about 1% in urine the remaining being eliminated by oxidation in the body. Hence they suggested that the elimination of ethanol from the body follows a mechanism for which one may write the equation,

$$-\frac{dC}{dt} = \frac{V}{K_m + C} C$$

V and K_m are constants.

Thus at very high concentrations where K_m is assumed very small compared to C, $-\frac{dC}{dt}$ is nearly equal to V. This accounts for the pseudo-zero order elimination at relatively high blood levels of ethanol.

On the other hand at very low blood levels, C is small compared to K_m and hence the elimination becomes pseudo-first order with $k_e = (V/K_m)$.

The integration of the original differential equation gives,

$$\frac{C_1 - C_2}{t_2 - t_1} = V - \frac{K_m}{t_2 - t_1} [\ln (C_1/C_2)]$$

In the experiments carried out by Lundquist and Wolthers[34] with human subjects after ingestion of ethanol the analysis of alcohol concentration in blood at various intervals seemed to confirm the suggested mechanism. The values of V were also close to the values of $V_{max.}$ for crystalline liver alcohol dehydrogenase under the physiological conditions of pH, ionic strength and DPN concentration.

The results of the investigations of Lundquist and Wolthers are listed below.

Constant	Range
K_m	1.52 to 2.53
V	54 to 77 mM./L./min.
Apparent half life period for the pseudo-first-order portion	13.5 to 20.5 min.

E. Studies on the Exhalation Kinetics of Ethanol

The object of these studies was to study the elimination kinetics of ethanol, particularly at very low concentrations where the previous methods of analysis are unsuitable due to limited sensitivity.

Procedure

Seventy-five milliliters of 33% v/v ethanol was orally ingested by the subject within one minute. Each subject then rinsed his mouth with 2 successive portions of 50 mls. of water in order to reduce the error which

would have otherwise been caused by the residual ethanol on the oral surface. At regular intervals the amount of ethanol in expired air was determined by the condensation method described before. A blank experiment was done without ingestion of ethanol, by just holding 33% v/v ethanol in mouth for one minute followed by rinsing the mouth twice with 50 mls. of water.

It was found that the error caused by the residual ethanol even after rinsing the mouth twice with 50 mls. of water was not insignificant in the beginning. However the extent of error dropped very sharply with time and for readings beyond 20 minutes the required correction was negligible. (Fig. 8)

Ethanol was administered as a 33% v/v solution because as indicated by studies of Lolli and Rubin[35] and in preliminary studies by this author the absorption of alcohol can be expected to be very rapid at this concentration. The same amount of alcohol if administered in higher concentration would produce gastric irritation and would delay absorption due to stimulation of secretion of gastric juice.

The determination of the concentration of ethanol in expired air was continued till it was below 1 µg./L.

Results and discussion:

A typical graph of the concentration of ethanol in the expired air following ingestion of 75 mls. of 33% v/v

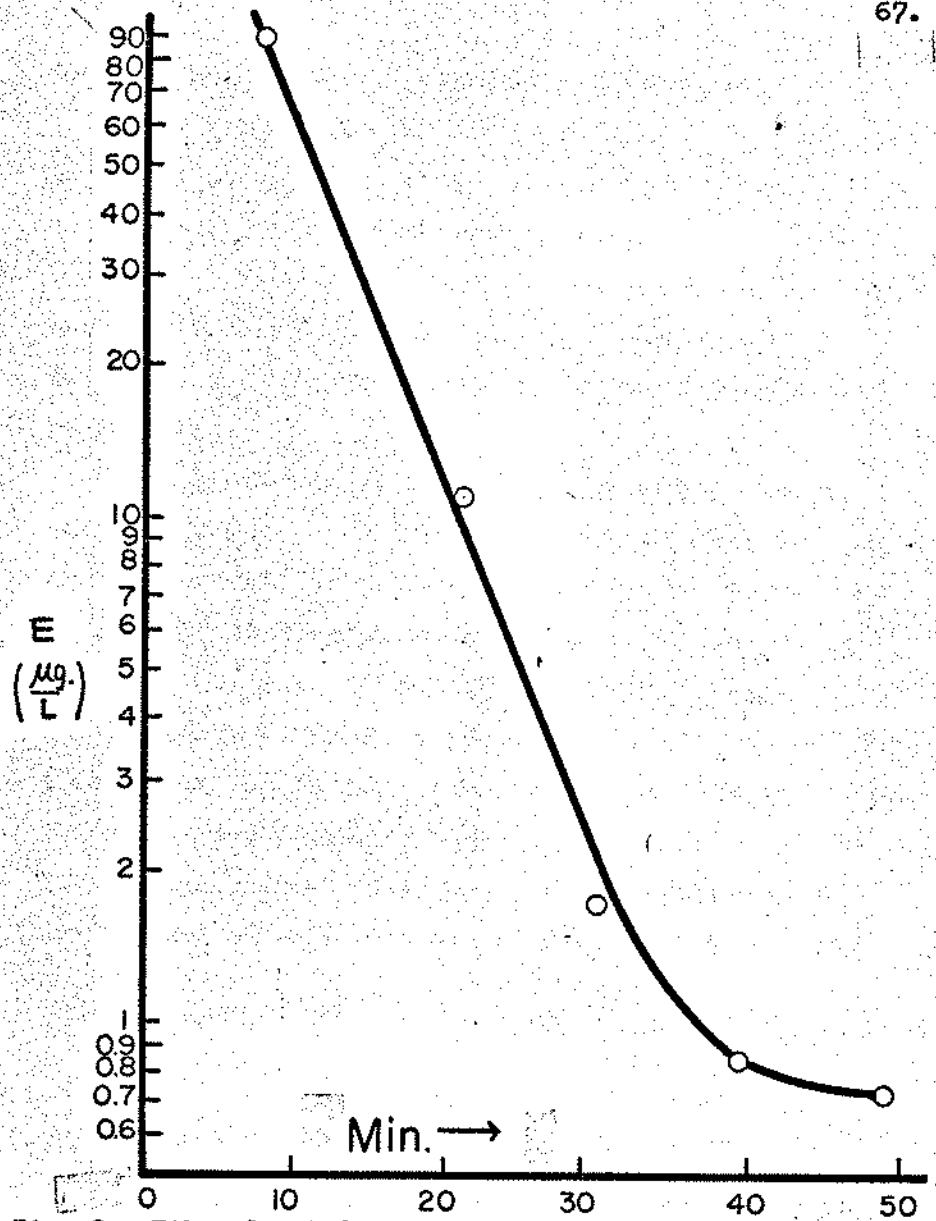


Fig. 8 Ethanol exhalation correction for oral administration.

E - Error in the concentration of ethanol in expired air caused by the oral administration of 33% v/v ethanol according to the procedure described.

ethanol by fasting subjects is indicated in Fig. 9.

The initial rise of the concentration of ethanol in expired air was very rapid. The maximum concentration was reached within 100 to 170 minutes.

The portion of the curve beyond the maximum could not be suitably fitted to the equation suggested by Lundquist and Wolther[34] based on Michaelis-Menten type mechanism.

The apparent half life values obtained from the semilogarithmic plots of ethanol concentration in expired air versus time for different subjects are listed in Table VII. They range from 20 min. to 30 min. as compared to 13.5 min. to 20.5 min. as would be expected from (V/km) values obtained from the results of the investigations of Lundquist and Wolthers.[34] Thus although it appears that the elimination of ethanol is not a simple first order process it does not seem to follow closely the kinetic model suggested by Lundquist and Wolthers.[34]

F. Determination of the Concentration of Acetone in Blood

The chemical determination of the concentration of acetone in blood is complicated by the presence of substances such as β-hydroxybutyric acid and acetoacetic acid which are collectively referred to as "acetone bodies." Hence although there are several methods for the determination of acetone bodies in blood reported in the literature there are very few methods reported for the

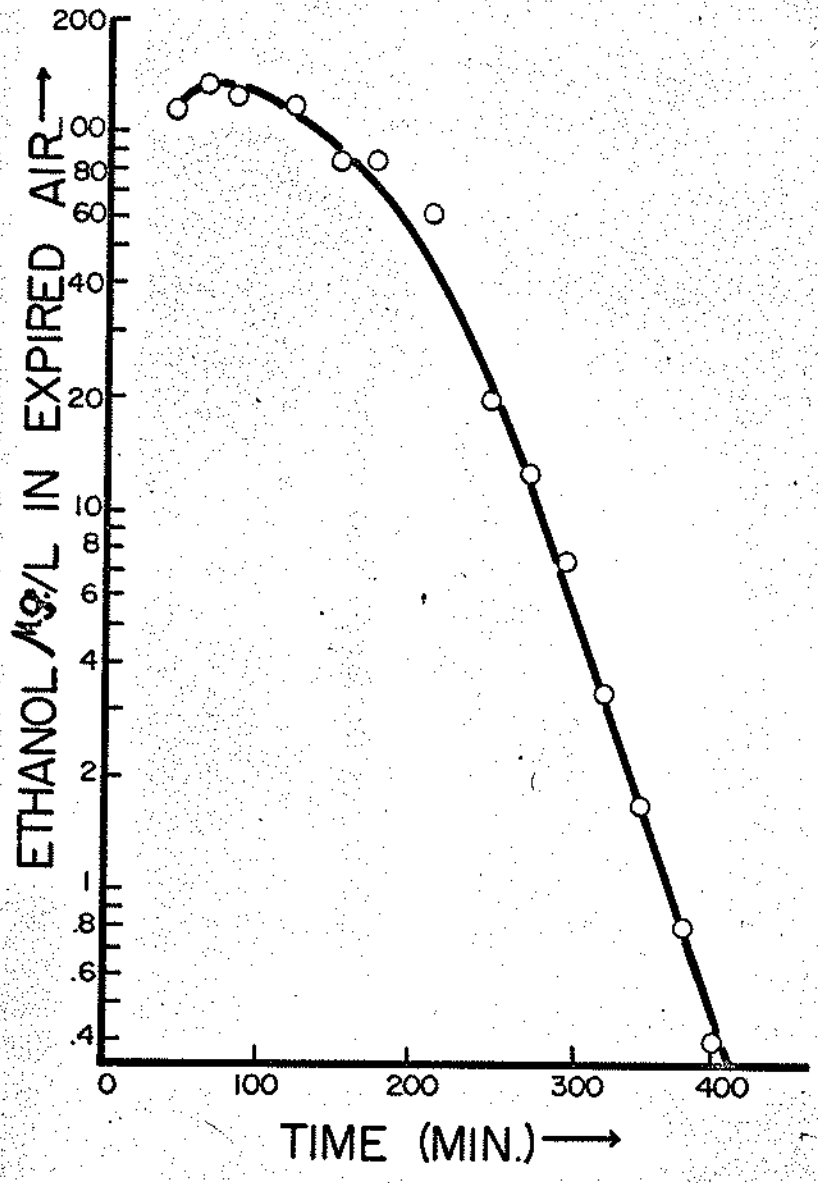


Fig. 9 Exhalation kinetics of ethanol following oral administration of 75 mls. of 33% v/v ethanol.

Table VII

EXHALATION KINETICS OF ETHANOL FOLLOWING ORAL ADMINISTRATION OF 75 mls. of 33% v/v ETHANOL TO FASTING SUBJECTS

Subject	Expt. no.	Apparent half life (min.)
A	1	30
	2	24
B	1	20
	2	28
C	1	26
	2	28
D	1	30
E	1	26

determination of only acetone in blood. The most important one from the point of view of sensitivity is probably the method reported by Dumazert and Garrigues.[37] This method based on conversion of acetone to difurfurylidene-acetone followed by colorimetric estimation at 5350 A° is reported to estimate amounts of acetone as small as 1 µg. with an accuracy of ±5% (when preceeded by microdistillation of sample). Thus this method can be considered to be effective for determination of acetone concentration in the blood as low as 1 mg./L.

With the gas-chromatographic method described in Section I concentrations of acetone in expired air as low as 0.01 µg./L. can be determined with comparable accuracy. Using the partition coefficient of acetone between blood and air as determined by Haggard, Greenberg and Turner[38] (330:1) this would correspond to a blood concentration of approximately 33 µg/L. Thus this method is about 300 times as sensitive as the method by Dumazert and Garrigues with the possibility of much higher sensitivity with better baseline control.

G. Studies on Exhalation Kinetics of Isopropanol and Acetone

Both acetone and isopropanol are widely used as industrial chemicals and therefore had attracted the attention of industrial toxicologists. Scopinuro, Gluani and Cecco[40] found that when acetone is administered

intravenously, a part of it is converted to aceto acetic acid and β -hydroxybutyric acid in the body and the other part is excreted in urine and in expired air. Price and Rittenberg[41] using radioactive acetone found that the activity was transferred to various different substances in the body such as fatty acids, urea, glycogen and cholesterol. This suggested that acetone was partly oxidized to acetate in the body and then underwent a variety of biochemical transformations involving various metabolic cycles. It was also known for a long time that isopropanol is oxidized in the body to acetone. However practically no information is reported in the literature about the elimination kinetics of acetone or isopropanol. In the investigations reported herein, small doses of acetone and isopropanol were administered orally and the kinetics of their elimination and interconversion was studied.

Procedure:

Acetone or isopropanol (2 mls.) was administered orally in hard gelatin capsules with a small quantity of water to fasting subjects. At regular intervals the concentration of acetone and isopropanol in expired air was determined by the method described in Section I. The results of the investigation are reported in Tables VIII, IX and X. Fig. 10 and 11 indicate the typical graphs of variation of the concentration of acetone in expired air

Table VIII

APPARENT HALF LIFE VALUES OF ACETONE IN EXPIRED AIR FOLLOWING

(i) ORAL ADMINISTRATION OF 2 mls. OF ACETONE, AND

(ii) ORAL ADMINISTRATION OF 2 mls. OF ISOPROPANOL TO FASTING HUMAN SUBJECTS

Subject	Expt. no.	Apparent half life (min.) (i)	(ii)
A	1	235	159
	2	126	190
B	1	140	175
	2	160	170
C	1	100	140
	2	125	138
D	1	160	120
	2	165	135

Table IX

RELATIVE CONCENTRATIONS OF ACETONE AND ISOPROPANOL IN EXPIRED AIR AFTER ORAL ADMINISTRATION OF 2 mls. OF ACETONE TO A FASTING HUMAN SUBJECT

Time After administration (Hrs.)	Concentration in expired air µg./L.		Ratio of Acetone/Isopropanol in expired air*
	Acetone	Isopropanol	
6.5	648	21.3	30.4
9	487	20.3	24
11	314	11.5	27.4
13.5	166	8.5	19.5
16.5	40.2	0.71	56.5

*Average ratio of the concentration of acetone to the concentration of isopropanol in expired air = 31.6.

Table X

RELATIVE CONCENTRATIONS OF ACETONE AND ISOPROPANOL IN EXPIRED AIR AFTER ORAL ADMINISTRATION OF 2 mls. OF ISOPROPANOL TO A FASTING HUMAN SUBJECT

Time after administration (Hrs.)	Concentration in expired air µg./L. Acetone	Isopropanol	Ratio of acetone/isopropanol in expired air*
6	425	8.34	51
8.25	421	9.35	45
10	289	9	32.1
12.25	211	7.85	26.9
14.5	68	2.3	29.6
16.2	43	1.3	33.2

*Average ratio of the concentration of acetone to the concentration of isopropanol in expired air = 36.1.

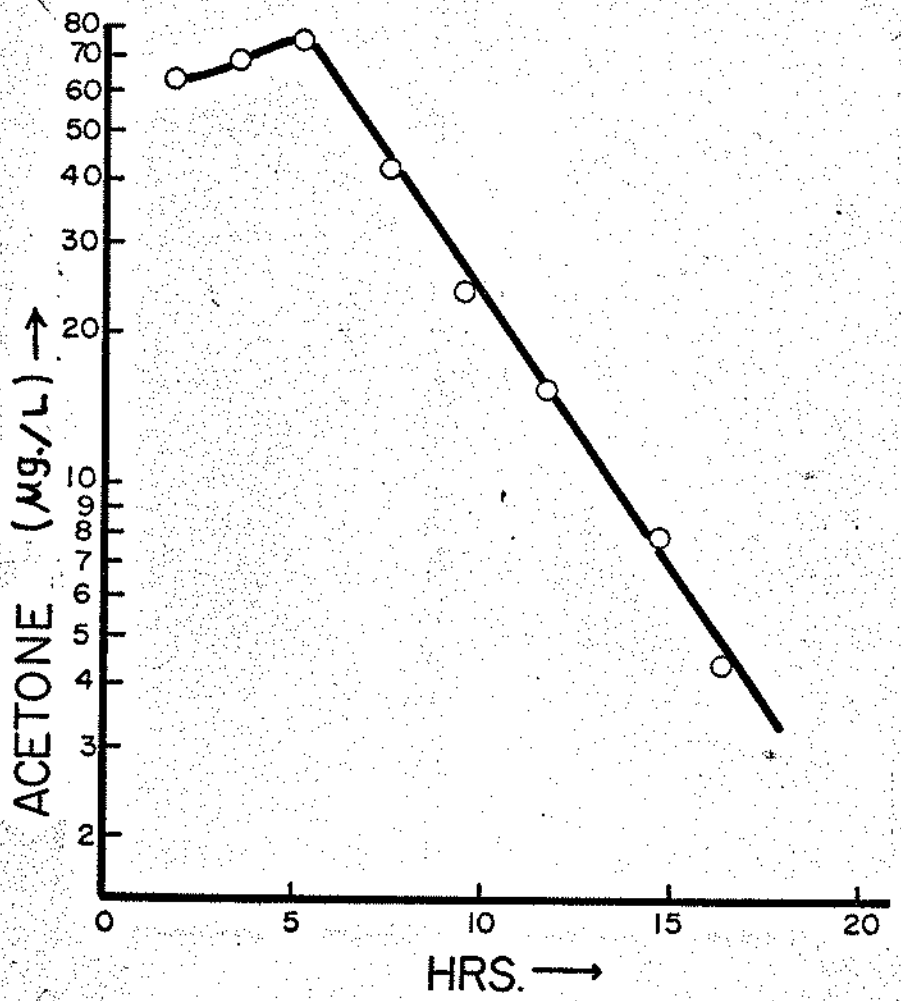


Fig. 10 Concentration of acetone in expired air following oral administration of 2 mls. of acetone to a fasting subject.

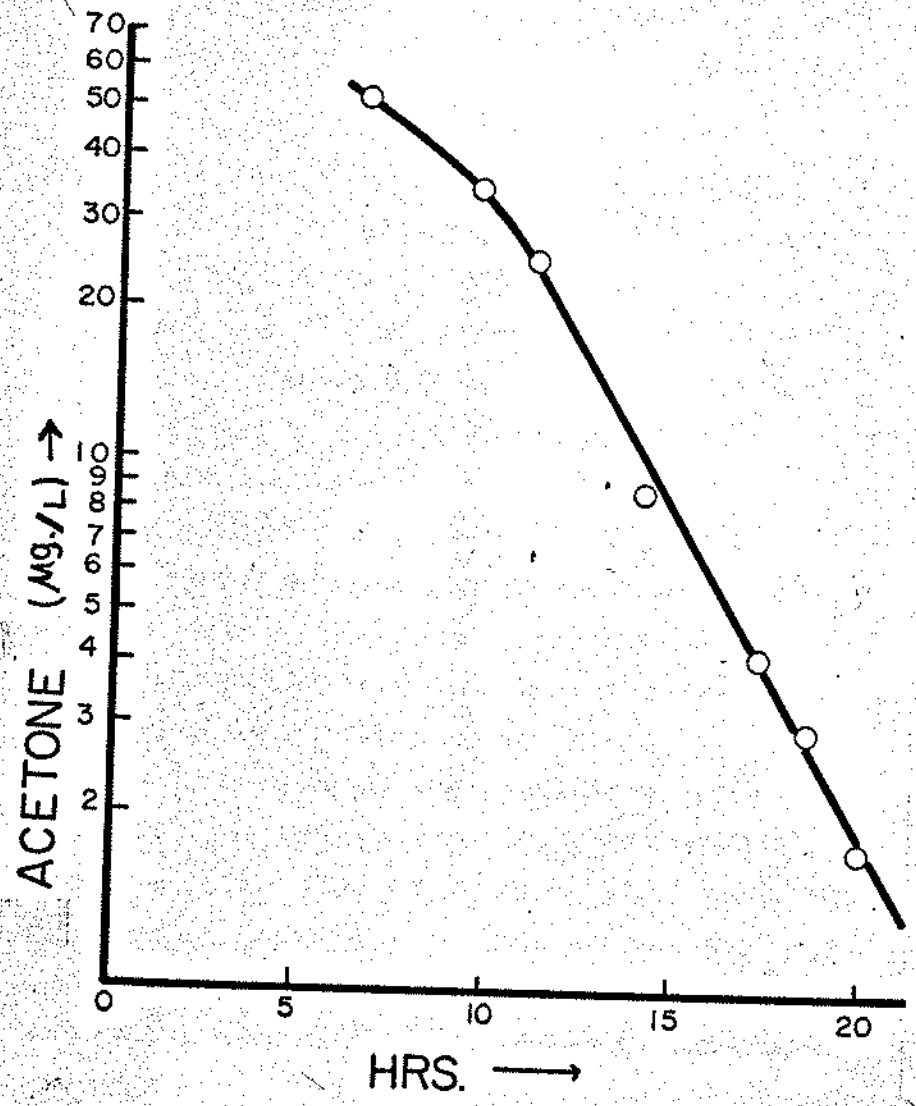


Fig. 11 Concentration of acetone in expired air following oral administration of 2 mls. of isopropanol to a fasting subject.

following oral administration of acetone and isopropanol respectively to fasting human subjects.

Results and discussion:

Both isopropanol and acetone are absorbed very rapidly.[39] The results show that both undergo interconversion in the body as clearly demonstrated in Tables IX and X. In both cases the concentration of acetone is much higher than that of acetone in expired air as well as in blood. Thus isopropanol is mainly eliminated in the form of acetone in expired air and in urine rather than as unchanged isopropanol.

The increase in the concentration of acetone in expired air was very sharp after the administration of either acetone or isopropanol. The maximum concentration is reached within 3 to 6 hours the period varying greatly in different experiments.

The decrease in the concentration of acetone in expired air was soon observed to become exponential with respect to time. There was considerable variation in the apparent half life values obtained in different experiments. The extent of the variation was larger than in case of the apparent half life values for elimination of paraldehyde or ethanol as reported in the earlier part of this section. The apparent half life of acetone in expired air following oral administration of acetone varied from 100 min. to 235 min. with an average value of

151 min. Following oral administration of isopropanol the apparent half life of acetone in expired air varied from 120 min. to 190 min. with an average value of 152 min. The semilogarithmic plots of the concentration of acetone in expired air following oral administration of acetone and of isopropanol with respect to time are nearly the same.

Thus it appears that the process of interconversion of acetone and isopropanol in the body is extremely rapid compared to the process of the elimination of either of the two and that the elimination of both occurs by the same pathways, namely,

1. Excretion in the form of acetone in expired air and urine.
2. Excretion in the form of isopropanol in expired air and urine, and
3. Oxidation to aceto acetic acid and acetate.

It was therefore logical to investigate whether isopropanol and acetone were in equilibrium concentrations in the body during the later parts of the experiment when sufficient time had elapsed after the absorption of all (or practically all) of the administered substance. The results are listed in Tables IX and X. The estimated ratios of the concentration of acetone and isopropanol were not constant, but varied from 19.5 to 56.5. The variation was found random and had no apparent relationship

to the blood-level of acetone (or isopropanol). The average values of the ratio however were 31.6 after the administration of acetone and 36.1 after the administration of isopropanol.

In view of relatively large standard deviation in the determination of acetone and isopropanol concentrations particularly at lower values, the variation in the estimated values of the ratio of the concentration of acetone to that of isopropanol may be considered to be within the limits of biological variation.

Thus the results suggest that acetone or isopropanol when administered in small doses undergo interconversion reaching an equilibrium with each other and follow the same pathways of elimination.

CONCLUDING REMARKS AND THE SCOPE OF FUTURE WORK

The study of exhalation of organic compounds in human expired air has assumed increasing importance in recent years. It has attracted the attention of workers whose primary interest lies in widely different fields, such as clinical investigation, industrial toxicology, biochemistry and even space-biology. In view of its very high sensitivity, convenience and speed the method of analysis of microquantities of organic compounds in expired air described in Section I can be considered to be a major contribution for investigations in the fields mentioned above. Its scope might be significantly increased if used in conjunction with radio-isotope technique in the investigation of metabolism of volatile compounds.

In the fields of pharmacy and pharmacology the technique described in Section I suggests many uses, especially for the investigation of absorption and excretion kinetics of drugs.

Another interesting possibility may be its application to studies of the <u>in vivo</u> disintegration of enteric coated and sustained release dosage forms such as those containing a substance that on absorption is rapidly transformed in the body to a volatile organic compound.

Finally it may also be possible to use this method for the investigations of pulmonary absorption of organic compounds for which the sensitivity of the method has to be extremely high.

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ABSTRACT

Exhalation Kinetics of Some Volatile Organic Compounds

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(Under the supervision of Associate Professor Stuart Eriksen)

The analysis of expired air for the determination of blood concentrations of administered compounds that are relatively volatile offers several advantages over direct analyses of blood or urine samples. Due to extremely large surface of the pulmonary membranes, the volatile organic compounds are distributed between the blood and the alveolar air in a manner closely approximating their partition coefficients. Since alveolar air is also saturated with respect to water, a flash condensation of alveolar air results in an aqueous solution the analysis of which can be used for the calculation of the concentration of the volatile organic compounds in alveolar air and thus in blood. For experimental purposes the use of vital capacity air equilibrated by holding the breath at the point of maximum inspiration for a short period can be shown to give the same results as alveolar air.

Since the vapor pressure of water is 47.1 mm. at body temperature the flash condensation technique gives a condensate in which the concentration of the volatile organic compounds originally present in expired air samples is increased approximately 22,800 times their original concentration in the expired air. This coupled

with the high sensitivity of gas-chromatographic analysis offers a highly sensitive method for the analysis of volatile organic compounds in expired air at very low concentrations. It also has the advantage of specificity as compared to microchemical methods of estimation.

Gas chromatographic separation of a dilute aqueous solution containing a number of organic compounds such as ethanol, methanol, acetone, acetaldehyde and paraldehyde is complicated by the broadening of the chromatographic peaks of the individual compounds by the large excess of water present as solvent. The stationary phases recommended for gas-liquid chromatography of alcohols, ketones etc. were found very unsatisfactory.

After a series of exhaustive investigations columns prepared from a combination of stationary phases (N,N,N',N',tetraethylsebacamide and behenyl alcohol and other closely related combinations) were found to give satisfactory separation of the compounds mentioned before. The analysis was done using flame ionization detection and the peak height method for quantitative estimations.

An investigation of peak height studies for analysis of solutions of ethanol, isopropanol, paraldehyde and acetone at different concentrations indicated that the peak height method of quantitative estimation has good reproducibility and that peak height for a given substance is proportional to its amount over a wide range under identical conditions of analysis.

The sensitivity of the method was found to be sufficient to estimate compounds such as ethanol, methanol, acetone and paraldehyde in expired air in concentrations as low as 0.001 μg./L.

Analysis of expired air of several normal human subjects indicated that ethanol, methanol and acetone are always present in human expired air. Their concentrations in expired air varied from 0.01 to 1.01 μg./L., from 0.06 to 0.49 μg./L. and from 0.08 to 3.5 μg./L. respectively. The presence of methanol in expired air cannot be attributed to dietary source alone because expired air of subjects after long period of fasting did not show the expected large decrease in the concentration of methanol in expired air upon fasting. A subject after extensive antibiotic treatment of gastrointestinal tract showed no significant change in the methanol concentration in his expired air thereby suggesting that the presence of methanol in the body cannot be attributed to its formation in gastrointestinal tract by bacterial action.

Elimination kinetics of paraldehyde, ethanol, acetone, and isopropanol in human subjects following ingestion of relatively small doses was studied.

After administration of 2 mls. of paraldehyde in hard gelatin capsules, the apparent half life of paraldehyde in 10 subjects varied from 280 min. to 435 min. The variation in absorption was however much greater.

After oral ingestion of 75 mls. of 33% v/v ethanol, the absorption was very rapid reaching the maximum level of ethanol in blood in less than one hour in almost all cases. The semilogarithmic plot of ethanol concentration in expired air versus time showed a distinct convex nature even after sufficient time after the time when absorption can be assumed to be completed. However this portion of the curve did not obey the equation suggested by Lundquist and Wolthers based on the Michaelis-Menten mechanism. The decrease in the concentration of ethanol then became exponential with an apparent half life varying from 20 min. to 30 min. in different subjects.

Acetone and isopropanol show clear evidence of interconversion in the body. The apparent half life values of acetone concentration in expired air, and the average ratio of the estimated concentration of acetone to that of isopropanol in blood at different times following the administration of acetone and following the administration of isopropanol were nearly the same. This suggests that the process of interconversion of acetone and isopropanol in the body is much faster than the process of elimination of either one and that both isopropanol and acetone thus share the same pathways of elimination.

Approved: Stuart Eriksen

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