Acetaldehyde concentrations in alveolar air following a standard dose of ethanol in man

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SUMMARY A method for serial gas chromatographic determinations of acetaldehyde, acetone, and ethanol in alveolar air is described. The concentration of alcohol in alveolar air increased rapidly and then declined linearly following ingestion of ethanol, 0.5 ml/lb body weight, drunk as a 10% solution in 30 min. Acetaldehyde in alveolar air at first increased rapidly to a maximum concentration, then decreased only slightly for several hours despite rising or falling ethanol concentrations. The concentration of acetaldehyde then declined rapidly, beginning at the time when the ethanol concentration had returned to 15–25 μg/100 ml alveolar air.

The concentrations of acetaldehyde in alveolar air appear to correspond to hepatic production. The plateau of acetaldehyde concentrations is probably due to the constant rate of ethanol oxidation over a wide range of ethanol concentrations.

Intra-individual variations of replicate tests are small. Comparison of a group of five healthy control subjects with two alcoholics suffering from alcoholic brain damage showed elevated acetaldehyde curves in the latter group. Acetaldehyde concentrations were in the upper normal range in a jaundiced alcoholic suffering from hepatic cirrhosis. Elevated acetaldehyde concentrations may possibly be causally related to alcoholic brain damage.

KEY WORDS ethanol · acetaldehyde · acetone · alveolar air · breath sampling · man · gas–liquid chromatography · alcoholism · alcohol tolerance test

ACETALDEHYDE IS THE FIRST and the only specific oxidation product of ethanol in the intermediary metabolism and is present in the blood after the ingestion of alcohol (1). It has been postulated (2) that acute and chronic exposure of the brain to acetaldehyde may be responsible for brain damage incurred by chronic alcoholics. In equivalent concentrations acetaldehyde is approximately 200 times more effective than ethanol in inhibiting potassium-stimulated brain respiration in vitro (3). A change of cerebral metabolism in alcoholics compared with normal subjects is suggested by the work of Sutherland, Burbridge, Adams, and Simon (4). However, knowledge concerning the metabolism of acetaldehyde in man is limited because the determination of serum acetaldehyde in the physiological range without premedication with disulfiram (Antabuse) has been technically difficult and unreliable (5, 6).

The presence of acetaldehyde in expired air after premedication with Antabuse and alcohol was established by Hald and Jacobsen (7). Since the boiling point of acetaldehyde is 21° and acetaldehyde diffuses readily through tissue (10), equilibration of the venous blood entering the lung with alveolar air is expected to be rapid, so that alveolar air concentrations would essentially reflect the production of acetaldehyde in the liver. Peripheral venous blood is probably less useful in this respect because tissues are known to be capable of metabolizing acetaldehyde, and venous acetaldehyde concentrations are the result of both hepatic production and tissue utilization. Furthermore, when acetaldehyde is determined in alveolar air, other less volatile aldehydes which usually interfere with the classical color reactions in blood are avoided.

The purpose of this publication is to describe a gas chromatographic method for the rapid, serial determination of acetaldehyde and ethanol in expired air and the application of this method to normal subjects after a standard dose of oral ethanol. Preliminary findings in three alcoholic patients are presented.

MATERIALS AND METHODS

All subjects, after fasting for at least 12 hr, except where specifically mentioned, drank a 10% solution of ethanol (USP) in unsweetened orange juice during 30 min. The standard total dose of ethanol was calculated as 0.5

ml/lb body weight. This amount was varied for the purpose of one experiment as described. The healthy nonalcoholic subjects were males ranging in age from 31 to 52 yr. One subject drank an acetaldehyde solution without ethanol. All subjects remained in a chair or in bed for the duration of the test. Exercise, where indicated, consisted of six deep knee-bends with outstretched arms at the beginning of each minute. All subjects abstained from smoking during the experiment unless stated to the contrary.

Alveolar air samples were collected by having the subject breathe at the end of expiration into a 5 × 7 cm plastic tube occluded at one end by a rubber septum perforated by a 1-inch piece of $^1/_8$ inch o.d. metal tubing. Through the septum approximately 5 ml of gas was drawn at the end of deep expiration into a 10 ml gas-tight syringe (Hamilton Co., Whittier, Calif.). Chamber and syringe were rinsed with nitrogen after each collection. Four milliliters of this sample were injected into the flash heater. A control air sample was analyzed before the alcohol was given. The subject's mouth was rinsed with water immediately after all the alcohol was consumed (rinsing of the mouth with ethanol without actual ingestion of it can result in measurable amounts of ethanol in the exhaled air for 2–3 min thereafter).

Gas–Liquid Chromatography

An F and M Model 400 gas–liquid chromatograph with a hydrogen flame detector was used. The 10-ft column, of $^1/_8$-inch o.d. stainless steel, was packed with 40% castor wax on 60–80 mesh Gas Chrom R P (firebrick). The operating temperatures were: column, 103°; flash heater, 140°; detector, 220°. The carrier gas was nitrogen ("Zero Gas," Matheson Co., Inc. Rutherford, N. J.) with a flow rate of 30 ml/min. The chart speed was 0.5 inch/min. The range setting was 1 × and the attenuation varied during each analysis from 2 × for the acetaldehyde to a maximum of 64 × for the ethanol. Each day several standardizations with 50 mμg of ethanol were performed as follows: 1.3 ml of absolute ethanol was diluted to 100 ml with distilled water. Ten microliters of this aqueous solution was injected into a rubber-capped, nitrogen-filled, 1000 ml foil-wrapped flask, to give a standard containing 0.1 μg of ethanol per ml of gas. Five hundred microliters (50 mμg) were injected into the flash heater with the same 1 ml gas-tight syringe (Hamilton) for each standardization, resulting in a peak height of approximately 5 inches at an attenuation setting of 2 ×. When compared with freshly prepared vapors this ethanol standard remained stable at room temperature for at least 6 months. The linearity of response was established in the appropriate ranges. The retention times (initial) in minutes were: acetaldehyde, 2.1; acetone, 3.1; unknown (ethyl acetate), 3.8; ethanol, 5. A typical graph is shown in Fig. 1. Acetic acid was not detectable in the column used.

Under the conditions described above, ethanol vapors gave rise to only one peak, whereas commercially available acetaldehyde (Matheson Coleman and Bell, Norwood, Ohio) produced several small peaks in addition to the main one, with longer retention times and a total area less than 5% of that of the acetaldehyde. The measurement of peak height was found to be as satisfactory as the measurement of peak area for the routine test. The peak height ratio of ethanol to acetaldehyde in a standard mixture (0.1 μg of each component per milliliter of nitrogen) remained constant and was 1.1:1 for these concentrations. The precision of replicate injections of 50 mμg of ethanol in the vapor standard (0.5 ml) was ±4 mμg at the 95% confidence level.

RESULTS

A 38-yr old healthy male drank the same amount of ethanol under identical conditions ten times during a period of 2 months. The resulting concentrations of ethanol and acetaldehyde in alveolar air are shown in Table 1 and in Figs. 2 and 3. It is evident that the height of the acetaldehyde "plateaus" varies slightly from day to day. Higher ethanol concentrations of a particular test period tend to be associated with slightly higher acetaldehyde concentrations.

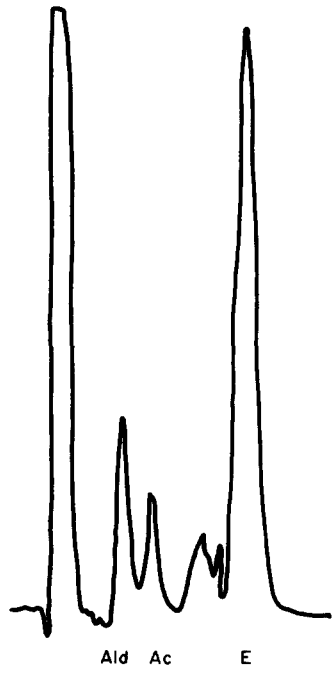

FIG. 1. Gas chromatogram. Analysis of alveolar air after oral ethanol. Column temperature 100°, 40% castor wax. *Ald*, acetaldehyde; *Ac*, acetone; *E*, ethanol (retention time 5 min).

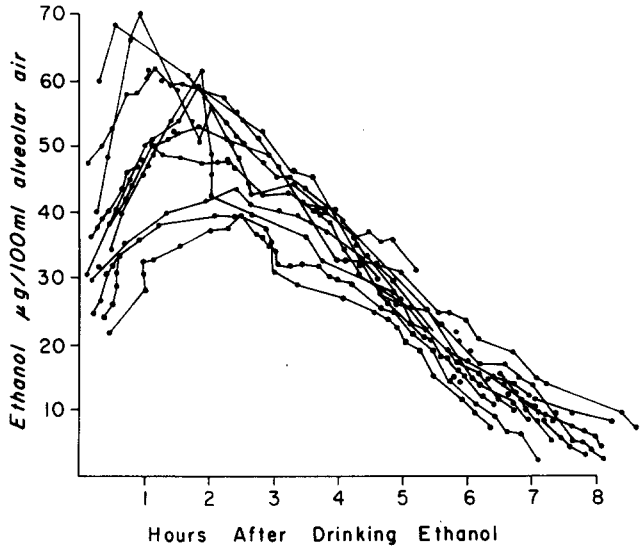

FIG. 2. Concentrations of ethanol in alveolar air in one healthy subject after drinking 0.5 ml of ethanol per lb body weight in 10 tests during a 2 month period.

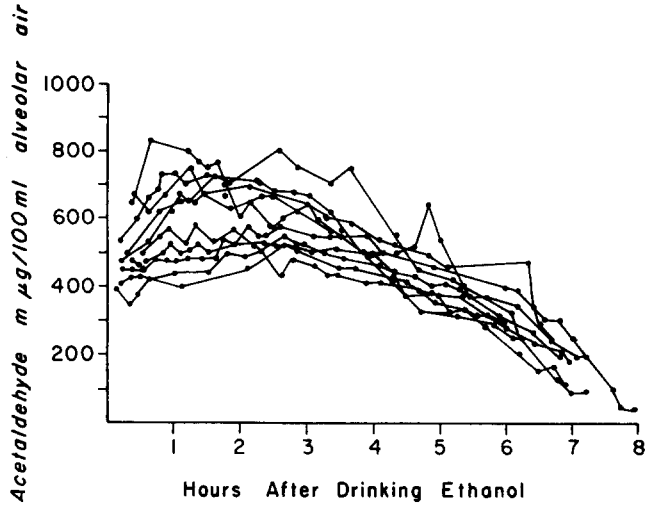

FIG. 3. Concentrations of acetaldehyde in alveolar air in one healthy subject after drinking 0.5 ml of ethanol per lb body weight in 10 tests during a 2 month period.

Acetaldehyde as a 1 ppm solution in 500 ml of chilled orange juice (the mixture had a strong acetaldehyde taste) was drunk over a period of 10 min. This concentration was greatly in excess of that found in commerical beverages. It did not produce detectable amounts of alveolar acetaldehyde, although it did cause nausea.

Eating shortly before the test lowered the maximal ethanol concentrations slightly because of delayed absorption (Fig. 4). The subject ate three meals, consisting of two meat sandwiches with butter on each, 6 and 2 hr before and 3 hr after drinking the ethanol. No effect was noted when only one meal was eaten, more than 4 hr after the alcohol. Sucrose or dextrose (100 g in 250 ml of water) alone, taken 2 hr before ethanol, had no appreciable effect on the alcohol and acetaldehyde concentrations.

Under the conditions of the experiment, 0.5 ml of ethanol per lb body weight was tolerated by most persons without nausea or vomiting. The effect of various doses of ethanol in one person is shown in a representative example in Fig. 5. When ethanol concentrations in alveolar air are less than 15–25 μg/100 ml, acetaldehyde concentrations rise in proportion to those of ethanol. Above this ethanol concentration, acetaldehyde values remain at a relatively constant level. When ethanol concentrations return to 25 μg/100 ml of alveolar air or below, acetaldehyde decreases rapidly.

Physical exercise during the test reduced acetone concentrations more than 50%, while acetaldehyde concentrations decreased 20–30%. Pathologic or voluntary hyperventilation diminished ethanol concentrations but acetaldehyde was not affected, apparently

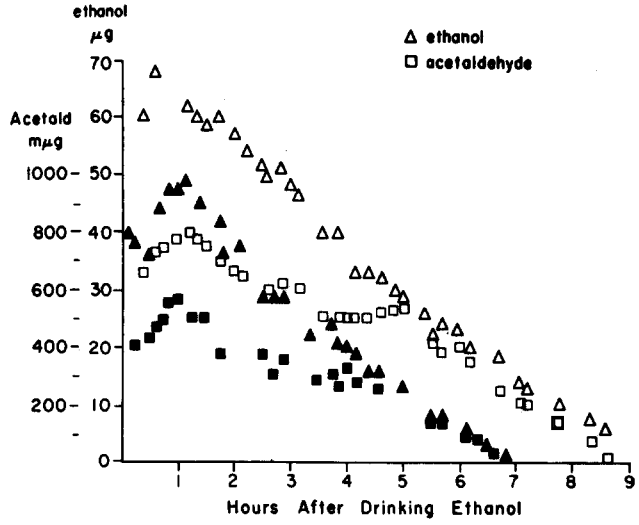

FIG. 4. A comparison of fasting (open symbols) with post-prandial (solid symbols) concentrations of acetaldehyde and ethanol in alveolar air after oral administration of identical amounts of ethanol in one subject.

because acetaldehyde equilibrates with alveolar air more rapidly than ethanol.

Cigarette smoking (filtered as well as unfiltered) in some subjects produced an additional peak in the analysis of expired air. The retention time of this unknown compound, which persisted for only a few minutes after smoking, was shorter than that of acetaldehyde. Smoking cigarettes increased acetaldehyde concentrations in alveolar air by up to 60% for several minutes. This may be due to increased pulmonary blood flow.

The variation of alveolar acetaldehyde and ethanol concentrations after giving the standard amount of al-

TABLE 1 ACETALDEHYDE AND ETHANOL CONCENTRATIONS IN ALVEOLAR AIR AFTER 10 STANDARD TESTS IN A SINGLE SUBJECT

A: Acetaldehyde mμg/100 ml — E: Ethanol μg/100 ml

	1		2		3		4		5		6		7		8		9		10	
Time	A	E	A	E	A	E	A	E	A	E	A	E	A	E	A	E	A	E	A	E
hr																				
0.2	500	40			475	30			225	20	400	50	500	40	525	52	450	36	475	41
4	500	48	650	68			675	51	300	26	625	52	525	43	500	38	450	39	450	38
6			800	68	525	40	625	53	475	31	650	52	500	48	550	42	475	40	475	42
8	625	66	675				675	58	400	36	625	56	500	52	575	47	475	43	475	45
1.0	625	70			675	50	625	58	450	37	600	56	525	51			475	46	475	47
2	650	56	800	62	650	51	650	61	400	38	625	60			525	50	475	50	425	48
4			775	60			750	62	450	40	650	58			575	49	475	51	500	51
6			750	59	725	54	675	58			650	60			525	48	475	52	500	50
8	650	54	700	61	700	59	625	60							575	47	550	53	450	50
2.0			650	57									575	58			525	53		
2	700	56	650	50					475	40			575	53	500	48	575	51	550	50
4	575	48	600	51			675	58			625	60	600	55			550	50	500	48
6	550	43	625	50	800	54	675	55			625	53			425	44	575	50	500	48
8			625	51	750	52					600	53			475	42	575	50		
3.0			650	48											450	35	550	45	500	43
2			600	46			575								425	42	550	45	500	40
4	600	44			700	46	575	40	450	32					425	42	550	43		
6			450	39	750	45			475	36			600	48						
8	500	40	500	39			500	40	450	32	525	43	575	46	400	38	550	40	450	38
4.0			500	32			500	41	450	28	500	50	550	40	400	34	500	38	450	38
2	525	36	500	32			450	35	425	28	500	34	550	40	425	30			475	37
4	525	37	500	33			375	34											450	34
6	325	35	525	32	450	27									375	28				
8	375	36	600	30							400	28			400	26			325	28
5.0			550	30			375	26	300	21	375	25			400	25				
2	325	30			425	25	325	23	325	20	375	21	375	24	350	22				
4					400	25	325	22	250	17			350	23						
6			375	23					200	14	300	19			300	18				
8			375	24											350	17				
6.0			400	23	325	14	275	15											325	21
2			350	20	250	13	200	13	125	10	225	15					400	16	300	17
4							150	11	125	8									300	14
6			250	18			175	9	125	6	175	10	325	19			300	16	250	12
8					125	11							325	16			300	14	125	13
7.0			200	14	100	8			75	5			300	15			250	13	125	10
2			200	13					75	2			275	10					100	10
4																				
6													125	5			100	4		
8													75	4			50	4		
8.0													50	4			50	3		
2																				
4			75	9																
6			0	7																
8																				

cohol to five healthy, nonalcoholic male subjects (31 to 52 yr of age) is shown in Table 2.

The maximal concentrations of acetaldehyde in alveolar air ranged from 650 to 1000 mμg/100 ml. The corresponding maximal concentrations of ethanol in alveolar air varied from 61 to 85 μg/100 ml.

The data in Table 3 illustrate the correlation between the concentrations of ethanol in blood and alveolar air in two individuals.

Acetone concentrations in alveolar air, in general, increased by 25–35% of the initial value 2–4 hr after alcohol intake. Baseline values were about 500 mμg/100 ml. The test results of three representative patients, hospitalized because of alcoholism, are as follows:

Mrs. E. R., a 37-yr old white housewife, gave a history of drinking heavily for only 4 yr (verified by husband). After an exhaustive neurological examination her persistent staggering gait, tremor, and change in personality were attributed to alcoholism. Liver function tests were normal. Results of the alcohol tolerance test indicated an acetaldehyde concentration almost twice as high as in most healthy subjects.

Mr. C. M., a 60-yr old automobile mechanic, admitted in coma, was an alcoholic for approximately 40

TABLE 2 STANDARD TESTS IN 5 HEALTHY NONALCOHOLIC MALES

A: Acetaldehyde in mμg/100 ml Alveolar Air — E: Ethanol in μg/100 ml

	O. H.		C. G.		F. G.		E. B.		S. S.	
Time	A	E	A	E	A	E	A	E	A	E
hr										
0.2										
4	700	75			650	44	825	55	625	42
6	725	78	925	66	650	50	850	60	650	52
8			1000	62	675	51	800	62	625	58
1.0	800	85	900	64	600	56	800	60	600	62
2	775	85			625	58			625	60
4			850	54	725	61			675	64
6			875	55	700	62				
8	750	64			650	58				
2.0	750	65					825	58		
2			875	47			800	59	625	61
4			950	51	650	60			600	62
6					650	60			600	60
8							775	41		
3.0	675	51	775	45			750	41		
2	650	50	775	43	550	39			525	45
4	675	51			550	40			550	43
6							725	33	550	39
8	625	35			475	41	675	30		
4.0	625	32			475	36	675	29		
2									450	36
4					425	34			425	34
6					400	34			400	34
8										
5.0	550	20	575	23	350	26	575	19		
2	525	18	575	22	375	23	550	17	325	24
4	525	18					525	17	350	23
6			375	14	325	22			325	22
8			300	12						
6.0	200	8			250	15	325	11	225	15
2							325	11	175	13
4	125	4	150	6	150	13	250	9	175	14
6	100	2	175	4	175	9				
8									150	9
7.0					100	5		0		

yr. One week after admission, the physical examination and liver function tests were normal. His intellectual ability was relatively well preserved. The ethanol and acetaldehyde concentrations as outlined in Table 4 were within normal limits.

Mr. M. J., a 61-yr old farmer, gave a history of drinking heavily for the past 25 yr. He was admitted because of jaundice, enlarged liver, and ascites, which were discovered 2 weeks before hospitalization. Because of nausea he had abstained from alcohol for 6 weeks before admission. The results of function tests indicating severe liver cell damage were as follows: total serum bilirubin 6.4 mg/100 ml (normal 0.1–1.2), total protein 6.7 (normal 6.7–8.3), albumin 3.0 g/100 ml, (normal 3.7–4.9), serum glutamic oxaloacetic transaminase 20 units (normal 10–40), and alkaline phosphatase 18 King-Armstrong units (normal 4–13 units). Liver biopsy was reported as showing "Laennec's cirrhosis with severe fatty infiltration."

The alcohol tolerance test was performed 2 weeks after admission to the hospital, following a course of diuretics, vitamins, and bed rest which had resulted in a weight loss of 8 kg. The total serum bilirubin had declined to 3.7 mg/100 ml. It is apparent from Table 4 that acetaldehyde concentrations are in the upper range of those of the healthy control subjects.

TABLE 3 ETHANOL CONCENTRATIONS IN BLOOD AND IN ALVEOLAR AIR, SAMPLED SIMULTANEOUSLY

Subject A		Subject B	
Blood	Alveolar Air	Blood	Alveolar Air
mg/100 ml	*mμg/100 ml*	*mg/100 ml*	*mμg/100 m*
130	50	120	48
100	44	115	48
45	15	25	9
30	10	120	50
125	50	100	40
95	35	60	22
65	25		

DISCUSSION

The results obtained in the present investigation indicate that acetaldehyde appearing in alveolar air can be determined accurately and reproducibly by gas–liquid chromatography. The concentrations of acetaldehyde in alveolar air probably correspond to the rate of formation of acetaldehyde in the liver, because acetaldehyde in the hepatic venous blood is carried directly to the lung, where it may be expected to equilibrate rapidly with the alveolar air since its boiling point is 21° and it readily diffuses through tissues.

It is apparent from the data presented that the concentrations of acetaldehyde in alveolar air follow a characteristic pattern when a standardized amount of ethanol is drunk. The results indicate that the concentrations of acetaldehyde in alveolar air rapidly reach a plateau despite increasing ethanol concentrations. This plateau is maintained at almost maximal concentrations until the ethanol concentrations in alveolar air decrease to approximately 15–25 μg/100 ml. At this latter concentration, acetaldehyde begins to decrease rapidly at a rate corresponding to that of ethanol decline. That acetaldehyde concentrations in a particular experiment remain relatively close to a maximum is apparently due to the constant rate of alcohol oxidation in the liver. This rate is limited at the higher concentrations of ethanol in the blood by the activity of ethanol dehydrogenase (8). The maximum rate of acetaldehyde formation in the liver appears to be relatively constant for a particular individual, as is evident from the data presented in 10 alcohol tolerance tests on a single subject. When several subjects are compared with one another, the acetaldehyde concentrations appear to be characteristic for a particular individual. Maximal acetaldehyde

TABLE 4 ACETALDEHYDE (A) AND ETHANOL (E) CONCENTRATIONS IN ALVEOLAR AIR IN 3 ALCOHOLIC SUBJECTS
After administration of 0.5 ml of ethanol per lb body weight.

	Mrs. E. R.		Mr. C. M.		Mr. M. J.	
Time	A	E	A	E	A	E
hr			*mμg/100 ml*			
0.2	500	38	350	26	500	50
4	675	43	500	27	450	54
6	675	42	475	26	550	50
8	725	44	500	27	500	50
1.0	800	42			500	51
2	1100	44				
4	1000	40	500	31		
6	1100	40	475	36		
8						
2.0	1150	34				
2						
4						
6	800	26	500	29	625	36
8						
3.0					550	34
2					575	30
4	720	17			625	32
6						
8	575	8	500	18		
4.0						
2	625	3	450	18		
4	575	2				
6					575	24
8			325	13	450	24
5.0						
2						
4			250	10		
6			175	9		
8			175	8		
6.0						
2			125	7	150	14
4			125	6	200	13
6					175	12
8			100	5		
7.0						
2						
4			0	0		
6						
8					0	0

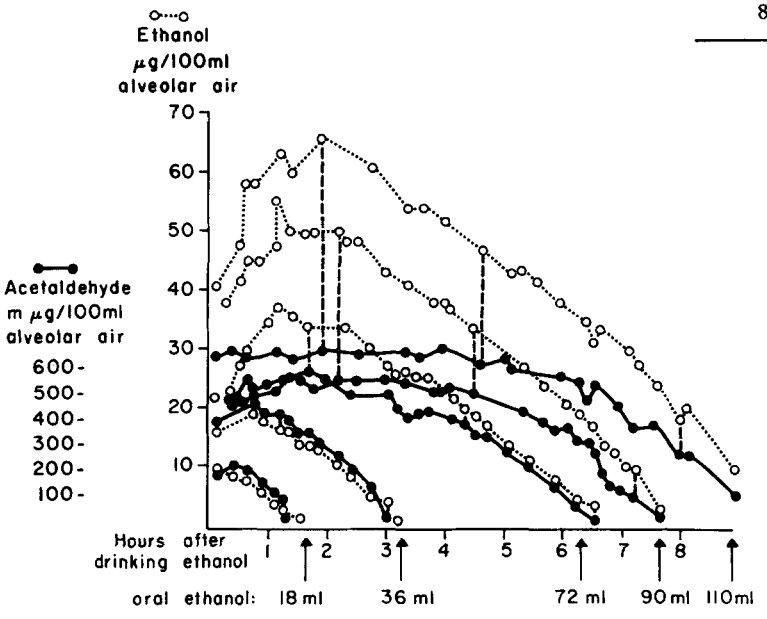

FIG. 5. The effect of increasing amounts of oral ethanol upon acetaldehyde and ethanol concentrations in alveolar air in one subject. The circles represent ethanol, the solid dots represent acetaldehyde concentrations. Each acetaldehyde curve is connected with its corresponding ethanol curve by three vertical lines.

concentrations in alveolar air in five normal subjects ranged from approximately 500 to 900 mμg/100 ml with the exception of two isolated concentrations higher than that. This is in contrast to concentrations in the 1100 mμg range obtained in a patient (E. R.) with neurological complications of alcoholism. The ability of the liver to form acetaldehyde appears to be preserved even in severe liver disease, as shown in one patient (M. J.), although quantitative aspects are not known at present.

Because of the toxic potentials of acetaldehyde (9) it has long been speculated that this substance may contribute to the complications of alcoholism. The method described here makes it feasible to investigate this possibility. If acetaldehyde does contribute to brain damage in chronic alcoholics, it may be expected that those individuals who develop high acetaldehyde concentrations are more prone to toxic complications than those with low concentrations. The preliminary evidence presented is compatible with this hypothesis but the data do not definitely establish that such a correlation exists. The investigation of many more subjects will be necessary before a final conclusion can be reached.

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