Pharmacology Biochemistry & Behavior, Vol. 18, Suppl. 1, pp. 141–150, 1983. © Ankho International. Printed in the U.S.A.

Human Blood Acetaldehyde Concentration During Ethanol Oxidation (Update 1982)

C. J. PETER ERIKSSON

Research Laboratories of the State Alcohol Monopoly (Alko), Box 350, SF-00101 Helsinki 10, Finland

ERIKSSON, C. J. P. *Human blood acetaldehyde concentration during ethanol oxidation (update 1982).* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 141–150, 1983.—A wide variety of levels of human blood acetaldehyde have been reported in the past. During the last few years, however, it has become increasingly evident that most, if not all, of the previously observed acetaldehyde concentrations during normal (i.e., no deficiency in, or inhibition of, aldehyde dehydrogenase activity) ethanol oxidation merely reflected artefactual acetaldehyde formed during the analytical procedures. The artefactual acetaldehyde formation, which occurs mainly during blood protein precipitation, is effectively minimized by the recently improved PCA method in which blood is immediately mixed with a perchloric acid-saline solution, and by the semicarbazide method in which blood is treated with a fresh isotonic semicarbazide solution before removal of the plasma. Nevertheless, a procedure involving control blood with ethanol added should be employed to control for any artefactual acetaldehyde still produced. Based on the improved analytical procedures, no detectable acetaldehyde was found in the venous blood of Caucasian subjects after acute ethanol intake.

Acetaldehyde Blood Ethanol oxidation

SURVEY OF EARLIER ACETALDEHYDE ANALYSES: METHODS AND LEVELS

The history of acetaldehyde analyses is outlined in chronological order in Table 1, with the various methods that have been developed being numbered sequentially.

Before 1950: Nonspecific Colorimetric Methods

In a very early investigation, from 1920, of blood acetaldehyde concentrations in human subjects drinking ethanol an average level of about 400 μM was determined [66]. The corresponding analytical procedures (method 1, Table 1) involved whole blood protein precipitation, distillation of the acidic supernatant and the colorimetric detection of acetaldehyde in the distillate reacting with iodine. The sensitivity was later improved by using a copper sulfate/sulfuric acid/p-hydroxybiphenyl reagent for the colorimetric reaction (methods 2 and 3). By using method 3 (without the distillation step), it was noted that human blood contained compounds other than acetaldehyde which also reacted with the p-hydroxybiphenyl reagent [30]. An open question remained to what extent the distillation could eliminate the interference of such blood contaminants.

1950–1959: Low Levels Determined in Blood Plasma

To shorten the rather time consuming method 2, method 4 was developed. The problem of specificity was not solved, however, as was later demonstrated by findings according to which acetone interfered with the spectrophotometric determination [46]. Similarly, the specificity of method 5 may be questioned. To overcome the specificity problem, enzymatic determination methods were developed (methods 6 and 7). With method 6, which involved whole blood protein precipitation with perchloric acid (PCA), acetaldehyde levels as high as with the earlier methods were found. However, with method 7, which determined acetaldehyde in the plasma, low levels (<10 μM) were reported. Moreover, the "endogenous" (without ethanol intake) acetaldehyde concentrations were close to those measured during ethanol oxidation. The discrepancy with the earlier methods was not discussed.

1960–1969: Development of GC Methods

The introduction of gas chromatography (GC) in the determination of blood acetaldehyde reduced the problems of specificity and improved the sensitivity over that of the earlier methods. The original GC method involved whole blood protein precipitation in a headspace vessel; after heating, a gas sample was transferred from the headspace to the GC (method 8). Method 9 involved direct GC injection of the blood supernatant. The acetaldehyde concentration obtained with these methods (8 and 9) varied from <4 to >200 μM. The possibility of artefactual acetaldehyde formation was recognized in some of these investigations [25,60].

1970–1979: Recognition of Artefactual Acetaldehyde Formation and Rediscovery of Plasma Methods

The original headspace method 8, which involved incubation of denaturated blood without removal of precipitated blood proteins, was soon found to be complicated by an ethanol-induced nonenzymatic formation of acetaldehyde during the incubation prior to the headspace analysis [74]. The method was, however, improved by removal of the proteins from the deproteinized blood samples (method 10). Acetaldehyde levels, thus determined, were very low (Table

TABLE 1

DETERMINATION OF HUMAN BLOOD ACETALDEHYDE CONCENTRATION DURING ETHANOL OXIDATION

Method	Acetaldehyde* (μM)	Ethanol* (mM)	Subjects and treatment‡	Reference
1. Stepp 1920 [66]: protein precipitation (whole blood+ phospho wolframate) centrifugation distillation (supernatant) colorimetric reaction (distillate + iodine)	365			Stepp 1920 [66]
2. Stotz 1943 [67]: protein precipitation (whole blood+ sodium wolframate/sulfuric acid)	320	39	Alcoholics	Stotz 1943 [67]
	158	16	Alcoholics, A	Brown, Knoblock 1951 [10]
centrifugation distillation (supernatant)	95	18		Mendeloff 1954 [51]
colorimetric reaction (distillate + copper sulfate/sulfuric acid/p-hydroxybiphenyl reagent)	386	54		Raby 1954 [59]
	+184	29	A	
	143	14		Clark, Hulpieu 1958 [13]
	+118	11	Charcoal	
	+180	82	Alcoholics	
	230	28	Japanese flushers	Akabane 1960 [3]
	390	40	Japanese	Ogata *et al.* 1966 [54]
3. Hald, Jacobsen 1948 [30]: as method 2 but without distillation	74	9		Hald, Jacobsen 1948 [30]
	+87	7	A	
4. Burbridge *et al.* 1950 [11]: protein precipitation (as method 2) diffusion into semicarbazide spectrophotometric determination	63	4	Alcoholics	Hine *et al.* 1952 [33]
	+104	5	Alcoholics, A	
	270	23		Forster 1956 [27]
	275		Alcoholics, A	Ezrielev 1973 [26]
	261		Alcoholics, A, Na-metabisulfite	
5. Lester, Greenberg 1950 [42]: distillation (whole blood applied on filter paper) colorimetric reaction (as method 2)	200	5	Alcoholics, A	Lester *et al.* 1952 [41]
6. Klein, Korzis 1958 [39]: protein precipitation (whole blood+ perchloric acid) centrifugation neutralization (supernatant) enzymatic assay (supernatant+ NADH + alcohol dehydrogenase)	200	4	A	Klein, Korzis 1958 [39]
7. Lundquist, 1958 [46]: centrifugation (whole blood) protein precipitation (plasma+ metaphosphoric acid)	7	9		Lundquist, Wolthers 1958 [47]

TABLE 1

DETERMINATION OF HUMAN BLOOD ACETALDEHYDE CONCENTRATION DURING ETHANOL OXIDATION
(CONTINUED)

Method	Acetaldehyde* (μM)	Ethanol* (mM)	Subjects and treatment‡	Reference
centrifugation neutralization (supernatant) enzymatic assay (supernatant + NAD + aldehyde dehydrogenase)				
8. Duritz, Truitt 1964 [16]): protein precipitation (whole blood + zinc sulfate/ barium hydroxide) headspace GC determination (suspension)	148 +31		Orientals	Ewing *et al.* 1974 [25]
	239 +16 +89	14 7 13	Chinese Ojibwa Indians	Reed *et al.* 1976 [60]
	1 3	2 3	Alcoholics, A Alcoholics, A, liver disease	Iber, Cohwdhury 1977 [34]
9. Roach, Creaven 1968 [61]: protein precipitation (as method 8) centrifugation GC determination (supernatant)	34	74	Alcoholics	Majchrowicz, Mendelson 1970 [49]
10. Truitt 1970, 1971 [74,75]: as method 8 but headspace analyses on supernatants (after centrifugation)	2.0 +0.5	13 22	Alcoholics	Truitt 1971 [75]
11. Coldwell *et al.* 1971 [14], Machata, Prokop 1971 [48], Kinoshita 1974 [38]: direct headspace GC determination (whole blood)	590	17		Machata, Prokop 1971 [48]
	48 +21	15 16	Japanese nonflushers Japanese flushers	Ijiri 1974 [35]
	30 +31 +16 +120	9 9 2 3	Japanese nonflushers Japanese flushers Japanese alcoholics Japanese alcoholics, A	Kinoshita 1974 [38]
	2 +7	9 10	Japanese nonflushers Japanese flushers	Kijima 1979 [37]
	2 +11	10 10	Japanese nonflushers Japanese flushers	Mizoi *et al.* 1979 [52]
	50 +30		Relatives to alcoholics	Schuckit, Rayses 1979 [64]
	6 +8	22 18	Japanese nonflushers Japanese flushers	Asakura 1980 [4]
	3 +38 +2 +62	17 17 16 10	Japanese nonflushers Japanese flushers Japanese alcoholics Japanese alcoholics, A	Maruyama 1980 [50]
	3 +7	10 10	Japanese nonflushers Japanese flushers	Mizoi *et al.* 1980 [53]
	230 +0	20 20	Nitroimidazole	Helmbrecht, Schweitzer 1981 [32]
12. Sippel 1972 [65], Brien, Loomis 1978 [6]: as method 10 but whole blood	27 +16	51 51	Alcoholics	Korsten *et al.* 1975 [40]

TABLE 1

DETERMINATION OF HUMAN BLOOD ACETALDEHYDE CONCENTRATION DURING ETHANOL OXIDATION
(CONTINUED)

Method	Acetaldehyde* (μM)	Ethanol* (mM)	Subjects and treatment‡	Reference
proteins precipitated with thiourea/perchloric acid	36	20		Lindros *et al.* 1977 [44]
	−7	21	Alcoholics	
	−12	17	4-MP	
	−14	15	Alcoholics, 4-MP	
	26	17		Salaspuro *et al.* 1977 [62]
	+ 3	18	4-MP	
	25	11		Brien, Loomis 1978 [6]
	+145	17	CC	
	30	12	Alcoholics	Brien *et al.* 1978 [9]
	300	17	Alcoholics, CC	
	60	15	Alcoholics	Brien *et al.* 1979 [8]
	250	19	Alcoholics, CC	
	170	9	Alcoholics (i.e. Alcoholics, CC)	Brien *et al.* 1980 [7]
	180	12	Alcoholics, CC	Peachey *et al.* 1981 [56]
13. Eriksson *et al.* 1975, 1977, 1980 [23, 24, 19]: as method 12 but without thiourea and with correction for artefactual acetaldehyde formation (based on control blood added to ethanol/perchloric acid)	40	45		Ylikahri *et al.* 1974 [79]
	23	55		Ylikahri *et al.* 1976 [80]
	22	22		Eriksson, Peachey 1980 [21]
	+1	20	Alcoholics	
14. Sauter *et al.* 1977 [63]: as method 8 but whole blood proteins precipitated with thiourea/perchloric acid	68	4	Alcoholics	Sauter *et al.* 1977 [63]
	164	4	Alcoholics, A	
15. Stowell *et al.* 1977, 1978, 1978 [70, 69, 71]: protein precipitation (as method 6) centrifugation distillation (supernatant) enzymatic assay (as method 7) correction for artefactual acetaldehyde formation (based on ethanol added to control blood before precipitation step)	10			Stowell *et al.* 1978 [71]
16. Pikkarainen *et al.* 1979 [58]: rapid centrifugation (whole blood) headspace GC (plasma)	0.4	19		Pikkarainen *et al.* 1979 [58]
	+0.6	60	Alcoholics	
	5		Alcoholics	Lindros *et al.* 1980 [45]
	2	10	Japanese nonflushers	Harada *et al.* 1981 [31]
	+33	11	Japanese flushers	
17. Stowell 1979 [68]: As method 13 but correction as method 15	35		CC	Stowell 1979 [68]
18. Stowell 1979 [68]: whole blood added to isotonic semicarbazide solution	33		CC	Stowell 1979 [68]
	8	20	Controls, alcoholics	Eriksson, Peachey 1980 [21]
centrifugation protein precipitation (plasma+ perchloric acid)	7	24		Stowell *et al.* 1980 [72]
	+69	6	CC	
centrifugation headspace GC (supernatant)	+3	6	CC, 4-MP	
	5	10		Christensen *et al.* 1981 [12]
	2	2	Nonflusher diabetics, CP	Jerntorp *et al.* 1981 [36]
	15	3	Flusher diabetics, CP	

TABLE 1

DETERMINATION OF HUMAN BLOOD ACETALDEHYDE CONCENTRATION DURING ETHANOL OXIDATION
(CONTINUED)

Method	Acetaldehyde* (μM)	Ethanol* (mM)	Subjects and treatment‡	Reference
19. Wartburg, Ris 1979 [78]: whole blood added to isotonic chloralhydrate solution	1	20	Controls, alcoholics	Eriksson, Peachey 1980 [21]
	22	23		Wartburg 1980 [77]
centrifugation protein precipitation (plasma) headspace GC (suspension)	4	1		Barnett *et al.* 1981 [5]
	+3	1	CP	
	+1	1	Nonflushing diabetics, CP	
	+27	1	Flushing diabetics, CP	
20. Abe *et al.* 1980 [2]: rapid distillation (whole blood) headspace GC (distillate)	23	12	Japanese nonflushers	Abe *et al.* 1980 [1]
	+95	11	Japanese flushers	
21. Eriksson, Peachey 1980 [21]: as method 19 but with correction (based on control blood treated with ethanol/chloralhydrate)	0	20	Controls, alcoholics	Eriksson, Peachey 1980 [21]
22. Eriksson, Peachey 1980 [21], Lindros *et al.* 1980 [45]:	0	20	Controls, alcoholics	Eriksson, Peachey 1980 [21]
as method 18 but with correction (based on control blood treated with ethanol/semicarbazide)	1			Lindros *et al.* 1980 [45]
	+109		Alcoholics	
	0	25		Present article, Fig. 2
23. Christensen *et al.* 1981 [12]: as method 10 but whole blood proteins immediately precipitated with sodium nitrite/sulphosalicylic acid	1.3	9		Christensen *et al.* 1981 [12]
24. Thomas *et al.* 1981 [73]: trapping with 2,4-dinitrophenylhydrazine (whole blood) extraction and separation of acetaldehyde derivative HPLC determination	(60)†			Thomas *et al.* 1981 [73]
25. Eriksson *et al.* 1982 [20]: as method 13 but immediate precipitation with saline/perchloric acid and correction as method 15	0	13		Present article, Fig. 3
26. Okada, Mizoi 1982 [55]: as method 25 but without correction	2	10	Japanese nonflushers	Okada, Mizoi 1982 [55]
	+28	11	Japanese flushers	
27. Tsukamoto *et al.* 1982 [76]: as method 26 but with water/perchloric acid	20		Japanese flushers	Tsukamoto *et al.* 1982 [76]
	4		Japanese alcoholics	

*Maximum acetaldehyde means or individual values, and the corresponding ethanol concentrations are listed. Deviations from normal conditions are marked + and −.

†The same acetaldehyde concentration was found with and without ethanol drinking: said possibly to represent "endogenously bound" acetaldehyde.

‡Only conditions that may affect blood acetaldehyde concentrations are listed. Antabuse=A, 4-methylpyrazole=4-MP, calcium carbimide=CC, chlorpropamide=CP. Subjects are Caucasians if not otherwise mentioned.

1). These low values were in accordance with the earlier plasma values determined with method 7 [47]. Moreover, these findings confirmed the artefactual nature of earlier high acetaldehyde concentrations determined with method 8.

Method 11, the direct headspace method, represents a simplification of the previously described headspace methods which had involved a protein precipitation step. In comparison with method 8, the direct headspace method seemed to produce less artefactual acetaldehyde [38,50]. However, as demonstrated in Table 1, the blood acetaldehyde concentrations determined with method 11 showed huge variations, with concentrations ranging from 2 to 590 μM. The problem in the direct headspace method is to preserve the original acetaldehyde while preventing ethanol-

induced artefactual acetaldehyde formation. At physiological temperatures acetaldehyde is known to be metabolized in human blood [16, 29, 67, 71]. At high temperatures, the rate of artefactual acetaldehyde formation increases ([38], Eriksson, unpublished observation), particularly if the blood becomes hemolyzed [23, 24, 38]. This problem seems to be insurmountable and thus it appears unlikely that reliable results will ever be obtainable from the direct headspace (without any blood treatment) method.

Thiourea has been suggested to inhibit artefactual acetaldehyde formation obtained with headspace analysis of blood PCA supernatants (method 12). Acetaldehyde levels ranging from 25 to 36 μM (Table 1) have been determined during normal ethanol oxidation with this method. Later it was demonstrated, however, that thiourea does not inhibit the artefactual acetaldehyde formation during the analytical procedures [23, 24, 70]. Instead thiourea reduces the acetaldehyde recovery [23,24], an effect which may be mistaken for an inhibition of the simultaneous artefactual acetaldehyde production. Thus, there are reasons to believe that the acetaldehyde concentrations determined with method 12 are too high. This should also be the case for the levels determined with method 14. Further studies on the nature of the artefactual acetaldehyde formation and the observation that the major portion of this reaction actually occurs during the whole blood protein precipitation led to the opinion that a reevaluation of most previous reports on human blood acetaldehyde levels was needed [17–19, 23, 24, 70]. The subsequent methods for determining human blood acetaldehyde (methods 13, 15 and 17) involved corrections for artefactual acetaldehyde formation and concentrations ranging from 10 to 40 μM were observed.

In contrast to the acetaldehyde levels determined with protein precipitation methods, considerably lower levels, 0.4–22 μM, have been obtained with new plasma methods (16, 18 and 19, Table 1) that involve initial removal of the cells which contain most of the capacity for artefactual acetaldehyde formation. These results confirm the early results with method 7.

1980–1982: Development of Improved PCA and Semicarbazide Methods

It was proposed that the contradiction between the acetaldehyde concentrations obtained with the plasma and the precipitation methods might be explained by rapid acetaldehyde binding or oxidation occurring in blood *in vivo* [21] or during blood sampling [19]. The possibility that the rapid acetaldehyde "disappearance" was merely an artefact, due to miscalculation of artefactual acetaldehyde formation [19], was, however, neglected until recently, when it was observed that the elapsed time between sampling and mixing of the blood in PCA affected the amount of acetaldehyde measured during ethanol intoxication [43]. In further experiments it was observed that pronounced mixing-time dependent acetaldehyde formation also occurred in ethanol-containing control blood added to PCA [20, 55, 76]. Mixing-time dependent but less active acetaldehyde production was found if blood was added to ethanol-containing PCA, instead of adding the ethanol to the blood before the PCA treatment [20]. These observations clearly indicated that earlier corrections (method 13) for artefactual acetaldehyde had been insufficient.

Because of the rapid acetaldehyde formation during blood protein precipitation, the mixing should be both immediate and efficient (method 27). However, after fulfilling these criteria by the use of a whirlmixer, artefactual acetaldehyde levels of about 5 μM were still obtained at blood ethanol concentrations of about 20 mM [20]. A decisive improvement was the finding [20,55] that by using immediate mixing in PCA made in saline, instead of water, the artefactual acetaldehyde formation could be reduced to about 0 to 2 μM, depending on the ethanol concentration (method 25 and 26). Correction is still needed for the remaining artefactual acetaldehyde, which is formed mainly during the incubation prior to headspace analysis (method 25). Method 23 represents another recent immediate precipitation method by which very low acetaldehyde levels have been determined. Mixing-time dependent artefactual acetaldehyde formation was, however, also observed with this method (Eriksson, unpublished observation).

The finding that both the semicarbazide (method 18) and chloralhydrate (method 19) procedures included some artefactual acetaldehyde led to the use of the correction procedures for these methods as well (methods 21 and 22). In preliminary studies using methods 21 and 22, no significant acetaldehyde was found during ethanol intoxication [21]. In addition, it was observed that freshly made semicarbazide should be used in order to avoid more pronounced artefactual acetaldehyde formation [21].

The two other recently reported methods remain to be checked for artefactual acetaldehyde formation (method 20) and for specificity (method 24).

REEVALUATION OF BLOOD ACETALDEHYDE CONCENTRATIONS DETERMINED WITH METHOD 13

The increase in measured blood acetaldehyde concentrations with the elapsed time between sampling and mixing in PCA is illustrated in Fig. 1. Also included in this figure are values (bars 1a, 1b and 1c) we observed in a previous study [19]. In this study the differences in blood acetaldehyde values (i.e., between c, b, and a) were interpreted as probably being caused by rapid disappearance. As demonstrated by the close fit between these values and the other points in Fig. 1, it is now apparent that this interpretation was incorrect, and that the differences in these previously reported values were instead a result of different degrees of artefactual acetaldehyde formation.

In another study we compared blood acetaldehyde concentrations during ethanol intoxication in alcoholics and controls [21]. After reconsidering the 20 sec between sampling and mixing of the blood in the PCA, it is clear that these values (bars 2a and 2b in Fig. 1) can be fully explained on the basis of artefactual acetaldehyde formation. Similarly, the blood acetaldehyde values observed in additional experiments with male volunteers following 1 g/kg ethanol (bars 3a and b in Fig. 1) fit within the frame of the artefactual acetaldehyde formation.

In summary, all of the acetaldehyde values observed in the studies depicted in Fig. 1 lie in a range that can be explained by artefactual formation. There is thus no evidence from these studies that the true *in vivo* concentration of acetaldehyde in human venous blood during ethanol intoxication is above zero.

IMPROVED SEMICARBAZIDE AND PCA METHODS: ZERO LEVELS OF ACETALDEHYDE

In some of the experiments depicted in Fig. 1, blood

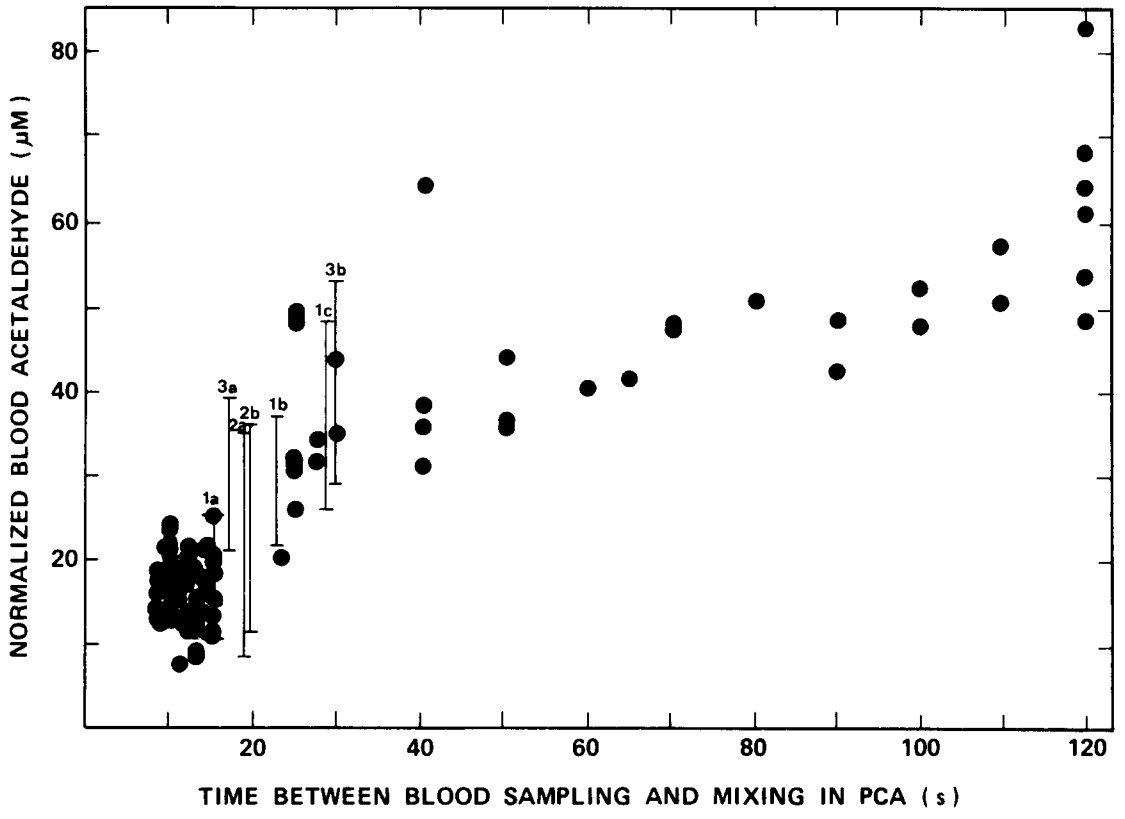

FIG. 1. Effect of the elapsed time between blood sampling and the mixing of the blood in PCA on blood acetaldehyde concentration during ethanol (1 g/kg) intoxication. All acetaldehyde values (method 13, Table 1, but noncorrected for artefactual formation) are normalized to a blood ethanol concentration of 20 mM, i.e., the depicted acetaldehyde concentration (μM)=measured acetaldehyde concentration (μM)×measured ethanol concentration (mM)/20 mM. Closed circles mark individual acetaldehyde values of 9 female and 11 male nonalcoholic subjects (no sex differences) determined 0.5–3 hr after the ethanol intake from blood directly dripped from the vein into PCA. Bars la, lb and lc mark the noncorrected ±SD range (male nonalcoholic subjects, n=6) from previously reported results [19] showing acetaldehyde determined from 3 (a, b and c) blood samples consecutively dripped into PCA 1 hr after the ethanol intake. Bars 2a (n=72 samples) and 2b (n=87 samples) mark the noncorrected ±SD range from previously reported results [21] showing acetaldehyde in blood dripped directly into PCA 1 to 7 hr after the ethanol intake for 9 male alcoholics and 9 male nonalcoholics. Bars 3a (n=53 samples) and 3b (n=9 samples) mark the ±SD range of acetaldehyde in blood dripped directly into PCA 0.5 to 1.5 hr (3a) or 1 hr (3b) after the ethanol intake in 9 male nonalcoholics.

acetaldehyde was also determined by the semicarbazide method (method 22, Table 1). The results are summarized in Fig. 2. Acetaldehyde concentrations, 0–10 μM, measured during ethanol intoxication were considerably lower than those measured with the PCA method (Fig. 1). As with the PCA methods, the acetaldehyde values measured with the semicarbazide method merely reflected artefactual acetaldehyde formation occurring with this analytical procedure.

Based on the improvements of the semicarbazide and PCA methods, another series of experiments were carried out. Ten male subjects received 0.5 g/kg ethanol (within 30 min) after overnight fasting. Blood samples were taken from an antecubital vein, 0.5 to 2 hr after the start of ethanol drinking, into EDTA-tubes, whereupon (<15 sec) a 0.5 ml blood sample was pipetted into 2 ml ice-cold 0.6 M PCA (made in saline) and shaken within 2 sec by a whirlmixer. A further 1 ml blood sample was taken into 1 ml semicarbazide buffer, which was made just prior to the experiments. No significant difference occurred in the recovery of acetaldehyde when PCA precipitation (or semicarbazide addition) was performed immediately or 30 sec after the addition of acetaldehyde to control blood at 37°C. Control blood samples containing various amounts of added ethanol were prepared for both methods before the start of drinking. Other analytical procedures were as described before. The results of the improved PCA and semicarbazide determinations are demonstrated in Fig. 3. In these experiments, very low (0–2 μM) acetaldehyde levels were observed with both methods. These levels were not significantly different from the corresponding artefactual levels found with the ethanol-containing control blood.

STATUS OF HUMAN BLOOD ACETALDEHYDE

The present review and experimental data suggest that acetaldehyde is not, at least with current analytical procedures, detectable in human venous blood during ethanol intoxication in subjects having neither a deficiency in, nor in-

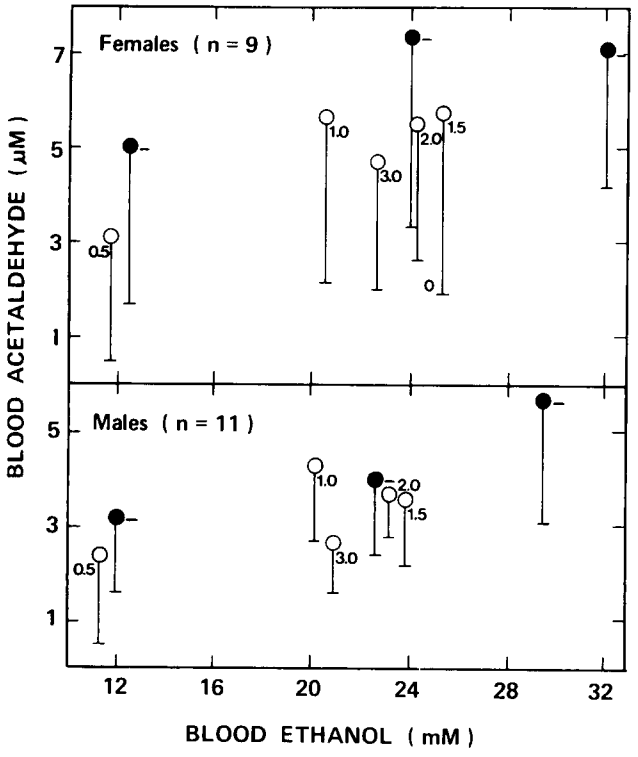

FIG. 2. Blood acetaldehyde (method 22, Table 1, semicarbazide solution: 0–3 days old) in male and female subjects during ethanol (1 g/kg) intoxication (same experiment as Fig. 1: individual values). Open circles mark noncorrected acetaldehyde means (−SD) and closed circles mark the mean (−SD) artefactual acetaldehyde from control blood added to semicarbazide containing ethanol. Numbers beside the circles mark the time (hr) from the start of the ethanol intake.

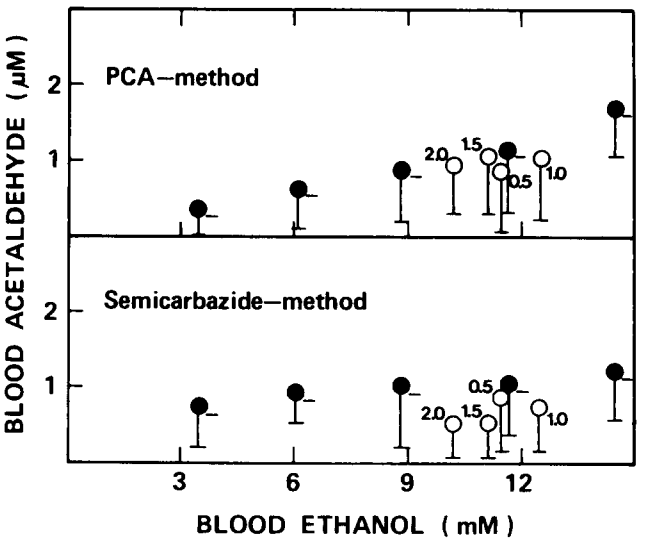

FIG. 3. Blood acetaldehyde (determined by improved PCA and semicarbazide methods, see text) during ethanol (0.5 g/kg) intoxication in male nonalcoholic subjects (n=10). Marking as in Fig. 2 with the addition that in PCA control experiments (closed circles above) blood containing ethanol was added to PCA.

hibition of, aldehyde dehydrogenase activity. This conclusion became more evident with improvement in the analytical procedures. Previously reported acetaldehyde concentrations are thus suggested to reflect merely acetaldehyde artefactually formed during the analytical procedures. However, as demonstrated in Table 1, factors such as genotypic aldehyde dehydrogenase deficiency causing flushing in many Orientals [31,55], treatments with the aldehyde dehydrogenase inhibitors antabuse, calcium carbimide and chlorpropamide, and in some cases chronic alcoholism, may cause elevated blood acetaldehyde concentrations during ethanol intoxication. To what extent these elevations have been affected by artefact reactions remains to be determined in future experiments.

The question of whether nondetectable acetaldehyde concentrations mean absolute zero, or whether during normal ethanol intoxication there may occur *in vivo* venous levels in the nanomolar range, is open. The latter alternative includes the possibility of rapid acetaldehyde turnover. Equally open is the question whether acetaldehyde exists as a normal endogenous metabolite in the absence of ethanol intake. To solve these questions future studies should always compare the blood taken during ethanol intoxication with control blood and control blood to which ethanol has been added.

It is important to emphasize that the absence of venous acetaldehyde during normal ethanol oxidation does not necessarily reflect the *in vivo* acetaldehyde concentration elsewhere in the body. In rat there is an extensive arterial-venous acetaldehyde concentration gradient [22]. Such a gradient was not noted in a previous report on human venous and arterial levels [19]. However, because most, if not all, of the acetaldehyde measured in this study was artefactually formed, the question of whether there exists low arterial levels during normal ethanol intoxication, is still open. In this connection, it would be interesting to know whether human breath acetaldehyde [15, 28, 57, 72, 81], which should not contain any major analytical artefact, possibly reflects arterial acetaldehyde levels, or merely the acetaldehyde formation in the mouth, airways or lung microsomes [57]. Until future studies have clarified these aspects it is difficult to settle the *in vivo* role, if any, of blood acetaldehyde during normal ethanol intoxication.

REFERENCES

1. Abe, H., K. Tanikawa and T. Yokoyama. Clearance of ethanol and acetaldehyde in blood in persons with suffered from so called "alcohol intolerance." *Jpn J Stud Alcohol* **15**: 325–334, 1980.

2. Abe, H., K. Tanikawa, T. Yokoyama and H. Arimoto. Determination of ethanol and acetaldehyde in blood using gas chromatograph equipped with gas sampler. *Jpn J Stud Alcohol* **15**: 335–344, 1980.

3. Akabane, J. Pharmacological aspects of manifestation of the acute after-effects of alcoholic beverages: A role of acetaldehyde in alcoholism. *Med J Shinshu Univ* **5:** 113–122, 1960.
4. Asakura, S. Determination of acetaldehyde level in expired air after intake of alcohol. *Jpn J Stud Alcohol* **15:** 43–73, 1980.
5. Barnett, A. H., C. Gonzalez-Auvert, D. A. Pyke, J. B. Saunders, R. Williams, C. J. Dickenson and M. D. Rawlings. Blood concentrations of acetaldehyde during chlorpropamide-alcohol flush. *Br Med J* **283:** 939–941, 1981.
6. Brien, J. F. and C. W. Loomis. Gas-liquid chromatographic determination of ethanol and acetaldehyde in blood. *Clin Chim Acta* **87:** 175–180, 1978.
7. Brien, J. F., J. E. Peachey and C. W. Loomis. Calcium carbimide-ethanol interaction. *Clin Pharmacol Ther* **27:** 426–433, 1980.
8. Brien, J. F., J. E. Peachey, C. W. Loomis and B. J. Rogers. The calcium carbimide-ethanol interaction: Effects of ethanol dose. *Clin Pharmacol Ther* **25:** 454–463, 1979.
9. Brien, J. F., J. E. Peachey, B. J. Rogers and C. W. Loomis. A study of the calcium carbimide-ethanol interaction in man. *Eur J Clin Pharmacol* **14:** 133–141, 1978.
10. Brown, C. T. and E. C. Knoblock. Antabuse therapy in the army. A preliminary report of fifty cases. *US Army Forces Med J* **2:** 191–202, 1951.
11. Burbridge, T. N., C. H. Hine and A. F. Schick. A simple spectrophotometric method for the determination of acetaldehyde in blood. *J Lab Clin Med* **35:** 983–987, 1950.
12. Christensen, J. M., H. Angelo and J. Knop. Determination of acetaldehyde in human blood by a gas chromatographic method with negligible artefactual acetaldehyde formation. *Clin Chim Acta* **116:** 389–395, 1981.
13. Clark, W. C. and H. R. Hulpieu. The disulfiram-like activity of animal charcoal. *J Pharmacol Exp Ther* **123:** 74–80, 1958.
14. Coldwell, B. B., G. Solomonraj, H. L. Trenholm and G. S. Wiberg. The gas chromatographic estimation of ethanol, acetaldehyde, and acetone in ethanol metabolism studies. *Clin Toxicol* **4:** 99–113, 1971.
15. Dannecker, J. R., E. G. Shaskan and M. Phillips. A new highly sensitive assay for breath acetaldehyde: Detection of endogenous levels in humans. *Anal Biochem* **114:** 1–7, 1981.
16. Duritz, G. and E. B. Truitt. A rapid method for the simultaneous determination of acetaldehyde and ethanol in blood using gas chromatography. *Q J Stud Alcohol* **25:** 498–510, 1964.
17. Eriksson, C. J. P. Elevated blood acetaldehyde levels in alcoholics and their relatives: A reevaluation. *Science* **207:** 1383–1384, 1980.
18. Eriksson, C. J. P. Problems and pitfalls in acetaldehyde determinations. *Alcoholism* **4:** 22–29, 1980.
19. Eriksson, C. J. P., M. E. Hillbom and A. R. A. Sovijärvi. Difficulties in measuring human blood acetaldehyde concentrations during ethanol intoxication. *Adv Exp Med Biol* **126:** 439–451, 1980.
20. Eriksson, C. J. P., Y. Mizoi and T. Fukunaga. The determination of acetaldehyde in human blood by the perchloric acid precipitation method: The characterization and elimination of artefactual acetaldehyde formation. *Anal Biochem* **125:** 259–263, 1982.
21. Eriksson, C. J. P. and J. E. Peachey. Lack of difference in blood acetaldehyde of alcoholics and controls after ethanol ingestion. *Pharmacol Biochem Behav* **13:** Suppl 1, 101–105, 1980.
22. Eriksson, C. J. P. and H. W. Sippel. The distribution and metabolism of acetaldehyde in rats during ethanol oxidation. I. The distribution of acetaldehyde in liver, brain, blood and breath. *Biochem Pharmacol* **26:** 241–247, 1977.
23. Eriksson, C. J. P., H. W. Sippel and O. A. Forsander. Factors influencing the determination of acetaldehyde in biological samples by head-space gas chromatography. *Finn Found Alcohol Stud* **23:** 9–18, 1975.
24. Eriksson, C. J. P., H. W. Sippel and O. A. Forsander. The determination of acetaldehyde in biological samples by head-space gas chromatography. *Anal Biochem* **80:** 116–124, 1977.
25. Ewing, J. A., B. A. Rouse and E. D. Pellizzari. Alcohol sensitivity and ethnic background. *Am J Psychiat* **131:** 206–210, 1974.
26. Ezrielev, G. I. Acetaldehyde and alcoholism. Pharmacogenesis of a disulfiram-alcohol reaction and its management by binding acetaldehyde with sodium metabisulfite. *Sov Neur Psychiat* **6:** 42–51, 1973.
27. Forster, B. Die Veränderungen des Acetaldehydspiegels im Blute nach Alkoholaufnahme. *Dtsch Z Gerichtl Med* **45:** 221–224, 1956.
28. Freund, G. and P. O'Hollaren. Acetaldehyde concentrations in alveolar air following a standard dose of ethanol in man. *J Lipid Res* **6:** 471–477, 1965.
29. Freundt, K. J. Zum Verhalten des Alkoholmetaboliten Acetaldehyd im Blut. *Blutalkohol* **12:** 389–392, 1975.
30. Hald, J. and E. Jacobsen. The formation of acetaldehyde in the organism after ingestion of antabuse (tetraethylthiuramdisulphide) and alcohol. *Acta Pharmacol* **4:** 305–310, 1948.
31. Harada, S., D. P. Agarwal and H. W. Goedde. Aldehyde dehydrogenase deficiency as cause of facial flushing reaction to alcohol in Japanese. *Lancet* **ii:** 982, 1981.
32. Helmbrecht, J. and H. Schweitzer. Aspects of acetaldehyde blood concentration after ingestion of alcohol and the trichomonas drug ornidazol. *Blutalkohol* **18:** 29–38, 1981.
33. Hine, C. H., T. N. Burbridge, E. A. Macklin, H. H. Anderson and A. Simon. Some aspects of the human pharmacology of tetraethylthiuramdisulphide (antabus)-alcohol reactions. *J Clin Invest* **31:** 317–325, 1952.
34. Iber, F. L. and B. Chowdhury. The persistance of the alcohol-disulfiram reaction after discontinuation of drug in patients with and without liver disease. *Alcoholism* **1:** 365–370, 1977.
35. Ijiri, I. Studies on the relationship between the concentrations of blood acetaldehyde and urinary catecholamine and the symptoms after drinking alcohol. *Jpn J Stud Alcohol* **9:** 35–59, 1974.
36. Jerntorp, P., H. Öhlin, B. Bergström and L.-O. Almér. Increase of plasma acetaldehyde. An objective indicator of the chlorpropamide alcohol flush. *Diabetes* **30:** 788–791, 1981.
37. Kijima, T. Alcohol sensitivity and urinary catecholamines. *Jpn J Stud Alcohol* **14:** 101–117, 1979.
38. Kinoshita, M. Gaschromatographic determination of blood acetaldehyde levels after ingestion of ethanol in man. *Jpn J Stud Alcohol* **9:** 1–34, 1974.
39. Klein, H. and J. Korzis. Der Acetaldehydgehalt des menschlichen Blutes. *Medizinische* **9:** 345–347, 1958.
40. Korsten, M. A., S. Matsuzaki, L. Feinman and C. S. Lieber. High blood acetaldehyde levels after ethanol administration. Difference between alcoholic and nonalcoholic subjects. *N Engl J Med* **292:** 386–389, 1975.
41. Lester, D., E. J. Conway and N. M. Mann. Evaluation of antidotes for the alcohol reaction syndrome in patients treated with disulfiram (tetraethylthiuram disulfide). *Q J Stud Alcohol* **13:** 1–8, 1952.
42. Lester, D. and L. A. Greenberg. The role of acetaldehyde in the toxicity of tetraethylthiuram disulfide and alcohol. With a method for the determination of acetaldehyde in 0.20 ml of blood. *Q J Stud Alcohol* **11:** 391–395, 1950.
43. Lindros, K. O. and C. J. P. Eriksson. Artefactual acetaldehyde formation during human blood perchlorate deproteinization. *Subst Alcohol Actions Misuse* **2:** 341–347, 1981.
44. Lindros, K., M. Salaspuro and P. Pikkarainen. Studies on the role of the ADH pathway in increased ethanol elimination after chronic alcohol intake in the rat and man. In: *Alcohol and Aldehyde Metabolizing Systems*, vol 3, *Intermediary Metabolism and Neurochemistry*, edited by R. G. Thurman, J. R. Williamson, H. R. Drott and B. Chance. New York: Academic Press, 1977, pp. 343–354.
45. Lindros, K. O., A. Stowell, P. Pikkarainen and M. Salaspuro. Elevated blood acetaldehyde in alcoholics with accelerated ethanol elimination. *Pharmacol Biochem Behav* **13:** Suppl 1, 119–124, 1980.
46. Lundquist, F. Enzymatic determination of acetaldehyde in blood. *Biochem J* **68:** 172–177, 1958.

47. Lundquist, F. and H. Wolthers. The kinetics of alcohol elimination in man. *Acta Pharmacol Toxicol* **14:** 265–289, 1958.
48. Machata, G. and L. Prokop. Alkoholabbau und Acetaldehyd. *Blutalkohol* **8:** 281–284, 1971.
49. Majchrowicz, E. and J. H. Mendelson. Blood concentrations of acetaldehyde and ethanol in chronic alcoholics. *Science* **168:** 1100–1102, 1970.
50. Maruyama, J. Studies on acetaldehyde formation from ethanol during the procedure of determination of blood acetaldehyde. *Jpn J Stud Alcohol* **15:** 19–36, 1980.
51. Mendeloff, A. I. Effect of intravenous infusions of ethanol upon estimated hepatic blood flow in man. *J Clin Invest* **33:** 1298–1302, 1954.
52. Mizoi, Y., I. Ijiri, Y. Tatsuno, T. Kijima, S. Fujiwara and J. Adachi. Relationship between facial flushing and blood acetaldehyde levels after alcohol intake. *Pharmacol Biochem Behav* **10:** 303–311, 1979.
53. Mizoi, Y., S. Hishida, I. Ijiri, J. Maruyama, S. Asakura, T. Kijima, T. Okada and J. Adachi. Individual differences in blood and breath acetaldehyde levels and urinary excretion of catecholamines after alcohol intake. *Alcoholsim* **4:** 354–360, 1980.
54. Ogata, S., T. Hosoi, H. Saji, M. Inukai, K. Morita, M. Morita and H. Oota. Studies on acute alcohol intoxication. Especially concerning its relation to the carbohydrate metabolism. *Jpn J Alcohol* **1:** 67–79, 1966.
55. Okada, T. and Y. Mizoi. Studies on the problem of blood acetaldehyde determination in man and level after alcohol intake. *Jpn J Alcohol Drug Depend* **17:** 141–159, 1982.
56. Peachey, J. E., J. F. Brien, D. H. Zilm, C. W. Loomis, M. F. Hemy and S. M. Maglana. The calcium carbimide-ethanol interaction in man. Effects of repeated ethanol administration. *J Stud Alcohol* **42:** 208–216, 1981.
57. Pikkarainen, P., E. Baraona, H. Seitz and C. S. Lieber. Breath acetaldehyde: Evidence of acetaldehyde production by oropharynzx microflora and by lung microsomes. *Adv Exp Med Biol* **132:** 469–474, 1980.
58. Pikkarainen, P. H., M. P. Salaspuro and C. S. Lieber. A method for the determination of "free" acetaldehyde in plasma. *Alcoholism* **3:** 259–261, 1979.
59. Raby, K. Relation of blood acetaldehyde level to clinical symptoms in the disulfiram-alcohol reaction. *Q J Stud Alcohol* **15:** 21–32, 1954.
60. Reed, T. E., H. Kalant, R. J. Gibbins, B. M. Kapur and J. G. Rankin. Alcohol and acetaldehyde metabolism in Caucasians, Chinese and Amerinds. *Can Med Ass J* **115:** 851–855, 1976.
61. Roach, M. K. and P. J. Creaven. A micro-method for the determination of acetaldehyde and ethanol in blood. *Clin Chim Acta* **21:** 275–278, 1968.
62. Salaspuro, M. P., P. Pikkarainen and K. Lindros. Ethanol-induced hypoglycaemia in man: its suppression by the alcohol dehydrogenase inhibitor 4-methylpyrazole. *Eur J Clin Invest* **7:** 487–490, 1977.
63. Sauter, A. M., D. Boss and J.-P. von Wartburg. Reevaluation of the disulfiram-alcohol reaction in man. *J Stud Alcohol* **38:** 1680–1695, 1977.
64. Schuckit, M. A. and V. Rayses. Ethanol ingestion: Differences in blood acetaldehyde concentrations in relatives of alcoholics and controls. *Science* **203:** 54–55, 1979.
65. Sippel, H. W. Thiourea, an effective inhibitor of the non-enzymatic ethanol oxidation in biological extracts. *Acta Chem Scand* **26:** 3398–3400, 1972.
66. Stepp, W. Über das Auftreten von Azetaldehyd im Körper beim Abbau des Äthylalkohols. *Arch Exp Pathol Pharmak* **87:** 148–152, 1920.
67. Stotz, E. A colorimetric determination of acetaldehyde in blood. *J Biol Chem* **148:** 585–591, 1943.
68. Stowell, A. R. An improved method for the determination of acetaldehyde in human blood with minimal ethanol interference. *Clin Chim Acta* **98:** 201–205, 1979.
69. Stowell, A. R., K. E. Crow, R. M. Greenway and R. D. Batt. Determination of acetaldehyde in blood using automated distillation and fluorometry. *Anal Biochem* **84:** 384–392, 1978.
70. Stowell, A. R., R. M. Greenway and R. D. Batt. Acetaldehyde formation during deproteinization of human blood samples containing ethanol. *Biochem Med* **18:** 392–401, 1977.
71. Stowell, A. R., R. M. Greenway and R. D. Batt. Stability of acetaldehyde in human blood samples. *Biochem Med* **20:** 167–179, 1978.
72. Stowell, A. R., K. O. Lindros and M. P. Salaspuro. Breath and blood acetaldehyde concentrations and their correlation during normal and calcium carbimide-modified ethanol oxidation in man. *Biochem Pharmacol* **29:** 783–787, 1980.
73. Thomas, M., C. K. Lim and T. J. Peters. Assaying acetaldehyde in biological fluids. *Lancet* **ii:** 530, 1981.
74. Truitt, E. B. Ethanol-induced release of acetaldehyde from blood and its effect on the determination of acetaldehyde. *Q J Stud Alcohol* **31:** 1–12, 1970.
75. Truitt, E. B. Blood acetaldehyde levels after alcohol consumption by alcoholic and nonalcoholic subjects. In: *Biological Aspects of Alcohol, Advances in Mental Science*, vol 3, edited by M. Roach, W. M. McIsaac and P. J. Creaven. Austin: The University of Texas Press, 1971, pp. 212–232.
76. Tsukamoto, S., T. Sudo, S. Karasawa, M. Kajiwara and T. Endo. Quantitative recovery of acetaldehyde in biological samples. *Nihon Univ J Med* **24:** 313–331, 1982.
77. Wartburg, J.-P. Comparison of alcohol metabolism in humans and animals. In: *Animal Models in Alcohol Research*, edited by K. Eriksson, J. D. Sinclair and K. Kiianmaa. New York: Academic Press, 1980, pp. 427–443.
78. Wartburg, J. P. and M. M. Ris. Determination of acetaldehyde in human blood. *Experientia* **35:** 1682–1683, 1979.
79. Ylikahri, R. H., M. O. Huttunen, C. J. P. Eriksson and E. A. Nikkilä. Metabolic studies on the pathogenesis of hangover. *Eur J Clin Invest* **4:** 93–100, 1974.
80. Ylikahri, R. H., T. Leino, M. O. Huttunen, A. R. Pösö, C. J. P. Eriksson and E. A. Nikkilä. Effects of fructose and glucose on ethanol-induced metabolic changes and on the intensity of alcohol intoxication and hangover. *Eur J Clin Invest* **6:** 93–102, 1976.
81. Zeiner, A. R., A. Paredes and H. D. Christensen. The role of acetaldehyde in mediating reactivity to an acute dose of ethanol among different racial groups. *Alcoholism* **3:** 11–18, 1979.