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Alcohol Sensitivity Related to Polymorphism of Alcohol-Metabolizing Enzymes in Japanese

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MIZOI, Y., Y. TATSUNO, J. ADACHI, M. KOGAME, T. FUKUNAGA, S. FUJIWARA, S. HISHIDA AND I. IJIRI. *Alcohol sensitivity related to polymorphism of alcohol-metabolizing enzymes in Japanese.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 127–133, 1983.—Normal Japanese subjects were divided into two groups, i.e., one with both low and high K_m isozymes of aldehyde dehydrogenase for acetaldehyde, and the other deficient in the low K_m isozyme. After intake of 0.4 g/kg alcohol, the deficient subjects showed high level of blood acetaldehyde, facial flushing and the other dysphoric symptoms, including increase of pulse rate, decrease of diastolic blood pressure, changes of pulse wave in the fingertip, and elevation of the arterial pressure and blood flow rate in common carotid arteries as well as increase of plasma catecholamines level. In contrast, subjects with normal ALDH did not show these changes. From the observation of liver specimens obtained at autopsy, the frequency of deficient phenotype of ALDH in Japanese was presumed to be about 36%.

Alcohol sensitivity Polymorphism Aldehyde dehydrogenase Alcohol dehydrogenase
Blood acetaldehyde Facial flushing Plasma catecholamine

SENSITIVITY to alcohol varies vastly from man to man in Japanese. More than about half of Japanese, following intake of an adequate amount of alcohol, as in most Caucacians, experience a general hot feeling, euphoria, talkativeness and excitation occur. On the other hand, the rest, even after drinking a small amout of alcohol, present marked facial flushing and experience palpitation and chest distress. Furthermore, some of them fall into severe drunken sickness. Individual difference in the metabolic pathway of alcohol may be responsible for the wide variability in the sensitivity to alcohol.

In the previous report [8], we clarified the relationship between blood acetaldehyde level and facial flushing in Japanese subjects after ingestion of a small amount of alcohol. The flushers showed increase of pulse rate, dilatation of periphral blood vessels and a rise of urinary catecholamines with conspicuous elevation in blood acetaldehyde levels.

The increase in blood acetaldehyde level may be due to a higher rate of alcohol oxidation to acetaldehyde by the activity of alcohol dehydrogenase (ADH) or a lower rate of acetaldehyde oxidation to acetate by low activity of aldehyde dehydrogenase (ALDH), or both.

Stamatoyannopoulos *et al.* [11] reported that atypical ADH discovered by von Wartburg *et al.* [12], which had several times higher activity at pH 8.8 than the normal type, was found in 85% of the Japanese liver specimens. Additionally, Wolff [13] reported that 83% of the Oriental subjects responded to alcohol with marked facial flushing. The close proximity of the incidence of atypical liver ADH to that of facial flushing in Orientals is consistent with the hypothesis that atypical ADH facilitated alcohol metabolism with a consequent elevation of blood acetaldehyde leading to facial flushing [11]. Against this supposition, we mentioned in our previous paper [9] that a facilitation of alcohol metabolism due to atypical ADH alone could not explain the elevation of blood acetaldehyde level in the flushers because the frequency of the flushers observed in our country was conjectured to be under 50%. We suggested that action of ALDH must be considered as a cause of the elevation of blood acetaldehyde level in the flushers. On the other hand, Harada *et al.* [5] have investigated the two main isozyme bands of ALDH in the Japanese liver specimens by electrophoresis and found that roughly half of the livers were deficient in low K_m isozyme of ALDH while the other half had both high and low K_m isozymes as in Caucasian liver. In accordance with these results, Goedde *et al.* [3] presented a

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TABLE 1

BLOOD ALCOHOL AND ACETALDEHYDE LEVELS IN USUAL AND UNUSUAL ALDH GROUPS AFTER INTAKE OF 0.4 g/kg ALCOHOL (MEAN ± S.D.)

	Before	30 min	1 hr	2 hr	3 hr	4 hr
Blood Alcohol (mM)						
Usual ALDH	0	10.17	9.88	6.37	2.92	0.57
		±2.27	±1.43	±1.41	±1.46	±0.66
Unusual ALDH	0	10.99	9.37	6.30	3.35	1.06
		±1.95	±1.08	±1.22	±1.34	±1.02
Blood Acetaldehyde (μM)						
Usual ALDH	0.25	1.98	1.56	1.39	0.81	0.52
	±0.22	±1.38	±0.96	±0.97	±0.62	±0.48
Unusual ALDH	0.23	32.82	33.27	18.84	14.84	6.26
	±0.29	±26.80	±26.50	±17.80	±13.59	±8.33

Usual ALDH group N=29; unusual ALDH group N=39.

new hypothesis that the alcohol sensitivity in Orientals was based on the polymorphism of liver ALDH.

In the present studies, the relationship between the polymorphism of ALDH and the symptoms and blood levels of acetaldehyde and catecholamines after alcohol ingestion was investigated in Japanese subjects. The blood acetaldehyde level noted in the previous report [8] was carefully reviewed with the new methods and the symptoms were also analyzed again in more detail. Furthermore, the frequency of the polymorphic variants of ADH and ALDH was investigated in liver specimens of Japanese individuals obtained at autopsy.

METHOD

Procedures of Alcohol Experiment

Sixty-eight healthy males, aged 20–32 years, of which over half were facial flushers were selected as the subjects. Each subject did not eat breakfast on the day of the experiment and ingested sake (Japanese rice wine with 16 v/v% alcoholic content), 0.4 g of alcohol per kg of body weight, between 10:00 and 10:10 a.m. The room temperature was maintained at 20–22°C. Blood was collected from the median cubital vein.

Determination of Blood Acetaldehyde and Alcohol

One ml of blood was instantly and throughly mixed with 6 ml of ice-cold 0.5 M perchloric acid made in saline. Soon after mixing, the sample was centrifuged at 4°C and 2 ml of the supernatant was delivered into a 22 ml vial tightly closed with a rubber stopper and an aluminum cap. The vial was incubated for 30 min at 65°C. The head space gas of the vial was analyzed with a Perkin-Elmer F45 Head Space Analyzer with a FID detector. A glass column (1.5 m × 2.4 mm inner diameter) packed with Chromosorb 101 (60–80 mesh) was used. The operating conditions were: column temperature, 130°C; detector temperature, 160°C; nitrogen (carrier gas) flow rate, 40 ml/min. The peak areas of acetaldehyde and alcohol were calculated with a digital integrator.

Determination of Plasma Catecholamines

Blood was drawn into heparinized tubes placed on ice and was centrifuged promptly. Three ml of plasma was deproteinized by 0.8 M perchloric acid and 3 ng of 3,4-dihydroxybenzyl amine (Aldrich) was added as an internal standard. The extraction was performed as followed by the modification of the method of Keller *et al.* [6]. Norepinephrine and epinephrine levels were determined using a high performance liquid chromatograph (Yanaco L-2000L) equipped with electrochemical detector (VMD-101).

Pulse Wave, Blood Flow Rate and Arterial Pressure

The pulse waves in the left fingertip and the flow rate of blood and arterial pressure in the common carotid arteries were measured by methods reported previously [8].

Estimation of the Phenotypes of ADH and ALDH

The liver and roughly 200 hair roots specimens was obtained at autopsy within 36 hr after death from 100 Japanese healthy adults selected randomly who had died suddenly due to accidental or homicidal causes. The normal and atypical phenotypes of ADH in the liver specimens were determined by activity assay carried out by the method of von Wartburg *et al.* [12], and the heterozygote and homozygote of atypical ADH were distinguished by high voltage starch gel electrophoresis with the method of Harada *et al.* [5]. Two kinds of ALDH phenotypes in the liver and hair roots specimens were determined by isoelectric focusing in the range of pH 3.5–10.0 according to the method of Harada *et al.* [4]. One was the usual ALDH which possessed both ALDH I and II isozymes with low and high K_m for acetaldehyde, respectively, and another was the unusual ALDH which was deficient in ALDH I isozyme.

For estimation of the ALDH phenotypes of the subjects in the alcohol experiment, roughly 20–30 hair roots were used for isoelectric focusing.

RESULTS

Table 1 shows the blood alcohol and acetaldehyde levels

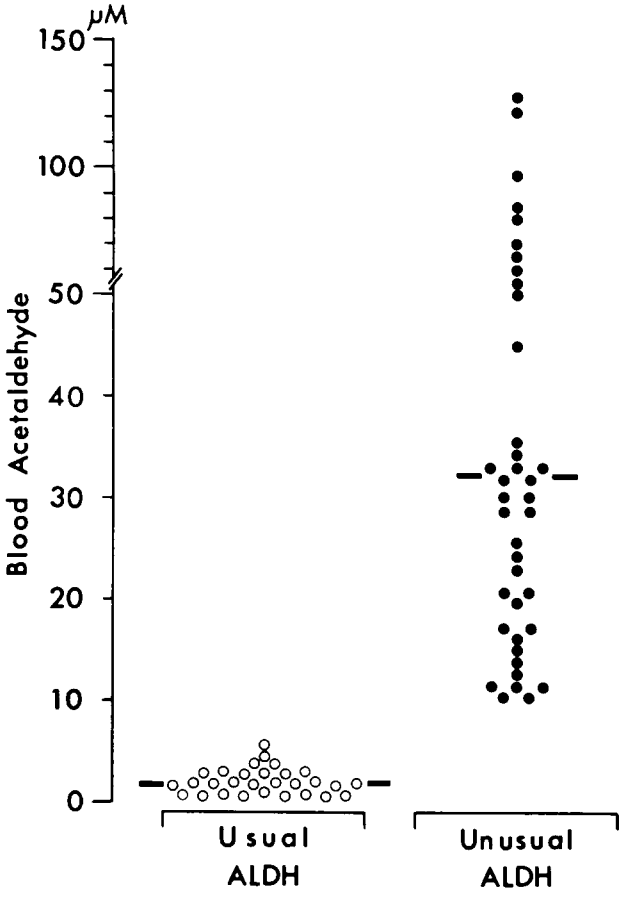

FIG. 1. Highest level of blood acetaldehyde in each subject of the alcohol experiment.

TABLE 2

AGE, BODY WEIGHT AND RATE OF ALCOHOL ELIMINATION IN THE SUBJECTS OF THE ALCOHOL EXPERIMENT (MEAN ± S.D.)

ALDH Phenotype	N	Age	Body Weight (kg)	Alcohol Elimination (mg/kg/hr)
Usual ALDH	29	23 ± 3	64 ± 7	106 ± 13
Unusual ALDH	39	22 ± 2	62 ± 7	98 ± 14
Significance		NS	NS	NS

NS:$p>0.05$.

of the subjects with usual and unusual ALDH phenotypes in the alcohol experiment. In both groups, the mean blood alcohol levels reached a peak value of about 10 mM 30 min after the initiation of the drinking. The time course of elimination was similar in both groups. The usual ALDH group showed under 2 μM acetaldehyde at every time of measurement, while the unusual group had over 30 μM at 30 or 60 min after drinking. Levels decreased gradually after that. Figure 1 shows the highest level obtained in each subject. While the highest levels in all the subjects with usual ALDH were less than 5 μM, the values ranged from 10 to 124 μM in those with unusual ALDH.

Means of ages, body weights and rates of alcohol elimination were not significantly different between the two groups as shown in Table 2.

Objective and subjective symptoms and signs after drinking in the subjects were depicted in Fig. 2. All of the subjects with unusual ALDH exhibited facial flushing from approximately 15 min after the drinking with restoration to normal color by 1–3 hr. On the other hand, none of the subjects with

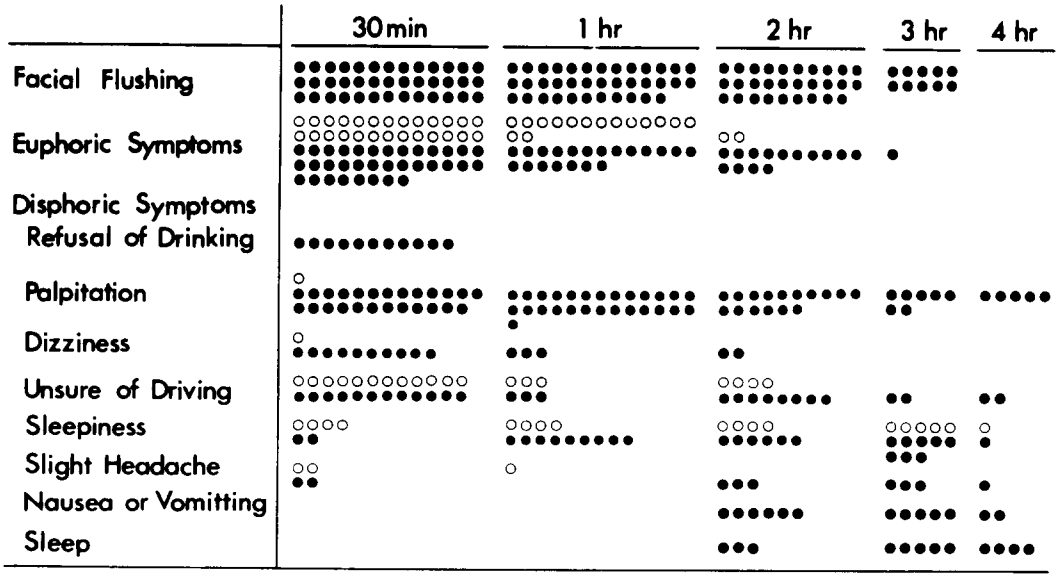

FIG. 2. Symptoms and signs in the subjects of the alcohol experiment. Usual ALDH group (○) N=29; unusual ALDH group (●) N=39.

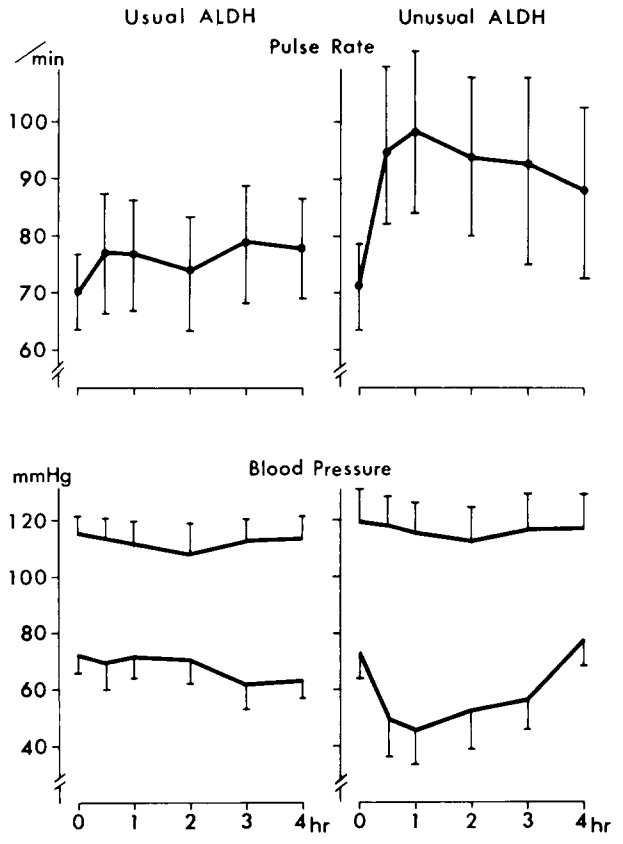

FIG. 3. Changes of pulse rate and blood pressure in the subjects of the alcohol experiment. (Mean±S.D.)

usual ALDH were flushers. Most of the subjects of both groups reported symptoms of comfort 30–60 min after the start of the drinking. After that time, the subjects of the usual ALDH group sobered up more quickly than the ones of unusual group. Many of the latter subjects complained of various discomforts, such as palpitation, dizziness, sleepiness, slight headache and so on, while few subjects with usual ALDH did. Six subjects with unusual ALDH showed very high blood acetaldehyde level at 30–60 min after alcohol intake and suffered from conspicuous drunken sickness with nausea or vomitting between 2 and 4 hr. They then fell asleep.

Figure 3 shows the changes of pulse rate and blood pressure. No difference was found in the values before alcohol intake between the two groups. After the ingestion of alcohol, the pulse rate increased conspicuously in the unusual ALDH group and slightly in the usual group. No change of the systolic blood pressure was seen in both groups after the drinking, while the diastolic blood pressure decreased only in the unusual ALDH group until 2 or 3 hr after the drinking. Sixty percent of the subjects with unusual ALDH showed more than 40% of the decrease in the diastolic pressure.

About 80% of the subjects in the unusual ALDH group showed dilated patterns in the pulse wave. The upper row in Fig. 4 shows the time course of changes of the pulse wave in a typical case where marked facial flushing occurred. The dilated pattern was most conspicuously found at 30 min after the drinking, and the normal pattern was not seen yet at 4 hr. On the other hand, the nonflushing subjects with usual ALDH presented no change as shown in the lower row.

Figure 5 (top) shows the arterial pressure and blood flow rate in the common carotid arteries before and after drinking in a typical case with unusual ALDH where marked facial flushing occurred. After drinking, both arterial pressure and mean blood flow rate increased correspondingly with the decrease of diastolic blood pressure and the dilatation of peripheral blood vessels shown in the finding of pulse wave at the fingertip. In the blood flow rate showed a diphasic pattern with sharp percussion and dicrotic wave. In a nonflushing subject with usual ALDH, as shown in Fig. 5 (bottom), scarcely any change was seen in both arterial pressure and blood flow rate after drinking. Figure 6 shows the time course of changes in these measured values of common carotid artery, pulse rate, blood alcohol and acetaldehyde levels and degree of facial flushing in the two subjects of Fig. 5. Changes are clear in only the subject with unusual ALDH. The blood flow rate, pulse rate and arterial pressure increased markedly corresponding with the manifestation of

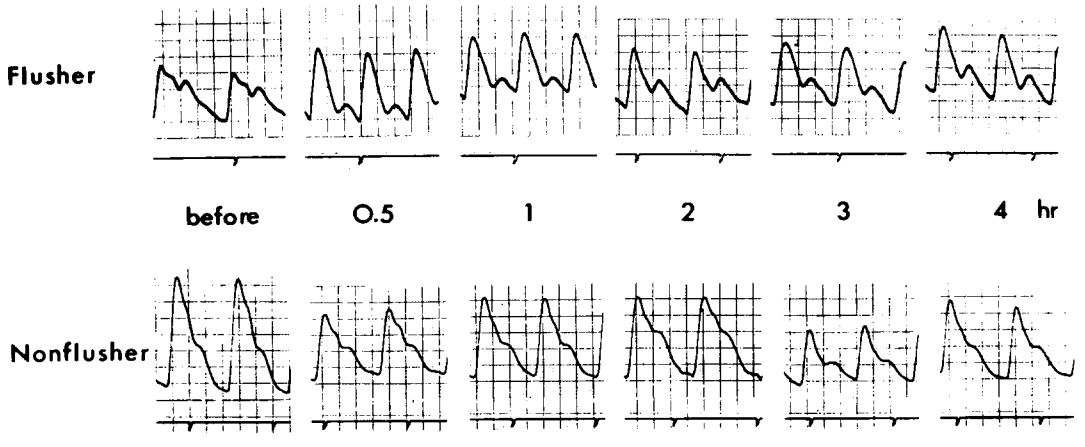

FIG. 4. Pulse waves of left fingertip before and after alcohol intake in a flusher with unusual ALDH and a non-flusher with usual ALDH.

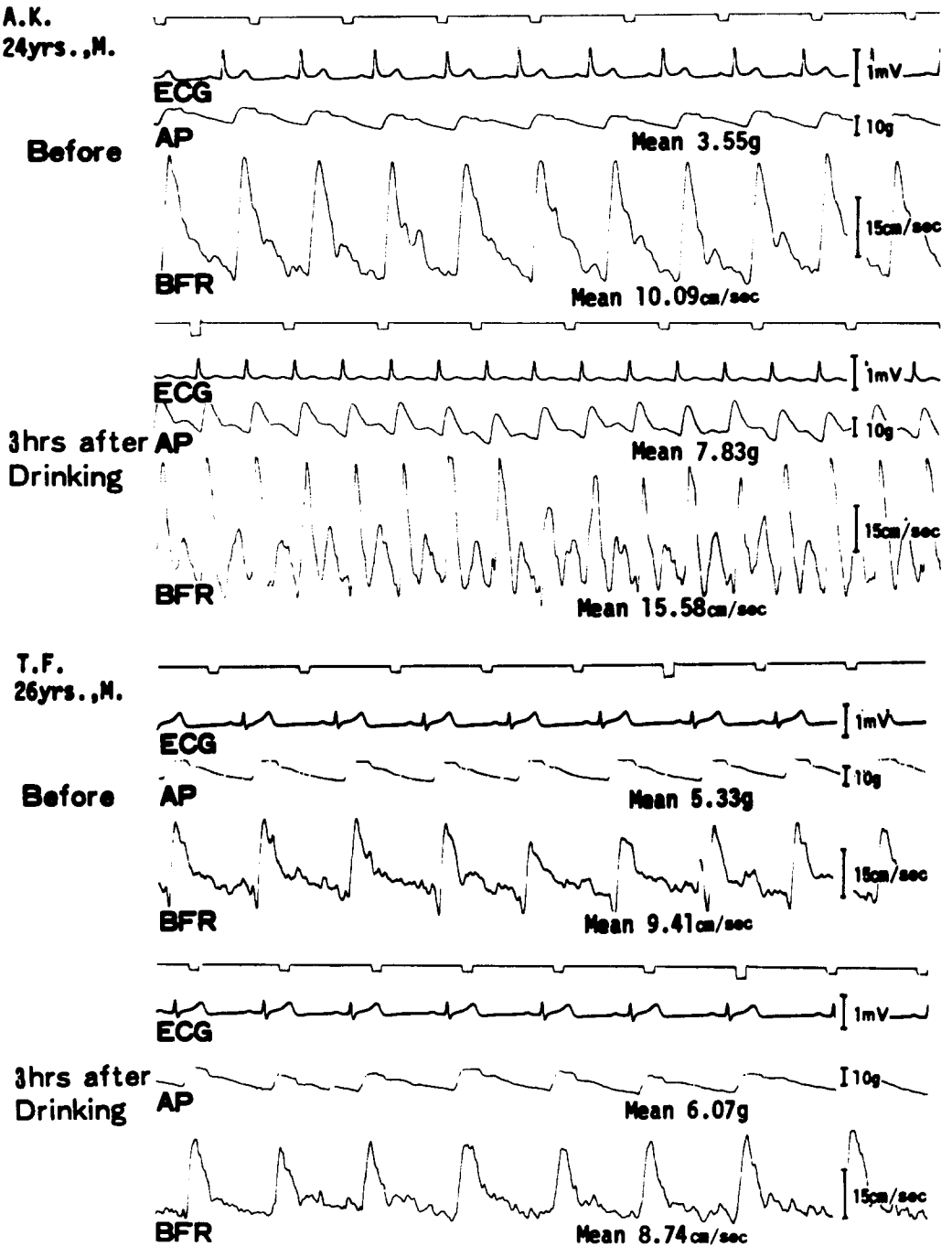

FIG. 5. Recordings of the electrocardiogram (ECG) and the arterial pressure (AP) and blood flow rate (BFR) in common carotid arteries before and after alcohol intake in a flusher with unusual ALDH (top) and a nonflusher with usual ALDH (bottom).

facial flushing and elevation of blood acetaldehyde level. These values did not return to the drinking baseline even when the flushing disappeared and blood acetaldehyde level decreased.

Figure 7 depicts the mean values of plasma catecholamines in the subjects with usual and unusual ALDH groups. In the control examination where each subject was given the same amount of water in place of alcohol under the same condition of the alcohol test on the other day, plasma norepinephrine and epinephrine did not change. However, both catecholamine levels in the unusual ALDH group roughly doubled at 45 min after alcohol intake and remained elevated about 1.5 times as high as the control at 3.5 hr. On the other hand, in the usual ALDH group, neither norepinephrine nor epinephrine level showed any increase.

The frequencies of ADH and ALDH polymorphism in the

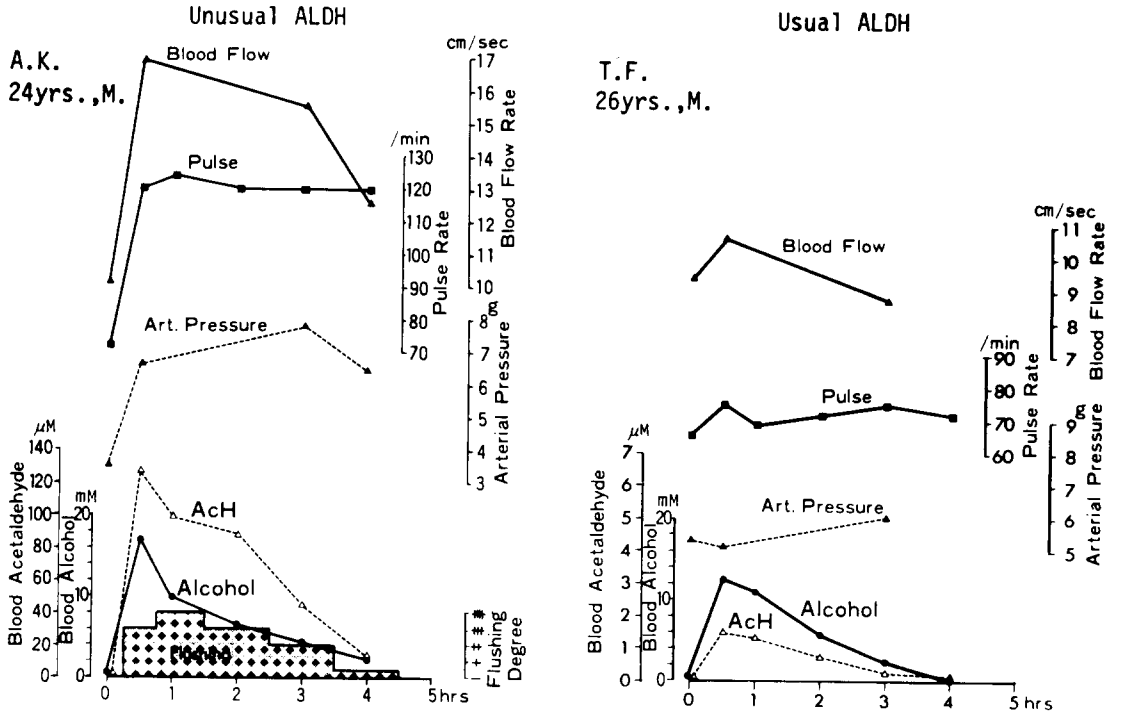

FIG. 6. Time course of changes of blood alcohol and acetaldehyde levels, degree of facial flushing, pulse rate, and the arterial pressure and blood flow rate of common carotid arteries in the same subjects as Fig. 5.

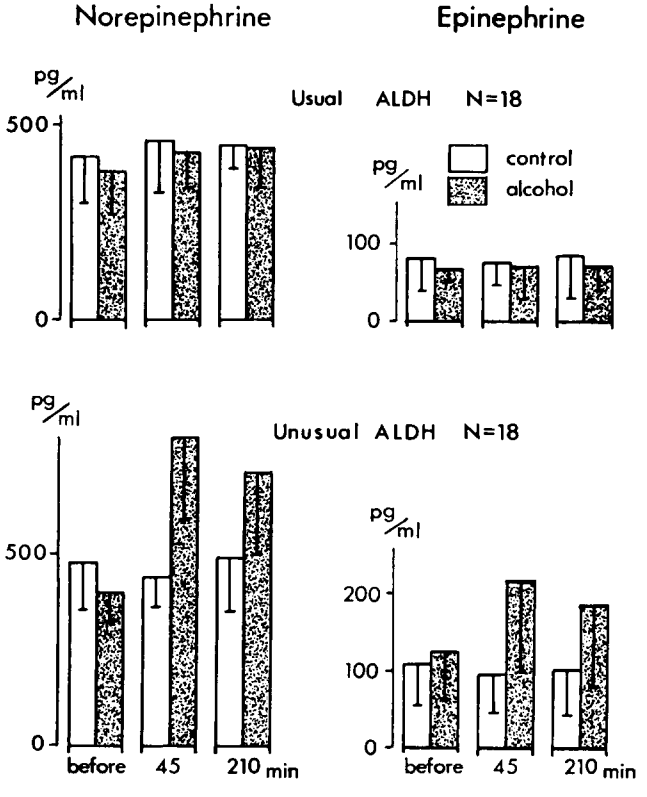

FIG. 7. Plasma catecholamine levels before and after intake of 0.4 g/kg alcohol.

TABLE 3

FREQUENCIES OF ADH AND ALDH POLYMORPHISM IN THE LIVER SPECIMENS OF JAPANESE (N=100)

	ADH			
ALDH	Typical	Atypical (T/AT)	(AT/AT)	Total
Usual	6	58 (31)	(27)	64
Unusual	3	33 (16)	(17)	36
Total	9	91 (47)	(44)	100

liver specimens obtained at autopsy were shown in Table 3. The typical form of ADH was found in 9% and the atypical form in 91% of the subjects, while the usual ALDH was found in 64% and the unusual one in 36%. No difference was recognized in the frequencies of usuul and unusual ALDH phenotypes among the individuals with typical and atypical forms of ADH. The heterozygote and homozygote of atypical liver ADH were found in 47% and 44% of the subjects, respectively. Both phenotypes of ALDH found in the hair root specimens of the same individuals whose liver enzymes were examined were in accord with the liver specimens.

DISCUSSION

Studies on determination blood acetaldehyde have developed markedly in recent few years [1,7]. In the method which we used in the present work, the artefactual formation of acetaldehyde from alcohol which may occur during the deproteinization of blood sample and the incubation prior to head-space analysis is diminished to less than 1 μM in a blood sample containing 10 mM alcohol [2,10].

The blood acetaldehyde levels of nearly all the subjects in the usual ALDH group remained at 1–2 μM. Therefore, it seemed that real blood acetaldehyde levels of most subjects with usual ALDH were approximately zero to 1 μM. The blood acetaldehyde levels of the subjects with unusual ALDH might also have an error of about 1 μM, but they were significantly higher (10 μM to 124 μM). From these results, it is clear that only the people who are lacking in the low K_m ALDH isozyme have elevated blood acetaldehyde following alcohol metabolism. It is conjectured that about 36% of Japanese are deficient in the low K_m ALDH from autopsy data. The frequency of facial flushing found in our daily observation is in accord with this percentage.

The blood acetaldehyde levels of flushing and nonflushing groups in our previous papers [8,9] were unfavorably affected by the artefactual reactions [10] though they depicted a significant difference between the two groups. Nevertheless, the flushing and nonflushing groups in those studies undoubtedly correspond to the unusual and usual ALDH groups in the present work, respectively.

The relation between the polymorphism of ADH and the elevation of blood acetaldehyde was not resolved in the present experiment due to the impossibility in classification of ADH phenotypes in the subjects. Considering the frequency of atypical ADH in the autopsy specimens, about 90% of the subjects with usual ALDH will certainly have atypical ADH. They would have shown a high blood acetaldehyde level if the atypical ADH with a higher activity for ethanol oxidation than the typical ADH made a contribution to the elevation of blood acetaldehyde. However, the usual ALDH subjects who possessed the low K_m ALDH did not show an elevation of blood acetaldehyde level. Only unusual ALDH subjects showed that. These facts point out that the absence of the low K_m ALDH surely causes on elevation of blood acetaldehyde. And yet, among the subjects with unusual phenotype of ALDH, blood acetaldehyde levels varied vastly in the present experiment. About 25% of them developed a very high level of blood acetaldehyde over 50 μM, conspicuous facial flushing and severe discomfort reactions. On the other hand, about 25% showed a relatively low acetaldehyde level (e.g., 20 μM) without any dysphoric symptoms except facial flushing. Such differences in blood acetaldehyde level of the subjects with unusual ALDH might be based on the polymorphism of ADH or the activity of ALDH.

In the present experiment, inspite of a small amount of alcohol ingestion, the subjects with unusual ALDH showed facial flushing, dilated pulse wave pattern, marked increase of pulse rate and decreased diastolic blood pressure with an elevation of plasma catecholamines. Those with usual ALDH did not exhibit these changes. The symptoms and signs may be due to the following: the plasma catacholamine is elevated as a consequence of the regulating mechanism against a decrease of blood pressure based on the dilatation of peripheral vessels. This dilatation leads to facial flushing and dilated pattern in the pulse wave and is caused either by a direct or an indirect influence of acetaldehyde. The increase of plasma catecholamines would elevate the pulse rate and blood flow rate in carotid arteries and prevent the decrease in systolic blood pressure.

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