The Effect of Ethanol on the Amino Acids of the Rat Brain with a Reference to the Administration of Glutamine

By HERTTA-MAIJA HÄKKINEN AND E. KULONEN

Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki, Finland

(Received 18 July 1960)

In an earlier investigation (Häkkinen & Kulonen, 1959*b*) it appeared that incorporation of the carbon atoms of ethanol into the brain proteins took place comparatively rapidly *in vivo*. Metabolism of the parent amino acids in the nervous tissue is well documented, and glutamic acid and its metabolic derivatives are known to vary with the functional state of the brain (Tower, 1956, 1957). In many convulsive conditions the concentration of γ -aminobutyric acid and glutamic acid in the brain is decreased (Elliott, 1959; Roberts & Baxter, 1959), and some psychotropic drugs increase the content of γ -aminobutyric acid in the brain (Woodbury & Vernadakis, 1958; Nauta, 1959). The glutamic acid content is also influenced by hypoxia, malonate and other inhibitors such as anaesthetics (Dawson, 1951; Klingmüller, 1955; Strecker, 1957; Tower, 1959).

It was expected that the content of the amino acids in the brain would be affected by ethanol intoxication. The changes observed in the amino acids are presented below, together with analyses of some relevant metabolites. A preliminary note on the γ -aminobutyric acid has already appeared (Häkkinen & Kulonen, 1959*a*).

Experiments with additional administration of glutamine were included because of the significance of this substance as the main nitrogenous nutrient passing the blood-brain barrier, and because of the favourable effect of glutamate in certain disorders of the brain (Weil-Malherbe, 1952). Glutamine is also known to decrease significantly the voluntary consumption of ethanol by rats (Rogers, Pelton & Williams, 1955, 1956).

EXPERIMENTAL

Animal experiments and sampling

Adult rats (average weight 150 g.) of the Wistar strain, previously starved for 24 hr., were used. To 19 rats 1.67 ml. of ethanol, i.e. 430 mg./100 g. of body weight as an aq. 33% (v/v) solution, was given by means of a stomach tube. To six rats the same relative amount of ethanol in which was dissolved 167 mg. of L(+)-glutamine (F. Hoffmann-La Roche and Co. Ltd., Basel, Switzerland) per 100 g. of body weight was given. Four rats received the same amount of glutamine alone as an aqueous solution and five rats an equal volume of water as a control. The rats which were given ethanol alone became stuporous and flabby, but not unconscious. The simultaneous administra-

tion of ethanol and glutamine alleviated these symptoms of intoxication. Three of the control rats were killed immediately after the administration of water, and the other rats after lapses of time varying from 20 min. to 24 hr. For the estimation of the variability between individuals an additional series of five ethanol-treated rats (killed after 60 min.) and two control rats (killed immediately) was included.

In all but four cases the brains were excised immediately after decapitation and homogenized with a Potter-Elvehjem homogenizer in ice-cold 75% (v/v) ethanol (12 ml./g. of fresh brain tissue) less than 5 min. after killing. Samples from four rats, two of which had received ethanol and two water, 1 hr. earlier, were fixed by rapid freezing. After a blow on the head (but with the circulation still intact) the rats were dipped into liquid air for 60 sec. Then all the heads were cut off with a saw and allowed to thaw in ice-cold absolute ethanol for 20 min. The brain could then be prepared and homogenized in 75% ice-cold ethanol.

When the analyses had been completed, Professor McIlwain kindly brought to our attention the work by Dawson & Richter (1950), who had used young rats weighing about 40 g. and avoided everything that might have excited the animals. Therefore additional experiments were performed with young rats, average weight 44 g., and the stunning was omitted. The effect of the method of killing is shown in Tables 1 and 2.

When the young rats were used, a fine precipitate appeared in the supernatant (containing 75% ethanol) of the brain homogenate, when it was stored at 4° for 24 hr. Additional precipitate was formed during the subsequent 24 hr. This precipitate was very soluble in chloroform, soluble in ether and slightly soluble in ethanol and acetone.

Reagents

The ethanol administered to the rats was purified, for spectrometric research, and manufactured by Oy Alkoholilike Ab, Finland. L(+)-Glutamine, L(+)-aspartic acid, L(+)-alanine, glycine, L(+)-serine and taurine, used for amino acid standards, were from F. Hoffmann-La Roche and Co. Ltd., Basel, Switzerland. γ -Aminobutyric acid, α -oxoglutaric acid and pyruvic acid were produced by Fluka AG., Buchs, Switzerland, and L(+)-glutamic acid and succinic acid by E. Merck A.-G., Darmstadt, Germany. Celite, Analytical Filter-Aid, was manufactured by Johns-Manville and Co. Ltd., London, S.E. 11. The rest of the chemicals used were all produced by E. Merck A.-G., Darmstadt, Germany, and they were either the 'pro analysi' or 'for chromatography' grade.

Analyses

The homogenates were kept for at least 1 hr. at 4° and then centrifuged at 24 000g for 1 hr. at 0°. The precipitate was suspended in 5 ml. of cold 75% (v/v) ethanol and

centrifuged again for 30 min. The combined supernatants were evaporated by using an air stream at room temperature. Some of the first samples were evaporated on a water bath. The dry residues were dissolved in water (1 ml./g. of original fresh brain tissue).

Amino acids. For paper-chromatographic analysis of γ -aminobutyric acid by a slight modification of the method of Roberts & Frankel (1950), 20 μ l. of this aqueous solution, representing 20 mg. of brain, was applied to each spot. Phenol-water-acetic acid (1000 g.:240 ml.:12.5 ml.) was used as the solvent and one-dimensional chromatograms were run at least in duplicate on Whatman no. 1 paper (size about 45 cm. $\times$ 60 cm.). The other amino acids were analysed by two-dimensional chromatography, with the phenol mixture as the first solvent and butan-1-ol-acetic acid-water (4:1:5, by vol.) as the second. The spots contained 30 μ l. of the same aqueous solution (representing 30 mg. of the fresh tissue). The sheets were dipped into a 0.1% ninhydrin solution (in propan-2-ol), and allowed to dry in the dark at room temperature for 23–25 hr.

The eight most intense spots in the chromatograms (corresponding to glutamic acid, γ -aminobutyric acid, glutamine, aspartic acid, alanine, glycine, serine and taurine) were measured. The spots were cut out (including the same total area in all of the samples), cut into narrow strips and eluted with 5 ml. of water for 1 hr. with occasional shaking. The paper strips were pressed down to the bottoms of the tubes with a glass rod. The colour of the eluate was measured at 570 m μ with a Beckman DU spectrophotometer.

For the preparation of the standard curves, known amounts of the individual amino acids were run through the whole chromatographic procedure. In later work occasional standards were run.

To illustrate the reproducibility of the determinations, the following standard deviations of duplicates from their respective means were calculated: γ -aminobutyric acid $\pm 5.3\%$ ($n = 19$), glutamic acid $\pm 1.5\%$ ($n = 19$), glutamine $\pm 4.8\%$ ($n = 19$) and aspartic acid $\pm 2.5\%$ ($n = 17$).

Succinic acid. For the enzymic determination with succinoxidase the interfering coenzymes had to be removed from the aforementioned aqueous solution. A portion (1 ml.) of this solution (representing 1 g. of the original fresh tissue) was acidified with sulphuric acid below pH 2, adsorbed in a Büchner funnel on 2 g. of dry Celite and eluted with about 200 ml. of dry ether (Swim & Utter, 1957). The ether was evaporated from the samples, the residues were dissolved in 1 ml. of 0.1M-phosphate buffer (pH 7.4), and 0.5 ml. of this solution was taken for each determination according to the manometric method of Cohen (1951).

α -Oxo acids. α -Oxoglutaric acid and pyruvic acid were determined spectrophotometrically as their 2:4-dinitrophenylhydrazones after paper chromatographic separation (Kulonen, Carpen & Ruokolainen, 1952). For each determination 0.5 ml. of the aforementioned aqueous solution (representing 500 mg. of the original fresh tissue) was used.

RESULTS

Amino acids

The amino acid contents of the whole brain (in μ moles/100 g. of fresh tissue $\pm$ S.D.) of the five adult control rats, decapitated at room temperature,

were: glutamic acid 912 ± 18.0 , γ -aminobutyric acid 422 ± 11.1 , glutamine 386 ± 30.5 , and aspartic acid 282 ± 7.3 . In addition, the following amino acids were determined in the brains of two of the control rats: alanine 260 (range 245–272), glycine 270 (range 263–280), serine 280 (range 277–288) and taurine 230 (range 222–232). The corresponding amino acid contents of the brain of the young rats were considerably smaller, with the exception of glutamine (Table 1).

The amount of glutamic acid (Fig. 1) rose in all three experimental series. The rats that received only ethanol showed the greatest increase (130 μ moles/100 g., 14%) at 90 min., and afterwards a decrease to 6% below the initial value. The maxi-

Fig. 1. Change in the glutamic acid content of rat brain after administration of the following substances: ethanol alone (●), ethanol and L-glutamine (■) and L-glutamine (▲). Each point on the curve with ethanol alone is derived from two animals, and on the other curves from a single animal.

Fig. 2. Change in the γ -aminobutyric acid content of rat brain after administration of the following substances: ethanol alone (●), ethanol and L-glutamine (■) and L-glutamine (▲). Each point on the curve with ethanol alone is derived from two animals, and on the other curves from a single animal.

imum increase of the glutamic acid appeared earlier in the other two groups of rats; it was only 7% and rapidly levelled off.

The amount of γ -aminobutyric acid (Fig. 2) rose rapidly in all three series and returned to the initial value in about 2 hr. The rats that received ethanol alone showed a maximum increase (163 μ moles/100 g., 38%) 60 min. after ethanol administration, whereas the rats that were given both ethanol and glutamine showed only an 18% increase, and those that received glutamine alone a 20% increase over the initial value, both 40 min. after the administration.

The glutamine level (Fig. 3) of the rats which were given ethanol alone fell by 68 μ moles/100 g., i.e. 18% of the initial value, in 40 min., and then slowly recovered in 3 hr. The rats that received

Fig. 3. Change in the glutamine content of the rat brain after administration of the following substances: ethanol alone (●), ethanol and L-glutamine (■) and L-glutamine (▲). Each point on the curve with ethanol alone is derived from two animals, and on the other curves from a single animal.

Fig. 4. Change in the aspartic acid content of rat brain after administration of the following substances: ethanol alone (●), ethanol and L-glutamine (■) and L-glutamine (▲). Each point on the curve with ethanol alone is derived from two animals, and on the other curves from a single animal.

both ethanol and glutamine as well as those that received glutamine alone showed a steep rise in the brain glutamine level, with maximum increases of 30 and 36%, respectively, followed by a comparatively slow decrease.

The rats that were given ethanol alone showed an increase of 50 μ moles/100 g. (18%) in the aspartic acid level between 1 and 2 hr. (Fig. 4), whereas there was no significant change in the other groups.

In the group that received ethanol alone, the maximum changes were thus observed in this chronological order: in the concentration of glutamine, γ -aminobutyric acid, glutamic acid and aspartic acid.

No notable changes occurred in the concentrations of alanine, glycine, serine and taurine. The changes in the glutamic acid, glutamine and γ -aminobutyric acid were not dependent on the killing conditions or on the age of the rats (Table 1).

Evaluation of the results. In Table 1 are listed the statistical probabilities of the chance occurrence of the changes observed in γ -aminobutyric acid, glutamic acid and glutamine in the brains of young rats 60 min. after ingestion of ethanol. The respective probabilities in adult rats (eight test animals, killed 60 min. after administration of ethanol, and five control animals, killed immediately after the administration of the same volume of water) are for γ -aminobutyric acid $P < 0.001$, for glutamic acid $P < 0.001$ and for glutamine $P < 0.005$. These statistical values in conjunction with the shape of the curves (Figs. 1-4) lead us to believe that the changes in these amino acids are significantly related to the action of ethanol. The change in the aspartic acid may be secondary only, but the 98% confidence limit of control values is $\pm 4.9\%$ and the observed maximal deviation ($+18\%$) is outside them.

The 98% confidence limits (based on the estimated standard error) of the amino acid analyses 60 min. after the ingestion of ethanol are for γ -aminobutyric acid $\pm 7.5\%$, for glutamic acid $\pm 1.5\%$ and for glutamine $\pm 2.7\%$. The values obtained by simultaneous ingestion of glutamine and ethanol fell outside these limits. We do not have data enough to decide whether there is a difference between the values obtained with glutamine and ethanol and with glutamine alone, or whether these values differ significantly from the normal level.

Succinic acid, α -oxoglutaric acid and pyruvic acid

The results are collected in Table 2: 60 min. after ethanol administration the succinic acid rose in the average by 22 μ moles/100 g. of fresh brain tissue ($P < 0.01$) above the level of the control rats. The keto acids fell, α -oxoglutaric acid in the

Table 1. *Effect of ethanol on the free amino acids of brain of young rats, when different methods of killing were employed*
Rats were killed 60 min. after the administration of ethanol or water. The figures refer to the concentrations in μ moles/100 g. of the fresh whole brain.

Substance administered	Method of killing	γ -Amino-butyric acid	Glutamic acid	Glutamine	Aspartic acid	Alanine	Serine, glycine and taurine (combined)
Water	Frozen in liquid air	272	559	521	150	64	220
		223	523	494	140	75	220
		214	545	521	161	59	250
	Mean	236	542	512	150	66	230
Ethanol	Frozen in liquid air	350	586	467	168	75	230
		242	545	418	147	51	230
		330	620	487	182	70	230
	Mean	307	584	457	166	65	230
Water	Decapitated at room temperature	+30	+7.7	-11	+11	-1.5	0
		242	518	467	138	62	210
		223	571	576	163	70	260
	Mean	233	544	522	151	66	235
Ethanol	Decapitated at room temperature	340	599	460	173	59	220
		291	588	521	166	81	260
		316	592	491	170	70	240
	Mean	316	+7.7	-6.0	+13	+6.0	+2.1
P of chance occurrence of the differences (water vs. ethanol)		<0.01	<0.025	Not significant	Not significant	—	—
Change in the brains of the adult rats decapitated at room temperature		+35 %	+10 %	-14 %	—	—	—

Table 2. *Succinic acid, α -oxoglutaric acid and pyruvic acid in the whole brain of ethanol-intoxicated rats*
Rats were killed 60 min. after the administration of ethanol or water. The figures refer to the concentrations in μ moles/100 g. of the fresh whole brain.

Substance administered	Method of killing	Age of rats	Succinic acid	α -Oxoglutaric acid	Pyruvic acid	
Water	Stunned, frozen in liquid air	Adult	41	60	74	
			53	67	81	
	Frozen in liquid air	Young	37	54	92	
			41	54	100	
Ethanol	Stunned, frozen in liquid air	Mean	42.8	59.0	86.6	
		Adult	58	45	58	
			88	60	72	
		Young	56	33	52	
	Frozen in liquid air		65	26	41	
			58	47	60	
		Mean	65.0	42.2	56.6	
			<0.01	<0.05	<0.005	
	Ethanol	Decapitated at room temperature	Adult	108	32	48
				109	33	49
Mean			108.5	32.5	48.5	

average by $17 \mu\text{moles}/100 \text{ g.}$ ($P < 0.05$) and pyruvic acid in the average by $30 \mu\text{moles}/100 \text{ g.}$ ($P < 0.005$).

In contrast to the observations on the amino acids, fixation of the brain in liquid air was essential for the analysis of these metabolites. During preparation at room temperature a conspicuous artifact increase was noted in the concentration of succinic acid and a decrease in both keto acids, as may be seen from Table 2.

DISCUSSION

Dawson (1951) found that in rats anaesthetized with a barbiturate (thiopentone) the free glutamic acid content of brain was lowered significantly and the glutamine slightly raised. We have no explanation for our findings, but believe that they are due to different mechanisms and levels of anaesthesia produced.

Kini & Quastel (1959) investigated the conversion of the uniformly labelled glucose into amino acids by cerebral tissues *in vitro*. Sodium amytal increased the radioactivity in alanine, glutamic acid and γ -aminobutyric acid. On simultaneous stimulation with potassium ions the radioactivity of the glutamine fell. The authors attributed these findings to inhibition of the reoxidation of reduced diphosphopyridine nucleotide by amytal. These changes closely resemble our experimental findings with ethanol, reported above, except as regards alanine. However, ethanol is not known to affect the respiratory enzymes in the brain, even if the oxygen uptake and glucose uptake of stimulated brain slices are decreased (Wallgren & Kulonen, 1960). Our preliminary assumption was that the increases in the glutamic and γ -aminobutyric acids were due to inhibition of the normal oxidation of α -oxoglutaric acid to succinic acid. The changes observed in these metabolites do not support this view, even though all the amino acids in which changes occurred are closely connected with the citric acid cycle.

It seems that the effect of ethanol on the amino acids of the brain is suppressed by glutamine, since the values obtained after combined ethanol and glutamine administration differ only slightly from those obtained with glutamine alone. If the total amount of amino groups bound in the glutamic acid, γ -aminobutyric acid and glutamine is considered, there seems to be a net increase of this bound ammonia during intoxication. In some unknown way the administration of glutamine appears to prevent this accumulation. Glutamine stimulates the adrenergic system (Weil-Malherbe, 1952; Weil-Malherbe & Bone, 1952), and it may not act at metabolic level. This point will be discussed later in connexion with relevant experiments *in vitro*.

The decrease in the keto acids may be due to the diminished utilization of glucose, which also may cause the impaired synthesis of glutamine. A deficit of oxygen may account for the increase of both the succinic and the γ -aminobutyric acid.

Whether the presence of ethanol induces these changes in the amino acids directly or indirectly is obscure. It seems improbable that the increase in the γ -aminobutyric acid is the direct cause of the manifestations of ethanol intoxication, since a notable increase is also observed on administration of glutamine. Since it is not necessary to employ rapid fixation in liquid air to obtain these results on the amino acids, it is suggested that the changes in the amino acid concentrations are not directly due to any imbalance in intermediates of the energy metabolism.

SUMMARY

1. During the period of ethanol intoxication an increase was observed in the content of γ -aminobutyric acid, glutamic acid and aspartic acid in the brain, and a decrease in glutamine. The alanine, glycine, serine and taurine remained unchanged. Simultaneous ingestion of glutamine suppressed these effects of ethanol.

2. Succinic acid increased and α -oxo acids decreased during intoxication.

3. When the brains were prepared at room temperature, the changes in the concentrations of succinic acid and α -oxo acids were more pronounced than after a fixation in liquid air. For the estimation of the glutamic acid, glutamine and γ -aminobutyric acid, fixation in liquid air was not necessary.

4. The content of the free amino acids in brain, with the exception of glutamine, was significantly lower in the young rats than in the adult rats.

We acknowledge our gratitude to our Chief, Dr Heikki Suomalainen, for encouragement and criticism, to Professor H. McIlwain for suggesting liquid-air fixation and for valuable comments, and to Dr O. Forsander and other colleagues for discussions and advice.

REFERENCES

- Cohen, P. P. (1951). In *Manometric Techniques and Tissue Metabolism*, p. 166. Ed. by Umbreit, W. W., Burris, R. H. & Stauffer, J. F. Minneapolis: Burgess Publishing Co.
- Dawson, R. M. C. (1951). *Biochem. J.* **49**, 138.
- Dawson, R. M. C. & Richter, D. (1950). *Amer. J. Physiol.* **160**, 203.
- Elliott, K. A. C. (1959). In *Biochemistry of the Central Nervous System*, p. 251. Ed. by Brücke, F. London: Pergamon Press Ltd.
- Häkkinen, H.-M. & Kulonen, E. (1959a). *Nature, Lond.*, **184**, 726.

- Häkkinen, H.-M. & Kulonen, E. (1959b). *Arch. int. Pharmacodyn.* **123**, 21.
- Kini, M. M. & Quastel, J. H. (1959). *Nature, Lond.*, **184**, 252.
- Klingmüller, V. (1955). *Biochemie, Physiologie und Klinik der Glutaminsäure*, p. 230. Aulendorf in Württemberg: Editio Cantor KG.
- Kulonen, E., Carpén, E. & Ruokolainen, T. (1952). *Scand. J. clin. Lab. Invest.* **4**, 189.
- Nauta, W. T. (1959). In *Biochemistry of the Central Nervous System*, p. 278. Ed. by Brücke, F. London: Pergamon Press Ltd.
- Roberts, E. & Baxter, C. F. (1959). In *Biochemistry of the Central Nervous System*, p. 268. Ed. by Brücke, F. London: Pergamon Press Ltd.
- Roberts, E. & Frankel, S. (1950). *J. biol. Chem.* **187**, 55.
- Rogers, L. L., Pelton, R. B. & Williams, R. J. (1955). *J. biol. Chem.* **214**, 503.
- Rogers, L. L., Pelton, R. B. & Williams, R. J. (1956). *J. biol. Chem.* **220**, 321.
- Strecker, H. J. (1957). In *Metabolism of the Nervous System*, p. 459. Ed. by Richter, D. London: Pergamon Press Ltd.
- Swim, H. E. & Utter, M. F. (1957). In *Methods in Enzymology*, vol. 4, p. 587. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Tower, D. B. (1956). In *Neurochemistry*, p. 169. Ed. by Korey, S. R. & Nurnberger, J. I. New York: Paul B. Hoeber Inc.
- Tower, D. B. (1957). *Clin. chim. Acta*, **2**, 397.
- Tower, D. B. (1959). In *Biochemistry of the Central Nervous System*, p. 213. Ed. by Brücke, F. London: Pergamon Press Ltd.
- Wallgren, H. & Kulonen, E. (1960). *Biochem. J.* **75**, 150.
- Weil-Malherbe, H. (1952). *Symp. biochem. Soc.* **8**, 16.
- Weil-Malherbe, H. & Bone, A. D. (1952). *J. ment. Sci.* **98**, 565.
- Woodbury, D. M. & Vernadakis, A. (1958). *Fed. Proc.* **17**, 420.

Biochem. J. (1961) **78**, 593

A Study of the Colorimetric Estimation of Oestradiol-17 β , Oestradiol-17 α , Oestrone, Oestriol and 16-*epi*Oestriol by the Kober Reaction

By W. NOCKE

*Chemische Abteilung, Chirurgische Universitäts- und Poliklinik, Bonn-Venusberg, Germany
and Frauenklinik, Medizinische Akademie, Düsseldorf, Germany**

(Received 6 July 1960)

In the course of applying the method of Brown (1955) for the estimation of urinary oestrogens, the Kober reaction employed in this method did not work well in our hands. The intensities of colours developed from pure crystalline oestrogens and their methyl ethers were considerably lower than those specified by Bauld (1954) and Brown (1955) (cf. Breuer, Nocke, Geissler & Mitchell, 1957). This was not rectified by addition of oxidizing agents to the reagents as recommended by Bauld (1954). In addition to the typical absorption maximum between 510 and 520 m μ there was a peak at 460–470 m μ , indicating that the yellow colour of the first stage of the reaction was not being completely converted into pink colour ('inhibition type II' of Bauld, 1954). Also, when compared with those of Brown (1955), there was a 4–6 m μ shift in the absorption peaks of the pink colours. The reproducibility of the results was satisfactory, but the sensitivity was low. The conditions of the reaction were therefore re-examined in an attempt to obtain the sensitivity reported by Bauld (1954) and Brown (1955). In this investigation, 16-*epi*oestriol

and oestradiol-17 α have been examined as well as the three 'classical' oestrogens, oestradiol-17 β , oestrone and oestriol.

MATERIALS AND METHODS

Steroids. Standard solutions of pure crystalline oestrogens and their 3-monomethyl ethers were prepared in ethanol (absolute, 'pro analysi'; E. Merck, Darmstadt) with a concentration of 10 μ g./ml. and stored at 4°.

Colour reagents. The Kober reagents contained 2.0% (w/v) of quinol ('puriss', E. Merck) dissolved in aqueous solutions of H₂SO₄ (sp.gr. 1.84; 'pro analysi', E. Merck). The final concentrations of H₂SO₄ were between 50 and 96% (v/v) and were calculated by assuming that the conc. H₂SO₄ was 96% (v/v). Solution of quinol was hastened by heating. The solutions were kept at least 1 week before use and stored in amber bottles at room temperature in the dark (cf. Brown, 1952b; Bauld, 1954); they were colourless and stable for months.

Kober reaction. The standard solutions (0.5–1.0 ml.) were pipetted into 'Kober tubes' (Jena glass; 175 mm. $\times$ 25 mm.) with standard NS 14.5 sockets fitted with cones. Quinol (4 mg.) in ethanolic solution (0.2 ml. of 2%, w/v) and two pieces of white Beauxilite abrasive (size no. 16; Universal Grinding Wheel Co. Ltd., Stafford) washed with ethanol

* Present address