more likely that the cation radical (*8*) is the rearranging species rather than *15*. Two additional points favor *8* over *15* for the intermediate: At high sweep rates the cyclic voltammogram of *6* shows R_4 which must be due to reduction of the intermediate. The radical *15* would reduce at negative potentials. Also R_2 is not observed in acetonitrile, most likely due to rapid deprotonation of *12*. Thus, we favor the reaction pathway involving the cation radical (*8*) for the reactions producing both *11* and *14*.

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Non-enzymic Oxidation of Lower Aliphatic Alcohols by Ascorbic Acid in Tissue Extracts

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It has been shown that ethanol is oxidized to acetaldehyde by ascorbic acid,[1] and since it is not oxidized further, the aldehyde accumulates. The ascorbic acid content of different tissues can vary considerably,[2] so that it can be assumed that the reaction proceeds at different speeds in different tissue extracts. As far as is known, no reports have been published on the non enzymic oxidation of other alcohols than ethanol.

Only small amounts of formaldehyde were formed from methanol when 20 mM alcohol was incubated in a citrate buffer, pH 4.0, containing 2.0 mM ascorbic acid (Table 1). The other normal and iso alcohols tested all formed more than 100 nmol aldehyde in a reaction mixture of 1 ml. With the exception of methanol the oxidation rate decreased with increasing chain length.

Table 1. Oxidation of some aliphatic alcohols by ascorbic acid. Alcohols (20 mM) in 0.1 M citrate buffer, pH 4.0, containing 2.0 mM ascorbic acid was incubated for 60 min at 65 °C. The aldehydes formed except formaldehyde were estimated gas chromatographically.[10] Formaldehyde was estimated spectrophotometrically by the Eegriwes' method.[11] The values given are the means of two experiments.

Alcohol added	Aldehyde formed	(μM)
Methanol	Formaldehyde	< 100
Ethanol	Acetaldehyde	530
Propanol	Propionaldehyde	310
Isobutanol	Isobutyraldehyde	230
Butanol	Butyraldehyde	220
Isopentanol	Isovaleraldehyde	120
Pentanol	Valeraldehyde	110

Extracts contained only a part of the ascorbic acid found in the intact tissue because of the dilution with perchloric acid (Table 2). Bovine liver has been reported to contain 1.7 mmol ascorbic acid/kg, kidney 0.7 mmol/kg, heart 0.3 mmol/kg and blood 0.1 mmol/kg.[2] Of the four tissue extracts examined the liver contained

Table 2. Formation of acetaldehyde from ethanol in tissue extracts. Ethanol 20 mM in PCA-precipitated tissue extracts, pH 4.0, was incubated for 60 min at 45 °C both without and with addition of ascorbic acid. The acetaldehyde formed was estimated gas chromatographically.[10] The values given are the means of two experiments.

Tissue extract	Without ascorbic acid addition		Ascorbic acid added	
	Ascorbic acid (mM)	Acetaldehyde produced (μM)	Ascorbic acid (mM)	Acetaldehyde produced (μM)
Liver	0.34	41.0	1.0	75.6
Kidney	0.15	65.9	0.91	97.9
Heart	0.03	9.0	0.94	108.4
Blood	0	3.4	0.89	66.4

the highest amount of ascorbic acid (Table 2). The kidney extract contained less than half the ascorbic acid present in the liver extract, but nevertheless formed the greatest amount of acetaldehyde from ethanol. Heart and blood extracts contained little ascorbic acid, and the non-enzymic aldehyde formation was low. The ascorbic acid content in the blood extract was so low that it could not be measured. The addition of a similar quantity of ascorbic acid to each of the extracts increased the formation of acetaldehyde in each case, but by an amount dependent on the tissue type (Table 2). This could be explained by the presence of varying amounts of substances in the extracts which can modify the rate of the oxidation. It has been assumed [1] that ethanol is oxidized by a semidehydroascorbate peroxy radical produced in the incubation solution when ascorbic acid is oxidized. Thiols and disulfides strongly inhibit this reaction by acting as free radical acceptors.[3,4] Liver is the tissue richest in thiols and disulfides, while their content in heart muscle and blood is rather low.[5,6]

The non-enzymic oxidation rate of other aliphatic alcohols in tissue extracts containing

Table 3. Formation of aldehydes from some aliphatic alcohols in tissue extracts. Aliphatic alcohols (20mM) were incubated in PCA-precipitated tissue extracts, pH 4.0, for 60 min at 45 °C after addition of ascorbic acid. The ascorbic acid content in the incubation solution after addition was 0.93 ± 0.04 mM. The aldehydes formed were estimated gas chromatographically.[10] The values given are the means of two experiments.

Alcohol added	Aldehyde produced (μM)			
	Liver	Kidney	Heart	Blood
Propanol	50.6	30.4	73.6	47.2
Butanol	31.2	38.6	46.7	24.3
Isobutanol	51.6	31.3	54.2	53.3
Pentanol	22.7	8.6	17.0	22.3
Isopentanol	11.2	10.9	21.8	25.7

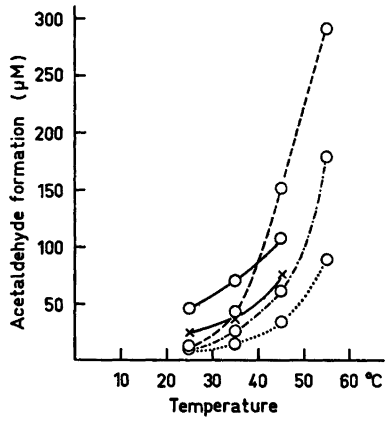

Fig. 1. Formation of acetaldehyde from ethanol at different temperatures and with different amounts of ascorbic acid. Ethanol (20 mM) in 0.1 M citrate buffer, pH 4.0, containing different amounts of ascorbic acid, and in liver and heart extracts (pH 4.0) to which ascorbic acid had been added, was incubated for 60 min at different temperatures. The acetaldehyde formed was estimated gas chromatographically.[10] Every point represents the mean value obtained from two experiments. - - - 2.0 mM, — · — 1.0 mM and ··· 0.5 mM ascorbic acid in citrate buffer; × liver extract containing 1.0 mM ascorbic acid, — heart extract containing 1.0 mM ascorbic acid.

0.9 mM ascorbic acid was lower than that of ethanol (Table 3). Propanol and isobutanol were oxidized at the same rate, while pentanol and isopentanol were oxidized slowly.

The acetaldehyde formed from a given amount of ethanol increased with increasing amounts of ascorbic acid (Fig. 1). With 20 mM ethanol and 1 – 2 mM ascorbic acid in citrate buffer, pH 4.0, rather much acetaldehyde was formed at 55 °C. At 37 °C, however, the aldehyde formation was low, especially at low ascorbic acid concentrations.

As shown in Fig. 1, more acetaldehyde was formed in tissue extract incubated at 25 °C than in ascorbic acid solution. An explanation for this may be that tissue extracts usually contain activators like Cu^{2+} and Fe^{3+} which catalyse the autoxidation of ascorbic acid, especially at low temperatures.[7] At physiological pH, however, the formation of acetaldehyde is much less since the reaction becomes much slower as the pH rises over 5.[1] In addition, ascorbic acid-rich tissues also contain a relatively high amount of SH groups,[8,6,5] which inhibit the non-enzymic oxidation *in vitro*.[1] It can be assumed, therefore, that aliphatic alcohols cannot be oxidized to aldehydes by ascorbic acid *in vivo* at such a speed that the products would produce harmful effects.

Experimental. Bovine liver, kidney, heart and blood were separately homogenized in 0.6 N perchloric acid (PCA) (35 g tissue/100 ml) and the homogenates filtered. Before use, the pH of the acidic filtrate was adjusted to 4.0 by adding 1.25 ml of 5 M KOH and 1 M citrate buffer (pH 4.0) to 10 ml of the filtrate. The samples were incubated for 60 min in a thermostated water bath in the presence of different aliphatic alcohols. After the incubation thiourea was added to prevent alcohol oxidation during the subsequent analysis of the aldehydes. Thiourea was added to the control samples before incubation. In two experiments 0.1 M citrate buffer, pH 4.0, containing different amounts of ascorbic acid was used instead of tissue extract. The ascorbic acid content was estimated by the method described by Roe.[9] The alcohols and aldehydes except formaldehyde were estimated on a Perkin-Elmer F 40 head-space gas liquid chromatograph, as reported previously.[10] Formaldehyde was estimated spectrophotometrically by the violet color which develops on heating with chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) in the presence of strong sulfuric acid. The absorbance was measured at 570 nm.[11]

The analytical procedure was standardized with samples of diluted formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, valeraldehyde, isovaleraldehyde, methanol, ethanol, propanol, butanol, isobutanol, pentanol, and isopentanol of analytical grade. Standard and unknown samples were determined by identical procedures.

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Correction of the Amino Acid Sequence of Soybean Leghemoglobin *a*

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A comparison of the sequence of soybean leghemoglobin *a* (Lb*a*) [1,2] with the sequence of soybean leghemoglobin *c* (*to be published*) and kidney bean leghemoglobin *a* [3] revealed a genetically inexplicable difference at the site 50 – 54 in the leghemoglobin *a* chain. The order of these amino acids in both soybean leghemoglobin *c* and kidney bean leghemoglobin *a* is *Gly-Val-Asp-Pro-Thr*, whereas *Pro-Thr-Asp-Gly-Val* was proposed for leghemoglobin *a* from soybean. This