bond. Studies of the interactions of hydroxamic acids with peroxidase and the very high reactivity of the enzyme with peroxy acids containing aromatic side chains,[10] indicate the presence of a non-polar region near the sixth coordination position of the hematin iron, which should repel the polar HMP more than the alkyl-hydroperoxides.

Materials and methods. H_2O_2, *p.a.* Perhydrol, Merck, HMP was prepared according to Marklund.[3] The concentrations of the peroxides were determined with titanium (IV).[3] Horseradish peroxidase, fraction IIIb,[5] $\varepsilon_{mM\,403}=100$ cm^2 mol^{-1}. Catalase,* beef liver, Boehringer, Mannheim. $\varepsilon_{mM\,405}$ was taken as 297 cm^2 mol^{-1}.[6] H_2O was double distilled from quartz vessels.

For spectrophotometry a Beckman Acta III was used. A Durrum Gibson stopped-flow spectrophotometer,[7] thermostatted at $25\pm0.05°$, and equipped with a 2 cm cuvette, was used for the study of rapid reaction kinetics. Dead-time was <5 msec.

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Detection of General Acid-catalyzed Hydrolysis in Buffer Solutions in Mixed Solvents

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One of the main problems in studies of the acid-catalyzed hydrolysis of acetals and ortho esters has recently been the timing of the proton transfer relative to the cleavage of the carbon-oxygen bond of the substrate. In some particular cases a change in reaction mechanism with structure has been established.[1-5] Conclusions about reaction mechanism have usually been based on whether or not the reaction is subject to general acid catalysis.

In most of the earlier experiments to detect general acid catalysis, reactions have been run in buffer solutions in dioxane–water mixtures.[1,2,6-8] However, the conclusions about general acid catalysis were drawn without studying the possible influence of specific salt effects which are pronounced in this solvent as illustrated by data for the hydrolysis of triethyl orthobenzoate.[9] The appearance of "general acid catalysis" depended on the electrolyte used to maintain the ionic strength constant. The observed differences in the hydrolysis rates were quantitatively accounted for by specific salt effects on the hydronium ion catalysis.

It is sometimes necessary to study reactions in buffer solutions in mixed solvents because the substrates are sparingly soluble in pure water. Therefore it is important to find a way by which salt effects can be eliminated. The absence of general acid catalysis in the hydrolysis of triethyl orthoformate was verified by using a great excess of neutral electrolyte.[10] Unfortunately, the possibility of detecting a very weak general acid catalysis is lost at the same time. The reduction of the rate of the hydronium ion-catalyzed reaction by the organic solvent is compensated for by the accelerating effect of the relatively high ionic strength.

The aim of this study was to find mixed solvents suitable for preparing buffer solutions in which specific salt effects would be eliminated as far as possible.

Table 1. First-order rate coefficients for the hydrolysis of acetophenone diethyl acetal in buffer solutions in DMSO–water mixtures. The ionic strength was maintained constant at 0.100 or 0.060 M with different electrolytes (E). The buffer component ratio [HA]/[A^-] was 1/1. Temperature 45°C.

Weight per cent DMSO	HA	[HA] M	E	10^2k s^{-1}
68.5	$ClCH_2COOH$	0.100	–	1.38
		0.080	$NaClO_4$	1.27
		0.060	»	1.51
		0.040	»	1.54
		0.020	»	1.59
		0.080	$NaNO_3$	1.47
		0.060	»	1.47
		0.040	»	1.47
		0.020	»	1.44
		0.080	NaCl	1.51
		0.060	»	1.43
		0.040	»	1.52
		0.020	»	1.43
81.5	$Cl_2CHCOOH$	0.060	–	2.73
		0.045	$NaClO_4$	2.70
		0.030	»	2.84
		0.015	»	2.67
		0.045	$NaNO_3$	2.75
		0.030	»	2.72
		0.015	»	2.67
		0.045	NaCl	2.78
		0.030	»	2.75
		0.015	»	2.71

Table 2. First-order rate coefficients for the hydrolysis of ethyl vinyl ether in dichloroacetic acid(HA)–sodium dichloroacetate buffers in DMSO–water mixtures. The ionic strength was maintained constant at 0.100 or 0.060 M with different electrolytes (E). The buffer component ratio [HA]/[A^-] was 1/1. Temperature 45°C.

Weight per cent DMSO	[HA] M	E	10^3k s^{-1}	10^2k_{HA} M^{-1} s^{-1}
68.5	0.1000	–	2.55	
	0.0667	$NaClO_4$	2.07	
	0.0333	»	1.65	1.35 ± 0.05
	0.0667	$NaNO_3$	2.01	
	0.0333	»	1.64	1.36 ± 0.15
	0.0667	NaCl	2.03	
	0.0333	»	1.69	1.30 ± 0.16
81.5	0.060	–	0.207	
	0.045	$NaClO_4$	0.176	
	0.030	»	0.161	
	0.015	»	0.120	0.184 ± 0.022
	0.045	$NaNO_3$	0.186	
	0.030	»	0.163	
	0.015	»	0.123	0.190 ± 0.012
	0.045	NaCl	0.187	
	0.030	»	0.171	
	0.015	»	0.132	0.161 ± 0.024

Experimental. Diethyl acetal of acetophenone was prepared from acetophenone, triethyl orthoformate and ethanol by the method first reported by Claisen.[11] Ethyl vinyl ether was a commercial product (Fluka AG, *purum*).

Buffer solutions were prepared by mixing measured volumes of aqueous buffer solutions and dimethyl sulfoxide (DMSO). The weight percentages of DMSO in the 2/1 and 4/1 v/v DMSO – water mixtures were calculated to be 68.5 and 81.5, respectively. The performance of the kinetic experiments has been described previously.[3]

A specifically hydronium ion-catalyzed reaction was studied to confirm that the rate of the reaction remains constant when the concentration of the acid buffer component is varied in buffer solutions in DMSO – water mixtures. Acetophenone diethyl acetal was chosen as the model compound for these studies.[12] Its hydrolysis rate was measured in chloroacetic acid – sodium chloroacetate and dichloroacetic acid – sodium dichloroacetate buffers where the molar ratio of acid to base was unity. When the concentration of the Brønsted acid was varied, three neutral electrolytes, $NaClO_4$, $NaNO_3$, and NaCl, were used to maintain the ionic strength constant. The kinetic data collected in Table 1 reveal that the rate coefficients are constant in each series of measurements. Thus it is reasonable to assume that these buffer solutions can be used to detect general acid catalysis. To show that this is really the case, the general acid-catalyzed hydrolysis of ethyl vinyl ether was studied in dichloroacetic acid – sodium dichloroacetate buffers in DMSO – water mixtures. The rate coefficients presented in Table 2 are plotted against the concentration of dichloroacetic acid in Fig. 1. The increase in the rate coefficient with increasing concentration of the undissociated acid points to general acid catalysis. The slopes of the plots were calculated for each series. The data in Table 2 show that the values of k_{HA} are equal within the limits of experimental error and thus independent of the nature of the electrolyte used to maintain the ionic strength constant. This confirms that the general acid catalysis is real and not due to specific salt effects.

The intercepts of the plots k_{obs} *vs.* [HA] represent the term $k_{H_3O^+}[H_3O^+]$; their values were also calculated. Values between 1.19×10^{-3} and 1.36×10^{-3} M^{-1} s^{-1}

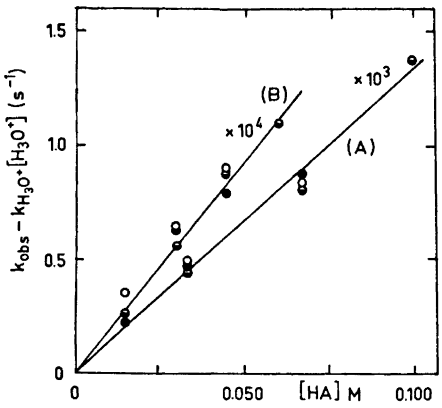

Fig. 1. Plot of $k_{obs} - k_{H_3O^+}[H_3O^+]$ *vs.* [HA]. for the hydrolysis of ethyl vinyl ether in dichloroacetic acid – sodium dichloroacetate buffers in (A) 68.5/31.5 w/w and in (B) 81.5/18.5 w/w DMSO – water solvent at 45°C. The ionic strength was maintained constant with the electrolytes ○ NaCl, ◒ $NaNO_3$, and ● $NaClO_4$. See also Table 2.

were obtained for the reactions in 68.5 wt % DMSO – water and values between 9.6×10^{-4} and 11.4×10^{-4} M^{-1} s^{-1} for the reactions in 81.5 wt % DMSO – water. When these values are compared, we find that the reducing effect of the organic solvent on the term $k_{H_3O^+}[H_3O^+]$ is about twice as great as its effect on k_{HA}. The assumption that general acid catalysis can be more easily detected in mixed solvents than in pure water is thus verified.

According to these studies, DMSO – water mixtures are, unlike dioxane – water mixtures, suitable solvents for buffers for kinetic experiments. Preliminary experiments were also made to investigate specific salt effects in acetonitrile – water mixtures. Salt effects appeared to be similar in this solvent system as in the dioxane – water system.

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Actinomycin D-Sensitive Increase in the Biotinidase Activity in Mouse Liver and Serum after Ethionine Feeding

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Biotinidase (biotinamide amidohydrolase EC 3.5.1.12) is an enzyme which hydrolyzes biotin esters and amides with release of biotin. It is present in several but not in all microbial sources studied, and in animal tissues, notably liver and blood serum.[1-6] The total activity of serum biotinidase in some animal species (*e.g.* hog, rat, and mouse) is of the same magnitude as the total activity in liver. There is also evidence that in these animals biotinidase is mainly produced in the liver and secreted into serum.[5] The present studies with ethionine and actinomycin D were made in order to confirm these earlier findings. Administration of ethionine is known to inhibit protein and ribonucleic acid synthesis, possibly owing to the decreased concentration of adenosine triphosphate caused by the formation of *S*-adenosyl ethionine.[7-10] Actinomycin D is supposed to inhibit protein synthesis mainly by inhibition of RNA synthesis.

Experimental. Adult female mice of 22–27 g were used and kept on the routine laboratory diet (Hankkija, Helsinki, Finland) unless otherwise stated. The diets for the experimental groups are given in the tables. DL-Ethionine, DL-methionine, adenosine, and adenosine triphosphate were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Ethionine and methionine were given mixed in the diet. The dosage of adenosine and adenosine triphosphate was 40 mg intraperitoneally every 12th hour during the experiment. Actinomycin D was purchased from Merck, Sharp & Dohme, Rahway, N.J., U.S.A., and the single dosis used was 30 μg per animal given intraperitoneally. Biotinidase activities were determined as described earlier.[5]

Results and comments. Preliminary studies indicated that there is no change in the biotinidase activities in any group during the first day after the beginning of ethionine feeding. A marked and temporally sharp increase is seen in the liver biotinidase activity 36 h after the beginning of the experimental diet in groups III and IV, which had received ethionine (Table 1). The increase varied from 30 to 150 % in different experiments. After two days activity in the liver decreased almost to control level. Activity in the serum, on the other hand, continued to increase. After one week the activity of liver biotinidase in the ethionine group seems to have decreased below control level. On the other hand, an increase of about 100 % is still found in serum biotinidase activities in the ethionine groups. It is also seen that methionine does not inhibit this effect of ethionine, but rather potentiates it. Similar results were also obtained when ethionine was given intraperitoneally. Intraperitoneal administration of adenosine or adenosine triphosphate had no effect. Thus it seems improbable that this unexpected increase of biotinidase activity is due to a decrease in methylation or in the nucleotide pool. These results indicate that ethionine and methionine may not be antagonistic in this case. Damage of liver cells is not a likely cause as administration of carbon tetrachloride is known to decrease the biotinidase level in rat serum and liver.[5] A similar methionine-resistant increase in the re-