(1100 ml). Gradient elution was then established by addition of 0.05M, pH 6.0 pyridine acetate buffer (1500 ml) through a mixing flask containing H_2O (300 ml). Individual fractions of 10 ml each were collected. The ninhydrin positive fractions in the gradient eluate (tube 51—121) were collected and the solvent was evaporated. Addition of EtOH to the residue afforded powders; yield 0.34 g (63%), mp 217—220°, $[\alpha]_D^{19}$ −7.7° (c=0.3, 10% AcOH). Rf_2 0.13. Amino acid ratios in an acid hydrolysate $Lys_{1.01}Ala_{1.00}$ $Gly_{1.00}$ (average recovery 87%). *Anal.* Calcd. for $C_{11}H_{22}O_4N_4 \cdot CH_3COOH$: C, 46.7; H, 7.8; N, 16.8. Found: C, 46.3; H, 7.9; N, 17.1.

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Reaction of Sodium Hypochlorite with Nucleic Acids and Their Constituents

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Sodium hypochlorite has been used as a disinfectant of drinking water. Bacteria and viruses are rapidly killed in hypochlorite solutions containing several-tenth parts per million (ppm) available chlorine. In order to elucidate the mechanism of the disinfection by hypochlorite, it seems important to know how nucleic acid molecules react with hypochlorite, since the mechanism still remains obscure.[2)] Such knowledge may also enable one to evaluate potential mutagenic toxicity of this chemical.

Little has been reported concerning the chemical reaction between nucleic acids and aqueous chlorine. Prat, *et al.*[3)] have isolated 5-chlorinated pyrimidines from an acid-hydrolyzate of bacteria that had been treated with hypochlorite. In this communication we wish to report a preliminary survey of the reaction of sodium hypochlorite with purines and pyrimidines constituting DNA and RNA.

Calf thymus DNA and yeast transfer RNA were treated with sodium hypochlorite at pH 7 and 37°, and the changes in their ultraviolet absorptions at 260 mμ were followed as a function of time of the treatment. As Fig. 1 shows, rapid decreases in the absorbance of both DNA and RNA were observed even with 10 ppm chlorine. On treatment with 100 ppm chlorine for 1 hr, the absorbance of either of DNA and RNA decreased to about half of the original value. No significant difference in the reaction rate was observed between double-stranded and heat-denatured DNA's. This indicates that a higher order structure does not protect the DNA from the attack of the agent. The decrease in the absorbance must have resulted from the breakdown of the purine and/or pyrimidine bases constituting the nucleic acids. A similar experiment was carried out using nucleotide or nucleoside as the substrate of the reaction, and the results are summarized in Table I. All of the bases re-

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3) R. Prat, C. Nofre, and A. Cier, *Compt. Rend.*, **260** (18) (Groupe 13), 4859 (1965); *idem*, *Ann. Inst. Past.*, **114**, 595 (1968).

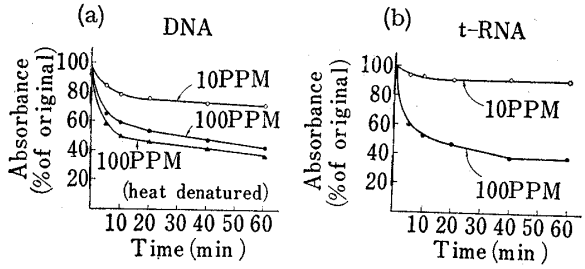

Fig. 1. Reaction of DNA and Transfer RNA with Sodium Hypochlorite

(a) —○—: Native DNA with 10 ppm chlorine;
—●—: native DNA with 100 ppm chlorine;
—▲—: heat-denatured DNA with 100 ppm chlorine
(b) —○—: tRNA with 10 ppm chlorine;
—●—: tRNA with 100 ppm chlorine
The reactions were carried out at pH 7 and 37°.
See text for details.

acted rapidly. The absorbance of purines decreased more extensively than that of pyrimidines. Examination of the entire absorption spectra revealed that after treatment with 100 ppm chlorine for 1 hr both guanosine 5′-phosphate and adenosine lost their absorption peaks at 250—260 mμ, giving only end absorptions. Hypochlorite seemed to react with uridine readily at the initial step, but the primary product that was formed appeared to be resistant to the attack of the reagent, as judged by the little absorption change observed after the initial rapid reaction.

TABLE I. Changes in UV Spectra of Nucleosides on Treatment with Sodium Hypochlorite

	Wavelength (mμ)	Absorbance (% of original)		
		10 ppm chlorine		100 ppm chlorine
		10 min	60 min	60 min
Adenosine	260	82	51	25
Guanosine 5′-phosphate	260	83	75	23
Cytidine	270	104	90	27
Uridine	260	84	83	80
Thymidine	267	87	85	74

Reaction conditions were similar to those employed for the experiment in Fig. 1.

In order to investigate the reaction products, purines and pyrimidines and their corresponding nucleosides were treated with one equivalent of hypochlorite, and the reaction mixtures were analyzed by paper chromatography, using four different chromatographic solvent systems. The *Rf* values in a solvent system and the characteristics in the ultraviolet absorptions of the reaction products are listed in Table II. Analysis by chromatography in the other solvents gave results consistent with those in this Table. The reactions of adenine- and guanine derivatives are apparently complex. Taking the results of Table I into account, one may deduce that from the purines the ultraviolet(UV)-absorbing products given in Table II must first be formed and subsequently the products are degraded into non-UV-absorbing substances. In the present experiment, elucidation of the structures of these products was not attempted.

The products obtained from uracil and uridine were identified as 5-chlorouracil[4] and 5-chlorouridine,[5] respectively, by comparison with authentic samples with respect to their *Rf* values in solvent systems 1 and 2 and their UV spectra in acid and alkali. The results of Table I regarding the uracil derivatives may therefore be interpreted as follows: Hypochlorite, at its chlorine concentration 100 ppm or less, converts uracil into a single product, 5-chlorouracil, and this compound ramains as such under the conditions employed. 5-Chlorocytosine[6] and 5-chlorocytidine[7] produced from cytosine and cytidine, respectively, were also identified by comparison with authentic samples. From either cytosine or cytidine, a

4) H.W. Barrett and R.A. West, *J. Am. Chem. Soc.*, **78**, 1612 (1956).
5) D.W. Visser, K. Dittmer, and I. Goodman, *J. Biol. Chem.*, **171**, 377 (1947).
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Table II. Analysis of Reaction Products by Paper Chromatography

Substrate	*Rf* values[a] of products	UV [H_2O] (λ_{max}, mμ)	*cis*-Hydroxyl groups[b]	Identification
Adenosine	0.07	268	+	
	0.12	250	+	
	0.20	260	+	adenosine
	0.33	264	±	
Guanosine	0.10	253	+	guanosine
	0.23	—	±	
	0.35	255	+	
	0.54	255	+	
Cytidine	0.12	271	+	cytidine
	0.21	287	+	5-chlorocytidine
	0.48	248, 272	+	dichlorocytidine
	0.50	—	+	
Uridine	0.18	261	+	uridine
	0.32	276	+	5-chlorouridine
Thymidine	0.15	270		
	0.44	267		thymidine
Cytosine	0.01	267.5		cytosine
	0.31	216, 282		5-chlorocytosine
	0.66	249, 280		dichlorocytosine
Uracil	0.34	259		uracil
	0.49	274		5-chlorouracil
Thymine	0.02	265		
	0.46	264		thymine

a) chromatographic solvent: *n*-butanol–water, 86:14 (v/v)
b) Detected by periodate–benzidine reagent according to Cifonelli and Smith (*Anal. Chem.*, **26**, 1132 (1954)).

UV-absorbing product having an *Rf* value larger than the 5-chloro compound was produced. From cytosine this product was obtained in a crystalline form. Results of elemental analysis indicated that it is a dichlorocytosine. UV and nuclear magnetic resonance (NMR) spectra suggested the presence of 5,6-double bond. On treatment with 0.1 N hydrochloric acid, this compound was readily converted into 5-chlorocytosine. The dichlorocytosine liberated iodine from potassium iodide in an aqueous solution, whereas 5-chlorocytosine did not do so. This result shows that the second chlorine atom in the dichlorocytosine molecule is a "combined available chlorine". Since cytidine also yields a corresponding dichloro derivative, the combined available-chlorine atom should be bonded to either N^3 or the exocyclic nitrogen atom.

Hypochlorite thus attacks nucleic acids very rapidly. It is possible that in the process of the disinfection by hypochlorite inactivation of nucleic acids occurs also rapidly. Hypochlorite reacts with nucleic acid bases nonspecifically. Therefore the possibility is remote that hypochlorite is highly mutagenic. Indeed, a preliminary experiment with phage λ did not show mutagenicity in this chemical.[8] The process of the degradation of the purines and pyrimidines by hypochlorite apparently involves extensive and complicated oxidation including chlorination. The presence of a product such as the dichlorocytosine which possesses a combined available chlorine would make the total reaction more complicated, because such a compound can by itself serve as a chlorinating agent.

8) A. Miura and H. Hayatsu, unpublished data.

Experimental

Materials and Methods——Sodium hypochlorite was purchased from Wako Chemical Co. Chlorine content of this chemical was found to be 9.8% by titration with sodium thiosulfate. Calf thymus DNA was a product of Sigma Chemical Co. Bulk transfer RNA was prepared from yeast according to Monier, *et al.*[9] Ascending paper chromatography was run in solvent systems, (1) *n*-butanol–water, 86:14 (v/v); (2) ethanol–1M ammonium acetate, pH 7,7:3; (3) *n*-propanol–ammonia–water, 55:10:35; (4) iso-propanol–conc. HCl–water, 65: 16.7:18.3.

Reaction Conditions——(a) Spectroscopic Studies: Ten ml of 0.09M phosphate buffer, pH 7.0, which contained 3 to 5 optical density units (at 260 mμ) of a substrate were warmed at 37°. The reaction was started by addition of 10 μl of the sodium hypochlorite reagent (for 100 ppm-reaction) or its ten-times diluted solution (for 10 ppm-reaction). The reaction mixture was maintained at 37° in a tightly stoppered test tube. At intervals samples were withdrawn and the absorbances were determined within 1 min after the withdrawal.

(b) Paper Chromatographic Studies: To a solution of 0.1 mmole of a substrate in 1 ml of 0.2M phosphate buffer, pH 7, 60 μl of 10% sodium hypochlorite solution (0.1 mmole) was added. After the solution was allowed to stand at 37° for 1 hr, it was submitted to the paper chromatographic analysis.

Dichlorocytosine——To a solution of cytosine (0.56 g) in water (about 35 ml) was added a 10% sodium hypochlorite solution (5 ml) which had been previously adjusted to pH 8 by addition of acetic acid. The reaction mixture was allowed to stand at 37° for 1 hr. Needle crystals of dichlorocytosine that precipitated was collected by filtration and recrystallized from ethanol; yield, 0.365 g (40%); mp 192° (decomp.). *Anal.* Calcd. for $C_4H_3ON_3Cl_2$: C, 23.34; H, 1.67; N, 26.68%. Found: C, 23.20; H, 1.78; N, 26.95%. By treatment with 0.1N HCl at room temperature for 1 hr, this compound was completely converted into 5-chlorocytosine, as detected by paper chromatography. The dichlorocytosine was stable in 0.1N NaOH at room temperature for 1 hr. The dichlorocytosine was electronically neutral at pH 7, as judged by paper electrophoresis in phosphate buffer. UV λ_{max} mμ (ε): 224 (7550), 280 (7900) [H_2O]; 233 (8700), 285 (7050) [0.1N NaOH]. NMR (d_6-DMSO) δ, 8.09 ppm (1-H, s, H-6).

Reaction with Cytidine——On treatment with hypochlorite, cytidine yielded a compound presumed to be dichlorocytidine. The paper chromatographic spot corresponding to this compound was eluted with water and some properties of this substance were examined. UV λ_{max} mμ: 226, 272 [H_2O]; 250, 270 [0.1N NaOH]. Whereas this compound was stable in alkali, it was completely converted into 5-chlorocytidine on treatment with 0.1N HCl at room temperature for 1 hr. The dichlorocytidine liberted iodine (detection by starch) from potassium iodide in its aqueous solution.

9) R. Monier, M.L. Stephenson, and P.C. Zamecnik, *Biochim. Biophys. Acta*, 43, 1 (1960).