The reason for the disagreement with LEDOUX must be looked for in the excessively large amounts of inhibitor and the long incubation periods used in his experiments. Under those conditions all the reagents, with the exception of ethylmaleimide, combine with amino groups. With iodosobenzoate, for example, at a ratio of reagent:enzyme of 100,000:1 he required an incubation of 30 minutes at pH 7 to produce an inhibition of 30%[3]. As emphasized by HELLERMAN *et al.*[6] iodosobenzoate is a reliable –SH reagent *only* when a slight excess is used. On addition of increasing amounts of iodosobenzoate, the utilization by the protein increased, an indication that other groups, such as OH and NH_2 groups, were oxidized (Table II). With H_2O_2 LEDOUX had to increase the ratio to two million to one. It is known[7,8] that H_2O_2 produces deamination of amino acids when used in excess. On addition of $1 \cdot 10^{-4}$ *M* H_2O_2 and $5 \cdot 10^{-5}$ *M* $FeSO_4$, to $1 \cdot 10^{-4}$ *M* amino acid, there was deamination of histidine (50%), tryptophan (50%), and tyrosine (38%)[9].

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1 E. S. G. BARRON, *Adv. in Enzymology*, 11 (1951) 201.
2 L. LEDOUX, *Biochim. Biophys. Acta*, 11 (1953) 517.
3 L. LEDOUX, *Biochim. Biophys. Acta*, 13 (1954) 121, 537.
4 L. LEDOUX, *Biochim. Biophys. Acta*, 14 (1954) 267.
5 C. B. ANFINSEN, R. R. REDFIELD, W. L. CHOATE, J. PAGE AND W. R. CARROLL, *J. Biol. Chem.*, 207 (1954) 201.
6 L. HELLERMAN, F. P. CHINARD AND P. A. RAMSDELL, *J. Am. Chem. Soc.*, 63 (1941) 2551.
7 H. D. DAKIN, *J. Biol. Chem.*, 1 (1906) 171.
8 C. NEUBERG, *Biochem. Z.*, 20 (1909) 531.
9 E. S. G. BARRON, J. AMBROSE AND P. JOHNSON, *Radiation Res.*, 2 (1955) 145.

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A fluorescent derivative of guanine formed during the hydrolysis of DNA

LEVY AND SNELLBAKER[1] have described a fluorescent compound (W.S.), which they have isolated from DNA's from several sources and have suggested it might be a naturally occurring base. Evidence presented below shows that W.S. is a product formed from guanine during the hydrolysis of the nucleic acid.

It was confirmed that the hydrolysis products of thymus DNA formed by the action of *N* HCl for 1 hour at 100° contained a compound with the properties of W.S. This was separated from the other products by 2 dimensional chromatography in *iso*propanol-water-hydrochloric acid[2] and *iso*propanol–water–ammonia[3]. (W.S. has the same R_F as cytosine in the first solvent and a slightly smaller R_F than cytosine in the second.). For estimation, cytosine and W.S. were eluted simultaneously; portions of the eluate were concentrated and W.S. and cytosine were separated by paper electrophoresis at pH 3.5. At this pH in ammonium formate buffer, W.S. remained stationary (*cf.* ref. 1) and cytosine moved as a cation. As shown in the table, the absorption at 258 mμ of the W.S. formed by the hydrolysis was equal to 17% of the absorption at 250 mμ of the liberated guanine. Hydrolysis with *N* HCl at 55° for 1 hour, which releases the purines guanine, adenine and 6-methyl-aminopurine[4], did not release any W.S. from thymus DNA. Apurinic acid prepared by this hydrolysis was dialysed against 0.1 *N* HCl and then against water. After hydrolysis in *N* HCl at 100° and separation on the same solvents, apurinic acid showed only 4% of the amount of W.S. present in an equivalent amount of DNA hydrolysed in the same way. The apurinic acid was found to contain residual amounts of purines sufficient to account for the presence of the small amount of W.S.

The properties of W.S.: fluorescence, $E_{max.}$ at pH 1 at 258 mμ, insolubility in alkaline solvents, p*K* values[1], suggested it might be derived from guanine. When 4 mg guanine were added to 4 mg of apurinic acid and the mixture hydrolysed with *N* HCl at 100°, the amount of W.S. formed was greatly increased, as shown in the table. Less guanine was obtained by *N* HCl hydrolysis at 100° of apurinic acid and thymus DNA, than was obtained by 72% perchloric acid hydrolysis. The difference was approximately proportional to the amount of W.S. formed. Adenine did not show any corresponding difference, as shown below.

In agreement with LEVY AND SNELLBAKER, I found guanine deoxyriboside, hydrolysed with

TABLE I

FORMATION OF BASES AND W.S. BY THE HYDROLYSIS OF THYMUS DNA AND OF APURINIC ACID, USING 72% PERCHLORIC ACID OR *N* HCl AT 100° FOR 1 HOUR

Values expressed as moles free bases/100 moles total bases in original DNA (or per 21.3 moles cytosine). Molar extinction coefficient of W.S. at 258 mμ and pH 1 assumed to be equal to that of guanine at 250 mμ (*i.e.* $11 \cdot 10^3$).

Hydrolysis	*Thymus DNA*		*Apurinic acid*		*Apurinic acid with added guanine*
	$HClO_4$	*N* HCl	$HClO_4$	*N* HCl	*N* HCl
Bases					
Adenine	28.2	28.5	0.18	0.18	
Guanine	21.5	19.1	0.43	0.21	21.5*
Cytosine	21.3	2.0	21.3	2.7	2.3
W.S.		3.2		0.13	11.2

* Added guanine calculated on molar basis.

N HCl at 100° for 1 hour, did not give any W.S., but, when phosphoric acid was added to the system, approximately 4% was converted to a fluorescent compound with properties similar to those of W.S. Also, guanine deoxyriboside, hydrolysed by *M* phosphoric acid, gave a similar quantity of W.S. Guanine riboside did not give any W.S. under the same conditions.

Although requiring phosphoric acid for its formation, W.S. does not contain phosphorus[1]. This was confirmed by ALLEN's method[5].

I conclude that W.S. is formed by a reaction between guanine and some degradation product of deoxyribose in the presence of phosphoric acid. This degradation product is not laevulinic acid[6], as guanine, phosphoric acid, *N* HCl and laevulinic acid heated together at 100° for 1 hour gave no detectable quantity of W.S.

Hydrolysis of W.S. with 72% perchloric acid or *M* phosphoric acid at 100° for 1 hour does not convert it to guanine, but to two or more compounds, the major component of which is non-fluorescent in acid and alkali and has the following characteristics: R_F in *iso*propanol–water–hydrochloric acid 0.35, $E_{max.}$ at pH 1 at 252 and 300 mμ and at pH 13 at 235, 258, 340 mμ.

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1 H. B. LEVY AND L. F. SNELLBAKER, *Arch. Biochem. Biophys.*, 54 (1955) 549.
2 G. R. WYATT, *Biochem. J.*, 48 (1951) 584.
3 R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 52 (1952) 552.
4 D. B. DUNN AND J. D. SMITH, *Biochem. J.*, 60 (1955) xvii.
5 R. J. L. ALLEN, *Biochem. J.*, 34 (1940) 858.
6 P. A. LEVENE AND T. MORI, *J. Biol. Chem.*, 83 (1929) 803.

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A buffer for spectrophotometric enzyme studies

The methods developed for the enzymic determination of purine nucleotides require buffer systems transparent at wavelengths corresponding to the ultra-violet absorption peak of the purines. KALCKAR[1] suggested the use of succinate buffer for the pH range up to 6.1, and glycylglycine and glycine for pH values above 7.3. The more general application of KALCKAR's technique has extended far beyond the estimation of nucleotides to such problems as the determination of reaction rates, the influence of activators and inhibitors, the determination of pH optima and other properties of enzymes. For these applications a greater range of buffers is needed, especially over the pH range 6–7. The specific requirements for these buffers are that they transmit in the ultraviolet and do not interfere with the course of the enzymic reactions. UMBREIT[2] lists the following buffers which can be used between pH 6 and 7: uric, carbonic, pyrophosphoric, maleic and phosphoric acids, and strychnine and quinine. Of these only carbonic and the phosphoric acids are transparent in the ultraviolet. However, carbonic acid is not suitable for an open system, and the deamination of adenylic acid by muscle deaminase is markedly inhibited by