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

phosphate (OSTERN AND MANN[3]). Veronal acetate buffer (MICHAELIS[4]) absorbs strongly in the ultraviolet above pH 6. Examination of the dissociation constants of various weak acids and bases suggested that alkyl substituted dicarboxylic acids would fulfil all requirements. A small amount of β,β':dimethylglutaric acid was kindly supplied by Dr. CYMERMAN-CRAIG of the Organic Chemistry Department and found to be satisfactory. A larger sample of β,β':dimethyl glutaric acid was prepared from dimedone (SHRINER AND TODD[5]) by oxidation with sodium hypochlorite (SMITH AND MCLEOD[6]). The product was contaminated with small quantities of β,β':dimethylacrylic acid which absorbed strongly in the 260 mμ region of the ultraviolet spectrum. This impurity was easily removed by dissolving the reaction product in acetone and oxidising the β,β':dimethylacrylic acid with potassium permanganate. The β,β':dimethylglutaric acid, after recrystallisation from benzene and drying over P_2O_5 under high vacuum, had a melting point of 100° C.

The ultraviolet absorption curve of a 0.02 *M* solution of disodium dimethylglutarate in water was determined in a Unicam SP 500 spectrophotometer. The optical density is sufficiently low to permit its use as a buffer at wavelengths down to 240 mμ (Fig. 1).

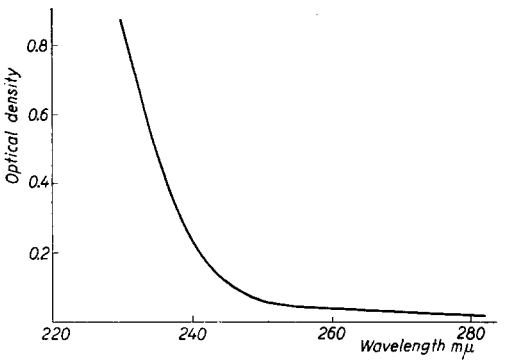

Fig. 1. Ultraviolet absorption spectrum of 0.02 *M* disodium dimethylglutaric acid in water.

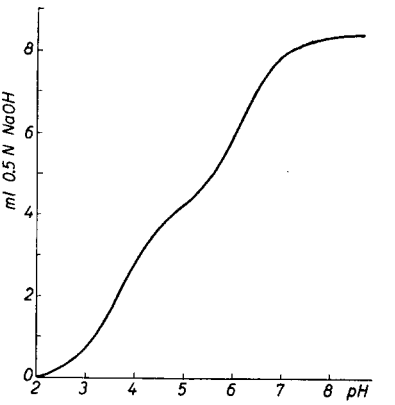

Fig. 2. The change in pH of 10 ml of 0.2 *M* dimethylglutaric acid on titration with 0.5 *N* NaOH.

The change in pH of 10 ml of 0.2 *M* dimethylglutaric acid solution on titration with 0.5 *N* NaOH is shown in Fig. 2. The only published value for the dissociation of the acid is given by AUWERS[7] as $K_a = 2.206 \cdot 10^{-4}$ for the first dissociation constant; this corresponds to a pK_a of 3.66 which is in good agreement with the maximum buffering capacity at the lower pH range shown in the figure. No value for the second dissociation constant has been found in the literature. Fig. 2 shows that dimethylglutaric acid buffers appreciably over the entire pH range from 2 to 7.5.

Dimethylglutaric acid was shown to have no effect on the activity of skeletal myosin adenylic acid deaminase or adenosine and adenylic acid deaminases from cardiac muscle.

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[1] H. M. KALCKAR, *J. Biol. Chem.*, 167 (1947) 429.
[2] W. W. UMBREIT, R. H. BURRIS AND J. F. STAUFFER, *Manometric Techniques and Tissue Metabolism*, 1949, Burgess Publishing Co., Minneapolis.
[3] P. OSTERN AND T. MANN, *Biochem. Z.*, 260 (1933) 326.
[4] L. MICHAELIS, *Biochem. Z.*, 234 (1931) 139.
[5] R. L. SHRINER AND H. R. TODD, *Organic Syntheses*, 15 (1935) 14.
[6] W. T. SMITH AND G. L. MCLEOD, *Organic Syntheses*, 31 (1951) 40.
[7] K. AUWERS, *Ann.*, 292 (1896) 132.

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