of the iron[13], and it is perhaps not without significance that both αα-dipyridyl and *o*-phenanthroline stabilize strongly the ferrous state[14]. Inhibition of the system by these two agents may, however, be due to hydroxylation of the complexing agent. The hydroxylating power of a combination of Fe^{++} and riboflavin must be of considerable biological interest.

3-hydroxypyrene is the phenol produced by the metabolism of pyrene[15] and the 3 position of pyrene is a site of free-radical attack[16]. It has been found during these experiments that naphthacene, chrysene, 1:2-benzanthracene and 3:4-benzopyrene also react under these conditions and work is now in progress to identify these products.

The 3-hydroxypyrene was identified by extracting the cooled reaction mixture with 3 × 50 ml. of ether, and chromatographing the dried ethereal extracts on alumina. On development with ether, any phenols present passed slowly down the column as bands fluorescing under ultra-violet illumination. Pyrene gave rise to a single bright-blue fluorescent band which after collection was evaporated to dryness under reduced pressure, and redissolved in spectroscopic ethanol. Its absorption spectrum (220–420 mμ) and that of its sodium salt proved identical with those of 3-hydroxypyrene and its sodium salt[17]. The yield was calculated from the absorption at 386 mμ and 366 mμ. Small losses occurred on the chromatography columns, and yields of less than 3γ could not be estimated satisfactorily.

F. Dewhurst
G. Calcutt

Department of Cancer Research,
Mount Vernon Hospital and the Radium Institute,
Northwood, Middlesex.

[1] Parke, D. V., and Williams, R. T., *Ann. Rep. Chem. Soc.*, **55**, 376 (1959).
[2] Conney, A. H., Miller, E. C., and Miller, J. A., *J. Biol. Chem.*, **288**, 753 (1957).
[3] Mitoma, C., Posner, H. S., Reitz, H. C., and Udenfriend, S., *Arch. Biochem. Biophys.*, **61**, 431 (1956).
[4] Udenfriend, S., Clark, C. T., Axelrod, J., and Brodie, B. B., *J. Biol. Chem.*, **208**, 731 (1954).
[5] Dalgliesh, C. E., *Arch. Biochem. Biophys.*, **58**, 214 (1955).
[6] Raper, H. S., *Biochem. J.*, **26**, 2000 (1932).
[7] Warren, F. L., *Biochem. J.*, **37**, 338 (1943).
[8] Calcutt, G., *Brit. J. Cancer*, **4**, 254 (1950).
[9] Harper, K. H., *Brit. J. Cancer*, **13**, 732 (1959).
[10] Mazelis, M., *Nature*, **189**, 305 (1961).
[11] Baldwin, E., *Dynamic Aspects of Biochemistry*, third ed., 160 (Camb. Univ. Press, 1959).
[12] Openheimer, C., and Stern, K. G., *Biological Oxidation*, 67 (W. Junk, The Hague, 1939).
[13] Emeléus, H. J., and Anderson, J. S., *Modern Aspects of Inorganic Chemistry*, second ed., 178 (Routledge and Kegan Paul, London, 1952).
[14] Emeléus, H. J., and Anderson, J. S., *Modern Aspects of Inorganic Chemistry*, second ed., 183 (Routledge and Kegan Paul, London, 1952).
[15] Harper, K. H., *Brit. J. Cancer*, **11**, 499 (1957).
[16] Norman, R. O. C., Thompson, G. A., and Waters, W. A., *J. Chem. Soc.*, 175 (1958).
[17] Friedel, R. A., and Orchin, M., *Ultra-violet Spectra of Aromatic Compounds* (Chapman and Hall, London, 1951).

Kinetics of the Enzymic Degradation of Deoxyribonucleic Acid into Sub-units

Recent reports from this laboratory[1,2] described the enzymic degradation of deoxyribonucleic acid (DNA) into rod-like sub-units having a molecular weight of 5–6 × 10^5 and a Watson and Crick[3] double helical structure. No production of dialysable nucleotides and no increase of the ultra-violet absorption coefficient at 260 mμ were detectable during the digestion. It is the purpose of this communication to report further information obtained by using a purified enzyme preparation.

The crude enzymic preparation from chicken erythrocytes already described[1,2] was purified several hundred times according to a procedure which will be reported elsewhere[4], and used at activity concentrations about 100 times higher than those of the crude preparation used before. Degradation of chicken erythrocyte DNA (which was exclusively used in this work) by the more active enzyme was characterized by: (1) a first phase in which the molecular weight of DNA (as determined by light scattering[5]) dropped rapidly from its starting value of about 6 × 10^6 to about 5–6 × 10^5 (this phase showed all the features already described for the degradation of DNA by the crude enzymic preparation); (2) a second phase in which the molecular weight continued to drop at a slower rate. In this phase, which had not been detected when using the crude enzyme, there was formation of acid-soluble nucleotides and an increase in the ultra-violet absorption coefficient at 260 mμ. A calf thymus acid deoxyribonuclease (DNase) preparation, to be described elsewhere[6], degraded DNA in much the same way as the purified chicken erythrocytes preparation and was used almost exclusively in further work.

As our previous results[1,2] strongly suggested, a non-random breakdown of DNA by the enzyme, and as a similar suggestion had been put forward by Oth *et al.*[7] in their work on the degradation of DNA by acid DNase, the kinetics of the enzymic degradation was investigated in detail. Digestions were carried out in the light-scattering cell at 20°; to 30 ml. of 5–10 mgm./100 ml. of DNA (molecular weight = 6 × 10^6) in acetate buffer *p*H = 5·4, μ = 0·15, 0·02 ml. of the enzymic preparation (generally containing 10γ protein) was added and molecular weights were determined at intervals of time. No time-lag was detectable, and the initial slope of log $(1 - R)$ versus log t ($R = M_w/M_w^\circ$; M_w = weight average molecular weight; M_w° = initial molecular weight; t = time) was near to 1. Therefore, making the reasonable assumption that $p_t = kt$ (p_t being the probability that any given bond will be cleaved during time t, and k a constant) for values of R between 1 and 0·8, it is concluded that the degradation occurs according to a 'single strand' degradation kinetics[8]. The same behaviour was displayed by DNA samples obtained by enzymic action and ranging in molecular weight from 6 to 1·1 × 10^6.

The degradation of DNA 'sub-units' was studied next. Several samples, obtained by enzymic action from different DNA preparations, and exhibiting molecular weights between 7 and 9 × 10^5 (in acetate buffer *p*H = 5·4, μ = 0·15), were deproteinized by shaking with chloroform *iso*amyl alcohol and then digested in the light-scattering cells as before (DNA concentrations were 10–20 mgm./100 ml.). A time-lag was evident and the initial slope of log $(1 - R)$ versus log t was close to 2. Therefore, making the same assumptions as before, degradation occurred according to a 'double strand' kinetics.

Finally, the transition phase between the two degradation kinetics was investigated; as expected, this was characterized by a plateau region, in which the molecular weight did not change.

These results show that two different types of degradation are going on at the same time. The 'single strand' degradation is the only one apparent in the first phase, because it is acting during the time-lag of the 'double strand' degradation. Whereas the latter

is a random degradation of the type already known for neutral DNase[8,9], the 'single strand' degradation as shown by this work is highly specific and unequivocally shows that (1) sites exist in DNA ($M_w = 6 \times 10^6$) where the enzymic action brings about the splitting of the molecule without any time-lag and (2) the number of these sites is discrete.

Giorgio Bernardi
Charles Sadron

Centre de Recherches sur les
Macromolécules,
Strasbourg.

[1] Bernardi, G., Champagne, M., and Sadron, C., *Nature*, **188**, 228 (1960).
[2] Bernardi, G., Champagne, M., and Sadron, C., *Biochim. Biophys. Acta*, **49**, 1 (1961).
[3] Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 737 (1953).
[4] Bernardi, G. (manuscript in preparation).
[5] Sadron, C., *The Nucleic Acids*, **3**, 1, edit. by Chargaff, E., and Davidson, J. N. (Academic Press, New York, 1960).
[6] Bernardi, G., *Biochim. Biophys Acta* (in the press).
[7] Oth, A., Frédéricq, E., and Hacha, R., *Biochim. Biophys. Acta*, **29**, 287 (1958).
[8] Schumaker, V. N., Richards, E. G., and Schachman, H. K., *J. Amer. Chem. Soc.*, **78**, 4230 (1956).
[9] Thomas, C. A., *J. Amer. Chem. Soc.*, **78**, 1861 (1956).

Relative Sweetness of α- and β-Forms of Selected Sugars

The mechanism of taste perception has been approached through work on the relationships between chemical properties of taste stimuli and their resultant taste responses. Ferguson and Lawrence[1,2] reported that the D-configurations of leucine, *iso*-leucine, valine, histidine, tryptophan and asparagine were sweet, whereas the L-forms were not. These authors observed that *iso*maltose (6-α-D-glucopyranosyl-D-glucose) was sweet, but its anomer, gentiobiose (6 - β - D - glucopyranosyl - D - glucose), was bitter. Cameron[3,4] found that a freshly prepared solution of 10 per cent α-D-glucose was definitely sweeter than a 16-hr.-old 10 per cent solution. The magnitude of the response (fourteen evaluations) suggested that the fresh solution had a sweetness equal to or more than that of a 10·5 per cent equilibrium solution.

The investigation discussed here was designed to repeat Cameron's work and to determine whether the α- and β-forms of other sugars differ in relative sweetness. The mutarotation-rates at room temperature of glucose, fructose, galactose, and lactose were determined by polarimetric measurements. Equilibrium between the α- and β-forms of these sugars was attained within 2·5 hr. after hydration. Consequently, to ensure having an equilibrium mixture, sugars were hydrated 3 hr. before tasting. Before this experiment, it was established that a freshly hydrated solution of sucrose, which does not undergo mutarotation, did not differ in sweetness from a 16-hr.-old solution.

The taste panel consisted of two women and three men, selected on the basis of their high acuity for detecting sweetness differences from an original group of eleven experienced tasters. Evaluations were made daily at 11.30 a.m. in individual, partitioned booths maintained at a controlled temperature. At each session every judge received four pairs of solutions, one of each pair having been hydrated 3 hr. previously, the other hydrated 100–200 sec. before tasting. The time between hydration of the fresh sample and tasting was kept to a minimum by adding freshly distilled water to the weighed compounds in individual volumetric flasks as each judge entered the booth. Judges were requested to circle the number of the sample within each pair which had the greater sweetness. The order of sample presentation was randomized. Distilled water was provided for oral rinsing at the judge's discretion. A minimum of forty separate evaluations per pair was collected. The method in ref. 5 was used to establish significant differences.

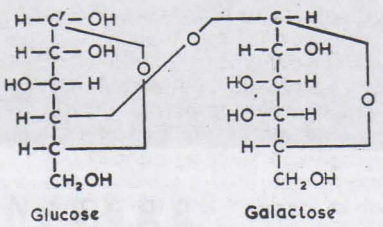

Fig. 1. α-Lactose (D-glucose-4(β-D-galactopyranoside))

As shown in Table 1, the differences in sweetness between the α- and β-configurations became greater as the concentration of the sugar increased. The α-configurations of fructose, glucose and galactose were sweeter than the β-forms, whereas the reverse was true for lactose. This observation is of interest, since the same asymmetric carbon atom (C–1) is responsible for the specific rotation of the lactose molecule as for the glucose molecule (Fig. 1). Rogers[6] suggested that the greater solubility of β-lactose ($55^{20°C}$. in 100 parts water) as compared with the α-form ($8^{20°C}$.)[7] contributed to its greater sweetness. However, β-D-glucose is more soluble but less sweet than α-D-glucose ($154^{15°C}$., $82^{25°C}$., respectively)[8]. When 10 per cent α-D-glucose was used as a sweetener in peach nectar, 76 per cent of 66 comparisons showed

Table 1. Relative Sweetness of α- and β-Forms of Four Sugars

Compound	Concentration (per cent)	No. of evaluations	Sec. after hydration	Response considering (per cent)		
				Alpha sweeter	Equilibrium sweeter	Beta sweeter
α-D-fructose[1]	0·09	40	101	65·0*	35·0	
	0·20	48	103	87·5‡	12·5	
	0·30	40	124	85·0‡	15·0	
	5·00	40	120	90·0‡	10·0	
	7·00	48	126	68·8*	31·2	
α-D-glucose[2]	0·30	40	121	50·0	50·0	
	5·00	40	107	77·5‡	22·5	
	10·00	44	137	88·6‡	11·4	
β-D-glucose[3]	5·00	40	121		75·0†	25·0
	10·00	40	127		85·0‡	15·0
α-versus β-glucose	5·00	40	135	95·0‡		5·0
	10·00	40	148	100·0‡		0·0
α-lactose[4]	0·30	40	117	70·0†	30·0	
	5·00	40	270	40·0	60·0	
	7·00	44	222	13·6	86·4‡	
β-lactose[3]	0·30	48	99		72·9†	27·1
	5·00	40	124		45·0	55·0
	7·00	40	140		35·0	65·0*
α- versus β-lactose	5·00	40	184	25·0		75·0†
	7·00	40	266	12·5		87·5‡
α-D-galactose[3]	5·00	40	130	62·5	37·5	
	10·00	40	185	72·5†	27·5	

* Significant at $P = 0·05$. † Significant at $P = 0·01$. ‡ Significant at $P = 0·001$.

[1] Eastman Organic Chemicals, Lot 679. [2] Eastman Organic Chemicals, Lot 84. [3] Nutritional Biochemicals Corp. [4] Eastman Organic Chemicals, Lot 357.