

ELECTROCHEMICAL OXIDATION-REDUCTION PATHS FOR PYRIMIDINE, CYTOSINE, PURINE AND ADENINE CORRELATION AND APPLICATION

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Summary—In order to evaluate the striking discrepancy between the experimental ease of polarographic reduction of adenine and cytosine, and that predicted by molecular orbital calculation, the electrochemical oxidation-reduction behaviour of pyrimidine, cytosine, purine, adenine and related compounds was investigated at both mercury and graphite electrodes. Information was obtained on the specific adsorption of reactant and product species on the electrode, the reversibility of the energy-controlling electron-transfer step, and accompanying chemical reactions. Triangular sweep voltammetry, a.c. and d.c. polarography, and electrocapillary data, in particular, were utilized. The first three techniques were critically examined for their potential analytical utility. The results were compared with previously obtained electrochemical data and the sequence of electron-transfer and various non-electron transfer steps was more firmly established. It became clear that in order validly to correlate quantum mechanically calculated data for the energy required to add or remove an electron to or from the outermost electron level of each molecule (in the gas phase), with electrochemical redox potentials (in solution), the effects of adsorption, electron-transfer reversibility and solvation energy must be considered.

CONSIDERATION of the conditions under which many electrochemical and biological transformations occur indicates that there should be a distinct parallelism between the two processes. Both electrolytic redox processes and, for example, enzymatic transformations involve heterogeneous electron-transfer as well as similar mass transport modes, and solvent, temperature and ionic strength effects; both processes also have stereochemical requirements that must be met before electron transfer can occur. Accordingly, it should be possible to correlate the pathways for the electrolytic breakdown of biologically important molecules (such as the purines and pyrimidines) with the characteristic potentials associated with these processes, with pathways for biological transformations, and with theoretically calculated energies for the energy-determining initial electron transfer in redox reactions. Struck and Elving¹ found that analogous mechanisms must be postulated to explain the enzymatic² and electrochemical oxidations of uric acid, and Dryhurst and Elving³ found that adenine (6-aminopurine) is electrolytically oxidized at the pyrolytic graphite electrode (PGE) by a mechanism that initially follows the enzymatic path.^{4,5} (The

* The purine and pyrimidine ring numbering advocated by *Chemical Abstracts* has been followed. The commonly accepted formulas are used; allowance may have to be made in some cases for alteration due to keto-enol and acid-base equilibria.

duration of the latter oxidation indicated that intermediate chemical or other non-electron transfer processes were involved; part of the work described in the present study is aimed at elucidating the roles of these effects.)

Quantum mechanical calculations^{6,7} indicate that, of the fundamental purines and pyrimidines found in nucleic acids, cytosine should be the most readily reduced. Predictions based on such calculations are, however, at best only in approximate accord with experimentally observed polarographic reduction half-wave potentials ($E_{1/2}$) or voltammetric oxidation half-peak ($E_{p/2}$) or peak (E_p) potentials. An obvious anomaly is the fact that cytosine (2-oxo-4-aminopyrimidine) is polarographically reduced at a more negative potential than is adenine. Because of this anomaly and the fundamental role that cytosine and adenine play in many biochemical processes, it was decided to examine in some detail the electrochemical behaviour of these compounds and their parent and related purines and pyrimidines. In particular, it was felt that the roles of adsorption of reactants and products at the electrode, reversibility of the electron-transfer step, and solvation energies should be at least qualitatively evaluated, since these effects influence the potential associated with the processes and probably, therefore, the mechanistic route.

In order more fully to understand electrochemical redox mechanisms and their relationship to biological transformations, it is necessary to ascertain qualitatively and, where possible, quantitatively the involvement of specific electrode-substrate phenomena, such as adsorption-desorption effects on the electrode surface and substrate-electrode reactions, which are not present in biological systems and which may, unless taken into account, invalidate correlations of biological and electrochemical data.

The electrochemical behaviour of purines and pyrimidines with stress on their polarography and voltammetry has been reviewed⁸. The compounds of interest in the present study have been investigated⁹⁻¹² by single-sweep direct-potential slow-scan polarography and voltammetry at the dropping mercury electrode (DME) and the pyrolytic graphite electrode (PGE). The present study utilized predominantly cyclic voltammetry at the PGE and at the hanging mercury drop electrode (HMDE). Alternating current polarography and electrocapillary curves at the DME were used to study adsorption phenomena; the former was also employed to evaluate the reversibility of various processes.

Cyclic voltammetric behaviour

Purine, adenine, pyrimidine and cytosine were examined; scan rates were generally 0.06 and 0.6 V/sec at the PGE and 0.026 and 0.26 V/sec at the HMDE.

Pyrimidine at HMDE. Between pH 0 and 4.6, pyrimidine gives a single pH-dependent peak (peak I, Table I). A second, less pH-dependent peak (II) appears between pH 4.6 and 5.5. Above pH 4.7 peak II is smaller than peak I, indicating a short-lived peak I product. Above pH 6, pH-dependent peak III appears, with current about twice that of peak I. At pH 8, peak IV appears, and at pH 9 peak V appears as a slight inflection on background discharge so that accurate evaluation of its potential and current is difficult; its current is about four times that of peak I.

At pH 1.05, i_p/AC , for peak I is constant at both scan rates (Table II); i_p is the peak current, A the electrode area and C the concentration.

Pyrimidine at PGE. Pyrimidine shows a single reduction peak in the pH range 0-8 at 0.06 V/sec and pH 0-6 at 0.6 V/sec scan rate. The pH-dependence of E_p at 0.06 V/sec scan rate seems to change at pH 4 with an additional shift at about pH 6; however, since these effects are small ($\Delta E_p/\Delta \text{pH} = -0.123$ for peak I and -0.091 for peak II) and reproducibility of E_p at the PGE is about ± 25 mV, E_p may be linearly dependent on pH. At the lower scan rate, i_p is constant between pH 1 and 4.6, but then gradually increases; at 0.60 V/sec, i_p is fairly constant. The apparent increase above pH 5-6 at both scan rates is due, at least in part, to the closeness to background discharge and the consequent difficulty in accurately evaluating the current.

TABLE I.—LINEAR E_p vs. pH RELATIONSHIPS FOR PURINE, ADENINE, PYRIMIDINE AND CYTOSINE ON CYCLIC VOLTAMMETRY

Electrode*	Scan rate V/sec	pH Range†	Wave‡	E_p , V
Pyrimidine (0.62mM)				
HMDE§	0.026	0-5.5	I	-0.52 - 0.125pH
		4.5-5.5	II	-1.11 - 0.036pH
		6-9	III	-0.73 - 0.082pH
		8	IV	-1.66
		8.5-13	V	-0.76 - 0.094pH
	0.26	0-5.5	I	-0.57 - 0.113pH
		4.5-5.5	II	-1.22 - 0.031pH
		6-9	III	-0.74 - 0.087pH
		8	IV	-1.71
		9-13	V	-1.90
PGE	0.06	0-4	I	-0.61 - 0.126pH
		4.5-6	II	-0.77 - 0.086pH
		7-8	III	-1.23 - 0.036pH
	0.6	0-3	I	-0.67 - 0.123pH
		3.5-6	II	-0.65 - 0.122pH
Cytosine (0.53mM)				
HMDE	0.026	3.7-7	I	-1.14 - 0.084pH
	0.26	3.7-7	I	-1.17 - 0.082pH
Purine (0.99mM)				
HMDE	0.026	0-9	I	-0.72 - 0.085pH
			II	-1.02 - 0.075pH
	0.26	0-9	I	-0.74 - 0.086pH
			II	-1.10 - 0.064pH
PGE	0.06	0-12	I	-0.84 - 0.077pH
			I _a	-0.88 - 0.089pH
	0.6	0-12	I	-0.80 - 0.083pH
Adenine (0.94mM)				
HMDE	0.026	0-5	I	-1.06 - 0.080pH
	0.26	0-5	I	-1.06 - 0.089pH
PGE	0.06	3.6-10	I _a	1.39 - 0.051pH
	0.6	3.6-10	I _a	1.45 - 0.056pH

* Electrode areas unless otherwise specified: PGE, 11 mm²; HMDE, 5 mm².

† Ionic strength 0.5M.

‡ Subscript a refers to an anodic wave; the other waves are cathodic.

§ Area of HMDE electrode was 0.6 mm².

No evidence for reversibility of the processes was seen on fast scan (36 V/sec) cyclic voltammetry. At pH 1.0, i_p/AC is constant (Table II).

Cytosine at HMDE. Cytosine shows a single reduction peak of almost constant i_p between pH 3.7 and 7; E_p varies linearly with pH (Table I). At 0.026 V/sec scan rate, point-by-point subtraction of background is necessary in order to characterize the peak; at the higher scan rate, the peak is remarkably well-formed. At pH 4.6, i_p increases linearly with concentration, although the i_p/AC ratio decreases (Table II).

There was no evidence for unstable electroactive intermediates at scan rates of up to 200 V/sec. Cytosine showed no electrochemical activity at the PGE.

Purine at HMDE. Purine shows two well-formed cathodic peaks of constant and very reproducible peak current between pH 1 and 8; peak II disappears at higher pH and peak I disappears above pH 9; peak potentials varied linearly with pH (Table I). No evidence for an anodic process was observed on the return scan at either rate. At pH 4.6, linear $i_p - C$ relationships were observed for both peak I and the sum of peaks I and II, although the i_p/AC ratio for peak II decreased somewhat with increasing concentration (Table II).

TABLE II.—VARIATION OF PEAK CURRENT WITH CONCENTRATION FOR PYRIMIDINE, CYTOSINE, PURINE AND ADENINE ON CYCLIC VOLTAMMETRY AT HMDE AND PGE

Electrode	Area, mm ²	Compound (medium)	Concn. range,* mM	Scan-rate V/sec	Average current range,† μA	i_p/AC range‡
HMDE	6.50	Pyrimidine (pH 1.05 chloride)	0.115–1.15	0.026	0.96–10.3	1.54 ± 0.12
	5.33	Cytosine (pH 4.6 acetate)	0.106–1.06	0.026	3.2–30.7	4.61 ± 0.10§
				0.26	8.8–58	5.5 ± 0.7§
	5.33	Purine (pH 4.6 acetate)	0.04–0.99	0.026	0.57–17.2**	17–11¶
				0.26	(1.64–33.3††)	3.3 ± 0.4
					(2.5–52.5**)	7.3 ± 0.8§
				0.60	(5.2–83††)	10.8 ± 1.1
	5.33	Adenine (pH 5.0 McIlvaine)	0.10–1.00	0.026	7.2–46.5	26 ± 17)¶
				0.26	19–120	38 ± 21)¶
	5.33	Adenine (pH 4.6 acetate)	0.10–0.98	0.026	9.2–52	11.3 ± 0.8§
PGE	11.4	Pyrimidine (pH 1.0 chloride)	0.115–1.15	0.06	2.8–25	30.1 ± 1.6§
				0.60	16–47	2.0 ± 0.1
	11.4	Cytosine		Neither oxidized nor reduced		
	11.4	Purine (pH 4.6 acetate)	0.04–0.99	0.06	3.2–39	7.0–3.4¶
					(2.1–15)	4.7–1.3)‡‡
					19–128	42–11¶
	11.4		0.05–0.93	0.26	(17–48)	38–4)¶‡‡
					5–18	23–5¶
	4.2				(3–6)	14–2)¶
					5–31	25–8¶
4.2				(2–11)	11–3)¶	
				12–107	11.9–9.3	
11.4	Adenine (pH 4.6 acetate)	0.10–0.96	0.06	72–336	66–31¶	

* Five concentrations in each range; four in a very few cases.

† Each average current was the mean of 3 replicate measurements on the same solution.

‡ Either the range of extreme values or the mean and standard deviation.

§ One current omitted because deviant, probably owing to larger than usual variation in area of HMDE.

¶ The ratio of i_p/AC decreased with increasing concentration.

** Peak I.

†† Peak I + peak II.

‡‡ Anodic peak.

At a scan rate of 36 V/sec, a very small anodic peak was observed after both cathodic peaks had been scanned; it did not appear if only cathodic peak I was scanned. This peak is probably due to oxidation of a small portion of the peak II reduction product; the size of the peak and the necessity for very fast scan rates indicate the instability of the peak II product.

Purine at PGE. On starting at zero and scanning initially toward negative potential, a single reduction peak appears at more negative potential than peak I at the HMDE; above pH 9, the peak disappears behind background discharge. On the return scan (towards positive potential), a well-formed oxidation peak appears at much more positive potential; this peak does not appear if the initial scan at a clean electrode is toward positive potential, *i.e.*, it is necessary to scan to potentials sufficiently negative to produce the reduction peak before the oxidation peak appears (Fig. 1). Above pH 9, where the reduction peak is masked by background discharge, the oxidation peak is still observed provided that the initial scan is continued some distance beyond the background discharge potential. The anodic peak appears over the whole pH range investigated (1–12). Hence, the anodic peak is due to the oxidation of the reduction product or some species derived from it.

At both scan rates, E_p for both the anodic and cathodic peaks varies linearly with pH (Table I); the peak currents are constant at each scan rate. At 0.06 V/sec scan, the anodic peak is about one-third

the height of the cathodic peak; at 0.6 V/sec it is much closer in height to the cathodic peak. Above pH 9, when the cathodic peak presumably occurs at more negative potential than background discharge, the observed anodic i_p decreases only slightly. At pH 4.6 i_p for both peaks increases linearly with concentration (Table II) at 0.06 V/sec, although there is a marked decrease in the i_p/AC ratio. The latter effect is so pronounced at 0.26 V/sec that the i_p-C plot is exponential with i_p approaching a limiting value at 1mM level (Fig. 2).

Such behaviour is evidence for adsorption of either purine or its reduction product, although at high scan rates current-limiting effects due to a slow chemical process, *e.g.*, structural rearrangement, or to diffusion-layer limitations may be contributory factors. Additional evidence for adsorption at the PGE is provided by the somewhat more negative potentials of the reduction peak at the PGE compared to peak I at the HMDE, assuming that the potential of zero charge on both electrodes is the same.

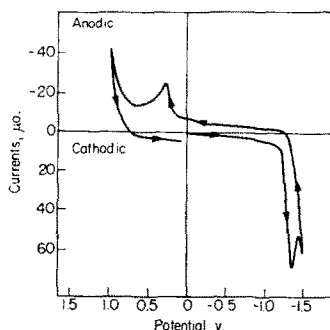


FIG. 1.—Cyclic voltammogram at PGE of 1mM purine in pH 7.0 McIlvaine buffer. Scan-rate 0.06 V/sec. Scan started at 0.0 V and run toward negative potential.

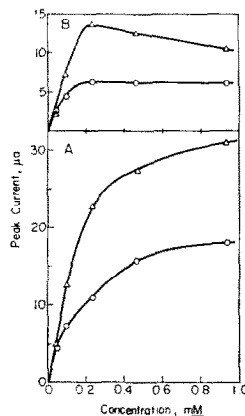


FIG. 2.—Variation of peak current with concentration on cyclic voltammetry of purine in pH 4.6 acetate buffer at a 2-mm PGE. A: reduction peak; B: oxidation peak. Scan-rates: circles, 0.26 V/sec; triangles, 0.6 V/sec.

Adenine at HMDE. Adenine shows a single cathodic peak and no anodic activity even at a 45 V/sec scan rate. Between pH 1 and 5.6, E_p varies linearly with pH at both scan rates (Table I); i_p is constant to pH 5.5 and then decreases very rapidly to vanish above pH 6.

At pH 4.6 (acetate), i_p-C plots are linear at both scan rates but the i_p/C ratio decreases with increasing concentration. The same general effects are noted with pH 5 McIlvaine buffer, although i_p is generally slightly lower than in acetate medium (Table II).

Adenine at PGE. Adenine shows no cathodic activity over the available potential range from pH 1 to 12. However, a large anodic peak appears between pH 3.6 and 10, and is often very poorly formed at the concentration studied (*ca.* 1mM); E_p varies linearly with pH (Table I). The i_p is approximately constant over the pH range. At pH 4.6, deviation from a linear i_p-C relation occurs at both scan rates (Table II); an exponential-type curve is produced at the faster scan rate.

As a qualitative test for adsorption of adenine at the PGE, a clean resurfaced electrode was immersed in a 0.02mM adenine solution for periods of 1–60 min, washed with water without the stream of water striking the surface directly, and then immersed in a previously deaerated pH 4.7 acetate solution; scanning between 0 and 1.3 V, gave a well-formed anodic peak at about 1.06 V.

Polarographic behaviour

The polarographic behaviour of pyrimidine, cytosine, purine and adenine at the DME was generally in conformity with past reports.^{8–10}

Polarography of purine. In view of the reduction of purine at the HMDE at pH \geq 6, contrary to a previous report,⁹ polarographic data for the pH range 1–9 are presented (Table III).

The relatively large magnitude of wave II compared to wave I is associated with the concomitant hydrogen reduction, which is catalysed by the fully reduced form of purine.⁹

$E_{1/2}$ data for purine fit the linear equations for the variation of $E_{1/2}$ with pH in acidic media.⁹

TABLE III.—POLAROGRAPHIC BEHAVIOUR OF PURINE AT THE DME

pH	Wave I			Wave II		
	$-E_{1/2}$, V	i_1 , μA	i_1/C	$-E_{1/2}$, V	i_1 , μA	i_1/C
1.0	0.73	7.1	7.8	1.00	11.3*	12.4
2.0	0.83	6.9	7.6	1.08	11.8*	12.9
3.0	0.95	6.8	7.5			
3.7	1.02	6.0	6.6	1.25	12.7	13.9
5.7	1.19	5.8	6.4	1.39	19.3	21.2
7.0	1.29	7.0	7.7	1.46	17.0*	18.7
7.6	1.32	6.0	6.6	1.49	9.8	10.8
7.8	1.35	5.8	6.4	1.52	7.7	8.5
8.0	1.38	7.8	8.6	1.55	8.4	9.2
9.0	1.46	8.1	8.9	1.58	6.6	7.3

* Maxima occurred on wave.

† Very poorly defined wave; difficult to measure $E_{1/2}$ and i_1 accurately.

Electrocapillary behaviour

Cyclic voltammetry at mercury and, more particularly, at graphite electrodes suggests that adsorption of purines and possibly pyrimidines, as well as of intermediates and/or products, might be a significant factor in the electrochemical reactions.

At pH 4.6, electrocapillary studies show that purine, adenine and cytosine are adsorbed at mercury, since solutions of each produce a depression in the electrocapillary curve, compared to that obtained for background alone, in the region of the electrocapillary maximum (ecm), which would indicate that the adsorbed species are probably uncharged (Fig. 3). The order of electrocapillary depression is: adenine > purine > cytosine.

Owing to erratic drop-times, caused by vibration in the work area, it was difficult to observe clearly the electrocapillary behaviour at potentials more negative than -1.2 to -1.4 V, the region of greatest interest, since faradaic activity frequently occurred at these or more negative potentials. The compounds were, therefore, examined at the DME by alternating current polarography.

Alternating current polarographic behaviour

Pyrimidine. Between pH 1 and 5, pyrimidine shows a single well formed a.c. peak (I, Fig. 4A), with a summit potential, E_s , which varies linearly with pH (Table IV) with a change in slope at *ca.* pH 5, which accounts for its designation as peaks I and II. The separation from background discharge is sufficient for accurate measurement of i_p , which has a minimum value at pH 3.0 (Table V). An additional small, very poorly formed peak may be seen at pH 3.0 ($E_s = 1.04$ V). At pH 3.7, i_p varies linearly with concentration (Table VI).

Between pH 1 and 7, the presence of pyrimidine shifts the background discharge to more positive potential. At pH 7.0, an inflection was noted on the background discharge, which resolved itself into a very well formed peak at pH 7.8 (III, Fig. 4B). Above pH 10–11, no peaks are observed. Over the pH range 1–10, pyrimidine shows no tendency to depress or elevate the base current.

Subsequent work by O'Reilly¹³ has shown that two additional small a.c. peaks can be observed. Between pH 3 and 4, a very small pH independent peak ($E_s = -1.25$ V) is observed. At pH values above 10.6, a fifth peak is seen as a slight inflection on the background discharge.

Clearly, the three peaks observed in the present study correspond to waves I, III and IV observed on DME polarography by Smith and Elving.⁹ The two less distinct waves observed by O'Reilly¹³ correspond to DME waves II and V.⁹

Cytosine. Cytosine shows a single small a.c. peak, with E_s varying linearly with pH (Table IV); i_p/AC is constant at pH 4.6, (Table II). The peak currents are much smaller than those for purine and adenine. Between 25° and 40° the temperature-dependence of i_p is high (3.2%/deg).

Purine. Two reduction peaks appear in the potential range from 0.0 to -1.8 V (Fig. 5). At pH 1 to 2, a very large maximum appears on peak II, similar to that observed on d.c. polarography;⁹ at higher pH, the maximum disappears and peak II is about half the height of peak I but considerably broader.

Both peaks show a linear pH-dependence of E_s between pH 1 and 6 (Table IV), and a linear i_p -C relation at pH 4.6. Between 25° and 40°, the temperature-dependence of both peak heights is high (1.2%/deg for I; 1.4%/deg for II).

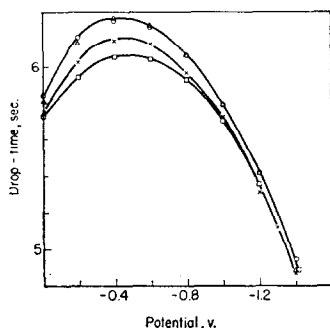


FIG. 3.—Electrocapillary curves for 2.5mM cytosine (triangles), purine (crosses) and adenine (rectangles) in pH 4.6 acetate buffer (background alone: circles).

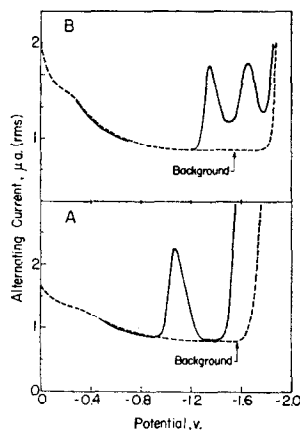


FIG. 4.—Alternating current polarogram of 2mM pyrimidine. A: pH 4.6 acetate buffer; B: pH 7.8 McIlvaine buffer. Where only the background trace is shown, that of the test solution merged with it.

TABLE IV.—LINEAR E_s vs. pH RELATIONSHIPS FOR PURINE, ADENINE, PYRIMIDINE AND CYTOSINE ON ALTERNATING CURRENT POLAROGRAPHY

Compound	Concn., mM	pH Range	Wave	E_s , V
Pyrimidine*	2.29	1-5	I	-0.58 - 0.108pH
		5-9	II	-0.85 - 0.064pH
		7-9	III	-2.01 + 0.036pH
Cytosine	0.99	1-6		-1.27 - 0.054pH
Purine	0.96	1-6	I	-0.72 - 0.080pH
			II	-1.11 - 0.041pH
Adenine	0.98	1-6		-1.09 - 0.067pH

* Two other a.c. waves have been reported for pyrimidine (*cf.* text).

TABLE V.—EFFECT OF pH ON a.c. SUMMIT POTENTIAL AND CURRENT FOR 2mM PYRIMIDINE

Buffer*	pH	E_s for peak, V		First peak† Δi_s , μA
		First†	Second‡	
Chloride	1.05	-0.680		1.76
Chloride	2.00	-0.765		1.62
Chloride	2.95	-0.870		0.67
Acetate	3.65	-0.975		1.18
Acetate	4.65	-1.070		1.45
Acetate	5.55	-1.210		2.03
McIlvaine	7.0	-1.310	-1.700	2.38
McIlvaine	7.82	-1.355	-1.665	1.31
Ammonia	9.1	-1.440	-1.860	1.47

* Ionic strength 0.5M.

† The first peak to appear on the polarogram (peak I between pH 1 and 4.7, and peak II between pH 5.6 and 9.1).

‡ The second peak to appear on the polarogram (peak III).

TABLE VI.—VARIATION OF PEAK CURRENT WITH CONCENTRATION FOR VARIOUS PURINES AND PYRIMIDINES ON a.c. POLAROGRAPHY

Compound	Concn. range, mM	No. of concns.	pH*	i_p † range	i_p/C ‡
Pyrimidine§	1.2–3.1	3	3.7	0.4–1.2	0.367 ± 0.015
Cytosine	0.5–2.5	4	4.7	0.1–1.4	0.46 ± 0.13
Purine	0.5–2.4	4	4.7	I 0.3–2.3 II 0.2–1.1	0.82 ± 0.16 0.41 ± 0.05
Adenine	0.3–2.4	5	4.7	0.3–2.5	1.16 ± 0.15
2-Amino-pyrimidine	1.0–2.4	3	3.7	0.3–0.9	0.35 ± 0.04
2-Hydroxy-pyrimidine	1.0–2.5	3	4.7	0.1–0.4	0.15 ± 0.03

* Acetate buffer, ionic strength 0.5M.

† This peak current is the root mean square value of the a.c. summit current, Δi_s .

‡ Mean and standard deviation.

§ Capillary characteristics: h 605 mm, m 0.89 mg/sec for pyrimidine; h 615 mm, m 1.05 mg/sec for the others.

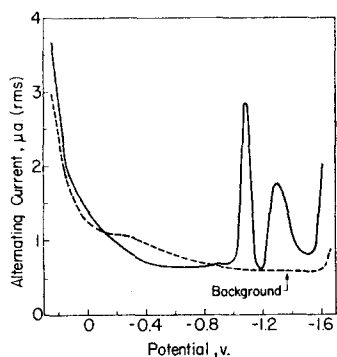


FIG. 5.—Alternating current polarogram of 2mM purine in pH 4.6 acetate buffer.

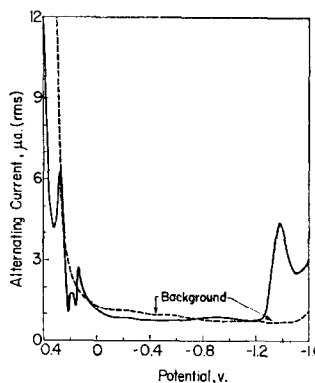


FIG. 6.—Alternating current polarogram of 2mM adenine in pH 4.7 acetate buffer.

Purine depresses the base current in the region of the ecm (Table VII; Fig. 5), although the effect is less than that for adenine. The extent of the depression increases with increasing pH at the values studied (1.05, 4.6 and 5.6). A slight elevation of the base current occurs at potentials immediately before the appearance of peak I. Base current depression and elevation are less at 40°.

With increasing pH, the background discharge in the presence of purine shifts to more positive potential.

Adenine. Over the potential range from 0.40 to -1.80 V, usually only a single well formed a.c. peak is observed (Table IV). At concentrations above about 0.5mM, two or, occasionally, three additional very closely spaced peaks appear at much less negative potential *e.g.*, ~ 0.27 , 0.19 and 0.13 V at pH 4.7 (Fig. 6).

Between pH 1 and 6, E_s of the faradaic peak at negative potential varies linearly with pH (Table IV). At low pH (1–2) i_p is much greater than at higher pH, *e.g.*, 6.8 μ A at pH 1 and 1.4 μ A at pH 4.7 for 1mM adenine; hence, an a.c. maximum probably occurs at low pH in agreement with that observed in d.c. polarography.⁹

A small well formed reproducible a.c. peak appears after the large maximum (at -1.30 V) at pH 1.05; its current is about the same as that for the single adenine peak observed at higher pH.

The faradaic peak at -1.40 V in pH 4.6 acetate buffer shows a more or less constant i_p/C ratio (Table VI). The heights of the closely spaced peaks at more positive potential are variable and show no simple concentration dependence.

In the presence of adenine, the base current is depressed between 0.4 and 0.27 V to about a quarter of its magnitude in the absence of adenine; $i-t$ curves in this region reveal that the a.c. current in the

TABLE VII.—EFFECT OF PURINE, ADENINE, PYRIMIDINE AND CYTOSINE ON THE A.C. POLAROGRAPHIC BASE CURRENT DEPRESSION AND ELEVATION

Compound	Buffer	pH	Depression, potential range V	Elevation, potential range V
Cytosine	Chloride	1.05	0.28–0.55	very small
	Acetate	4.6	0.18–1.09	very small
Purine	Acetate	5.6	0.10–0.75	0.75–1.47
	Chloride	1.05	0.10–0.34	0.34–0.85
	Acetate	4.6	0.10–0.83	about 0.9
Adenine	Acetate	5.6	0.10–0.80	about 1.0
	Chloride	1.05	0.25–0.69	0.69–0.89
	Chloride	2.03	0.24–0.68	very small
	Acetate	3.6	0.00–0.75	0.85–1.20
	Acetate	4.6	0.05–0.83	0.83–1.25
	Acetate	5.6	0.00–0.75	0.75–1.35

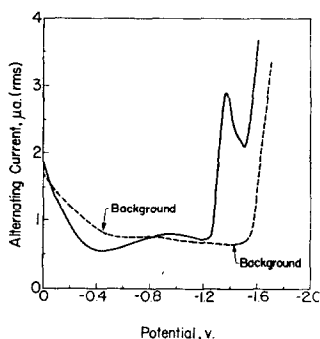


FIG. 7.—Alternating current polarogram of 2mM adenosine in pH 3.6 acetate buffer.

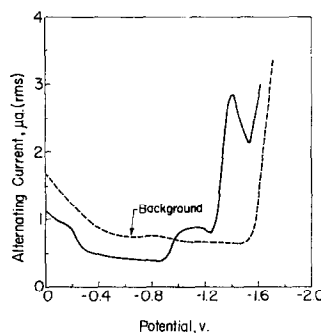


FIG. 8.—Alternating current polarogram of 2mM adenosine monophosphate in pH 3.7 acetate buffer.

presence of adenine is not proportional to $t^{2/3}$ as theoretically predicted for a diffusion controlled process but, after initial growth, becomes almost constant. At the first peak, a.c. $i-t$ curves are normal and the current is unchanged by the presence of adenine. Between 0.27 and 0.24 V, a.c. polarograms of adenine and of background alone coincide. A depression occurs at 0.21 V with a small peak at 0.20–0.18 V; $i-t$ curves in this potential range show an initial increase in current followed by a decrease; in the absence of adenine, normal curves are observed. If a capillary with a long drop-time is used in the region of 0.2 V, the decrease of current with time is very pronounced.

Adenine markedly depresses the base current in the ecm region (Table IX) in all of the background solutions employed; the effect is least pronounced at pH 3.6. At potentials more negative than that of the ecm, a region of base current elevation usually appears (Table VII; Fig. 6).

Below pH 3.7, the presence of adenine appears to have only slight, if any, effect on the background discharge potential. At pH 4.7 and 5.7, adenine causes an appreciable shift of background discharge to more positive potential.

Related compounds. In order better to identify the chemical structures involved in adsorption as well as for general orientative purposes, selected purines and pyrimidines were examined, especially with regard to their effect on the base current, *i.e.*, adsorption behaviour. Generally, each compound was investigated at only a single pH, where the d.c. polarographic behaviour was well known.

2-Aminopyrimidine gives a single small a.c. peak at -0.91 V at pH 3.6, with height linearly dependent on concentration (Table VI). No noticeable effects on the base current or discharge potential are evident.

2-Hydroxypyrimidine gives one small a.c. peak at -0.91 V at pH 4.7; the current, which is linearly dependent on concentration (Table VI), is the smallest for any compound examined. No appreciable effect on base current or onset of background discharge is observed.

At pH 3.6, adenosine gives a single a.c. peak at -1.37 V, which is slightly smaller than that observed for adenine at the same pH (at the 2.0mM level, i_p is 2.12 μ A for adenine and 1.74 μ A for

adenosine), Fig. 7; the background discharge is shifted about 100 mV more positive. In the region of the ecm, adenosine produces a large base current depression with a maximum effect at -0.4 V. At about -1.0 V the base current is elevated.

Adenosine monophosphate gives a single a.c. peak at -1.40 V at pH 3.6, close to the background discharge which is itself shifted to more positive potential (Fig. 8). A very large base current depression occurs between 0 and -0.9 V, which would clearly have continued to positive potentials. A large base current elevation occurs between -1.00 and -1.25 V.

Comparative behaviour. In summary, with increasing substitution of adenine at *N*-9, i_p decreases appreciably and E_s becomes slightly more negative (Table VIII).

Comparative results obtained under identical concentration, capillary and a.c. conditions, are summarized in Table IX. The pH values selected were those for which the d.c. polarographic processes are well documented and a good estimate of the number of electrons involved in the process

TABLE VIII.—ALTERNATING CURRENT POLAROGRAPHIC CHARACTERISTICS OF ADENINE AND DERIVATIVES IN pH 3.6 ACETATE MEDIUM

Compound	Concn., mM	E_s , V	Δi_s , μA
Adenine	2.0	1.380	2.12
Adenosine	2.0	1.370	1.74
Adenosine monophosphate	2.0	1.400	1.51
Adenosine triphosphate	2.0	1.420	0.85

* Capillary characteristics: h 700 mm; m 1.086 mg/sec.

TABLE IX.—COMPARISON OF a.c. POLAROGRAPHY AND LEMO VALUES FOR VARIOUS PURINES AND PYRIMIDINES

Compound*	pH	Reversibility factor†		LEMO‡ energy coeff. k_t	$-E_{1/2}$, V§	
		$n = 1$	$n = 2$		pH 0	pH 5.7
2-Hydroxypyrimidine	4.08	11.7				
	4.87	12.4		-0.670	0.530	0.975
	5.60	16.2				
Purine(I)	4.08	34.4	8.6			
	4.87	37.8	9.4	-0.739	0.697	1.170
	5.60	36.1	9.0			
Purine(II)	4.08	16.5	4.1			
	4.87	25.6	6.4		0.902	1.358
	5.60	20.5	5.1			
Cytosine	4.08	27.1	6.8			
	4.87	28.0	6.9	-0.795	1.125	1.540
	5.60	36.3	9.1			
Pyrimidine	4.08	20.7				
	4.87	24.1		-0.820	0.576	1.175
	5.60	34.2				
2-Aminopyrimidine	4.08	18.8				
	4.87	20.7		-0.820	0.685	1.115
	5.60	21.4				
Adenine	4.08	53.5	13.9			
	4.87	54.1	14.0	-0.865	0.975	1.488
	5.60	30.4	7.9			

* All compounds were examined at the 2mM concentration level. The numerals refer to the two purine waves.

† Method of calculation is described in the text.

‡ Taken from reference 6 except for the values for 2-hydroxy- and 2-aminopyrimidine, which were kindly furnished by Dr. A. Pullman.

§ Calculated from references 9 and 10.

could be made. A reversibility factor was calculated at each particular pH studied, by using the relation

$$\text{Reversibility factor} = \frac{(\Delta i_s)_{\text{expt}}}{(\Delta i_s)_{\text{theory}}} \times 100 \quad (1)$$

where $(\Delta i_s)_{\text{theory}}$ was calculated from the equation²⁹

$$(\Delta i_s)_{\text{theory}} = \frac{n^2 F^2 V A \omega^{1/2} D_0^{1/2} C}{4RT} \times 0.458 \quad (2)$$

where n is the faradaic number of electrons, F the Faraday, V the amplitude of the applied alternating voltage, A the maximum area of the mercury drop, ω the angular frequency, D_0 the diffusion coefficient of the purine or pyrimidine, C the millimolar concentration of the electroactive material, R the gas constant and T the absolute temperature. The expression was multiplied by 0.458 to correct for the much lower current observed for the reversible Fe(III)/Fe(II) system in oxalate media; the low current observed for even such a reversible system was due to the uncompensated capillary thread resistance and other instrumental effects. For those electrochemical reductions where it was known that only a single electron was involved in the d.c. process, $(\Delta i_s)_{\text{theory}}$ was calculated only for $n = 1$ e.g., pyrimidine and 2-aminopyrimidine. Several of the d.c. processes were known to involve two or more electrons, e.g., adenine, purine and cytosine. Because of the low reversibility of these processes, it was considered highly unlikely that n was greater than 2; indeed, it is more likely that n in all cases is 1. Nevertheless, reversibility factors in the latter cases were calculated based on $n = 1$ and $n = 2$ (Table IX). Britz and Bauer³⁰ have reported that although aromatic nitro compounds exhibit a single 4- or 6-electron d.c. wave, the a.c. wave corresponds to only a 1-electron process.

ELECTROCHEMICAL REDOX MECHANISMS

To facilitate detailed discussion of the electrochemical mechanistic pathways for the individual compounds, reversibility, faradaic n values and adsorption phenomena, as these affect all of the compounds involved, will first be considered.

Reversibility. The a.c. polarographic current observed for the various compounds examined is much less than would be expected for even a completely reversible 1-electron process. The fairly large temperature dependence of the faradaic a.c. waves is characteristic of processes having a low degree of reversibility.

Even where cyclic voltammetry showed an anodic-cathodic wave pair, e.g., oxidation of the product produced on reduction of the original species, the separation in peak potentials was very much greater than expected for a perfectly reversible redox couple.^{14,15}

Faradaic n values. In stationary electrode voltammetry, the peak current for an essentially irreversible electrode process at 25° is defined by

$$i_p = 3.01 \times 10^5 n(\alpha n_a)^{1/2} A D^{1/2} C V^{1/2} \quad (3)$$

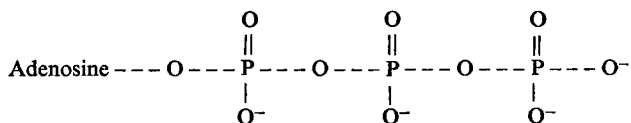
where i_p is in μA , n is the faradaic number of electrons transferred per molecule of reactant electrolysed, α is the transfer coefficient and n_a the number of electrons involved in the rate-determining step of the electrode process, A is the electrode area in mm^2 , C is the concentration of the electroactive species in mmole/l , D is its diffusion coefficient in $\text{cm}^2 \text{sec}^{-1}$ and V is the scan rate in V/sec .

The peak current should vary linearly with concentration and with the square root of scan rate. For the scan rates used in the present study at the PGE and HMDE the current ratio at each should be 0.316; generally, the agreement with this prediction is satisfactory.

Since n_a is generally 1 and α generally varies between 0.3 and 0.7, the term $(\alpha n_a)^{1/2}$ is usually between 0.55 and 0.84. If the not unreasonable assumption is made that α

would not differ by a factor of more than 1.5–2 for the different purines and pyrimidine processes observed, the faradaic n values would be approximately proportional to the peak currents, or to i_p/AC .

Adsorption phenomena. The a.c. results indicate that all of the purine-based compounds are adsorbed on mercury. With the exception of the phosphate derivatives, the compounds are adsorbed in the region of the electrocapillary maximum. The phosphate derivatives appear to be more strongly adsorbed at potentials positive to the ecm, which suggests adsorption of a negatively charged species formed by acidic dissociation of the phosphate grouping as, for example, with adenosine phosphates,



The fact that of the four pyrimidines examined only cytosine gave any evidence for adsorption at mercury, suggests that the site of adsorption of purines may be in the imidazole ring, at either position 7 or 8, since position 9 is occupied in the adenosines. Since position 7 is occupied by a heterocyclic nitrogen atom, adsorption may be more favourable at this position. It is more likely, however, that adsorption would involve π -bonding to the aromatic ring. Base current elevation immediately before the appearance of the a.c. peak suggests that in these potential regions the species are desorbed from the electrode surface.

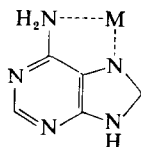
Vetterl^{16,17} used a bridge and a modified tensammetric method to study the effects of purines and pyrimidines on the differential capacity of a DME in 1M sodium chloride with a view to determining the relative magnitudes of various intermolecular forces. His results indicate strong intermolecular interaction of adsorbed molecules only with the usual DNA and RNA components such as adenine, guanine, thymine, cytosine and uracil. Generally, Vetterl sought pronounced depressions in the capacity curves, which only appeared at relatively high concentrations (5mM adenine or 39mM cytosine). Direct comparison of the present work and that of Vetterl is difficult because of the differences in techniques and concentrations employed; however, all of the capacity curves presented by Vetterl showed base current depressions in the presence of a purine or a pyrimidine as compared to background, followed in most cases by an elevation. The failure of Vetterl to report a.c. peaks may be explained on the basis of either confusion of the faradaic current with background discharge owing to the high concentrations employed or the electrochemical inactivity of some species at pH 7.

It was particularly noticeable in the present study that all of the adenine-based compounds are strongly adsorbed on mercury; cyclic and linear scan voltammetry indicates that purine and adenine are adsorbed on graphite. The a.c. peaks observed for adenine at the DME at positive potential suggest either that the compound is adsorbed or that a mercury-adenine compound is formed; the a.c. current-time behaviour agrees with either of these processes occurring, although, since adenine is subsequently adsorbed at potentials near that of the ecm, it is more likely that compound formation occurs. The almost constant height of the adenine peaks at positive potential and above 0.5mM concentration indicates a process which is dependent

mainly on the electrode surface area, *i.e.*, effective saturation of the available surface is reached at 0.5mM.

Failure of the peaks at positive potential to appear at concentrations below 0.4mM suggests their shift to more positive potential, as would be expected if adenine does react with mercury *e.g.*, similarly to riboflavin.¹⁸ The small magnitude of the peaks and the fact that often only two, rather than three, peaks were noted complicate the interpretation. There is further evidence that the peaks are not tensammetric in nature, *e.g.*, a marked base current depression at potentials more positive than even the most positive peak and *i-t* curves characteristic of an adsorption process. If the peaks were in fact tensammetric, the polarogram would be expected to coincide with the background polarogram at potential more positive than that of the most positive peak.

Adenine possess the steric configuration necessary for metal chelation; the *N*(7) and the extracyclic heteroatom attached to *C*(6), provide favourable sites for formation of a five-membered ring, representing a particularly stable configuration:



This expectation (*cf.* pp. 253-8 of reference 6), is borne out in experiment since bivalent metals chemically related to mercury, *i.e.*, copper, zinc and nickel, form metal complexes with purines.¹⁹ Formation constants for the reaction of metal ions with purine and substituted purines have been reported,²⁰ as has the reaction of mercury(II) acetate with purines, including adenine, in aqueous or acetic acid solutions.²¹

Pyrimidine. No evidence for reversibility was observed at the HMDE for any of the five peaks. The close agreement between the pH dependences by cyclic voltammetry (Table I) and slow single scan polarography¹⁰ indicates that identical mechanisms are operative in both cases. Thus, peak I results from the 1-electron reduction of the 3,4 bond to form a free radical, which dimerizes very rapidly, as indicated by the very small peak II currents obtained on cyclic voltammetry, which are often lower than those for peak I, or is reduced at more negative potential in a further 1-electron process (peak II) to 3,4-dihydropyrimidine. At higher pH, the 2-electron peak III, due to combined peak I and II processes, occurs. Peak IV represents the 2-electron reduction of dihydropyrimidine to tetrahydropyrimidine. The difference in the pH-dependence of waves III and IV results in their merging to form the 4-electron peak V.

The i_p/AC ratios for pyrimidine peaks I, III and V and purine peak I are in the ratios 1.0:2.0:3.6:2.5 at 0.026 V/sec and 1.0:1.7:3.6:2.5 at 0.26 V/sec, which are in reasonable agreement for the ratio of faradaic *n* values—1:2:4:2.

The single cathodic peak observed for pyrimidine at the PGE over the whole pH range corresponds most closely to the succession of peaks I, II and possibly IV, observed at the HMDE; the lower cathodic potential range at PGE results in peaks III and V (at HMDE) not appearing. The fairly linear current-concentration relation at pH 1.05 suggests that adsorption is not involved in the pyrimidine electrochemical processes on graphite, at least in acidic solution.

Cytosine. The single 3-electron cytosine peak is due to a succession of three processes: (1) 2-electron electrochemical reduction of the 3,4 N=C bond (this is a combination of the first two 1-electron processes seen in pyrimidine), (2) rapid deamination of the reduction product to 2-hydroxypyrimidine, and (3) 1-electron electrochemical reduction of the latter, similar to pyrimidine, to a free radical, which dimerizes under the experimental conditions before it can be further reduced.¹⁰

The single reduction peak observed at the HMDE gives no evidence for reversibility. The close agreement of the variation of E_p with pH with that of $E_{1/2}$ at the DME^{8,10} and the constancy of i_p over the pH range, which is in accord with the constant diffusion current constant at the DME, suggest identical mechanisms.

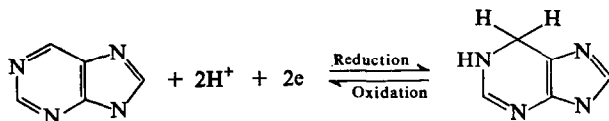
The i_p/AC ratio for cytosine, purine peak I and adenine are in ratio 1.7:1.0:3.5 at 0.026 V/sec; and 1.2:1.0:3.2 at 0.26 V/sec. These ratios are in fair agreement with earlier coulometrically measured faradaic n values of 3, 2 and 6 respectively, for cytosine, purine wave I and adenine.^{9,10}

Purine. Purine exhibits two pH-dependent polarographic waves in acidic solution (ca. pH 2–5.5) at the DME⁹. Wave I ($E_{1/2} = -0.697 - 0.083 \text{ pH}$) is due to 2-electron reduction to 1,6-dihydropurine, wave II ($E_{1/2} = -0.902 - 0.080 \text{ pH}$) is due to a further 2-electron reduction, probably to 1,2,3,6-tetrahydropurine, which hydrolyses to a 4-aminoimidazole.

The two cathodic peaks seen at the HMDE are due to identical processes as indicated by the close agreement between E_p and $E_{1/2}$. The cyclic voltammetric behaviour gives little evidence of reversibility, although oxidation of the unstable reduction product of peak II could be detected at very fast scan rates as a small anodic peak. The constancy of the peak currents to above pH 9, at which peak II disappears, contradicts earlier DME studies.⁹ Re-examination of purine at the DME, as previously described, showed no diminution of the limiting current for waves I and II up to pH 9; wave I persisted to even higher pH values.

The i_p for the single cathodic peak observed on cyclic voltammetry at the PGE is relatively constant to pH 9; i_p for the anodic peak, which is about one-third of that for the cathodic peak, is remarkably constant. Above pH 9, where the cathodic peak disappears beyond the background discharge, the anodic i_p drops slightly owing to the cathodic background reactions in the potential range involved.

The process giving rise to the single PGE cathodic peak most likely corresponds to the wave I process observed at the HMDE, as evidenced by the similar pH-dependence at both electrodes, although the peak always occurred at somewhat more negative potential at the PGE than at the HMDE. Such behaviour is indicative of adsorption or, at least, of more pronounced adsorption on graphite than on mercury, which would tend to inhibit the reduction process owing to the lower activity of the electroactive purine species in the adsorbed state.^{14,22} Smith and Elving⁹ reported that the product of the first 2-electron process at mercury is fairly stable in aqueous solution in the absence of oxygen; hence the oxidation peak observed on the reverse sweep at the PGE is due to the reoxidation of the product of the cathodic process and the overall cathodic-anodic process is



The exponential current-concentration relations at the PGE are further evidence for adsorption. The absence of a second cathodic peak at the PGE can be attributed to the smaller potential range available at the PGE.

Adenine. Reduction of adenine at mercury electrodes involves an overall 6-electron reduction: 2-electron hydrogenation of the 1,6 double bond, followed by 2-electron reduction of the 2,3 double bond, deamination at the 6-position, further 2-electron reduction of the consequently regenerated 1,6 double bond and hydrolytic cleavage at the 2,3 position to give the same product as the overall 4-electron purine reduction.¹⁰

Adenine gives a single anodic wave at the PGE, from 2*M* sulphuric acid medium to pH 9; the half-peak potential varies from 1.34 to 0.74 V.¹²

The single cathodic peak observed for adenine at the HMDE has no anodic counterpart. The pH-dependence and the large peak current suggest that the peak corresponds to the DME wave.⁹

The ratio of i_p/AC for adenine to that for purine peak I is 3.5 at 0.026 V/sec and 3.2 at 0.26 V/sec, which is in good accord with six electrons being involved in the adenine reduction and two in the purine reduction as discussed under *cytosine*.

The single oxidation peak observed for adenine on cyclic voltammetry at the PGE corresponds to that observed at slow single sweep voltammetry.¹² The absence of a reduction peak is again related to the smaller negative potential range available at graphite. The exponential decrease in the i_p/AC ratio with increasing concentration, especially at 0.6 V/sec, is indicative of adsorption. The i_p/AC ratio often decreased appreciably at the PGE, even at 0.06 V/sec, in agreement with the previously noted¹² slight decrease in the i_p/C ratio in pH 4.7 acetate medium from 79 to 72 $\mu\text{A.l.m mole}^{-1}$ over a 0.2–1.0*M* concentration range on single-sweep voltammetry. However, as with the purine results, the current-limiting effect could be due to a slow chemical step.

CORRELATION OF ELECTROCHEMICAL BEHAVIOUR AND QUANTUM MECHANICAL PARAMETERS

The facts that adenine and cytosine are adsorbed on electrodes and produce faradaic a.c. waves, *i.e.*, possess some degree of reversibility, indicate two possible causes for their apparently anomalous relative $E_{1/2}$ potentials, as compared to the order predicted from quantum mechanical calculations.

For the energy of the lowest empty molecular orbital (LEMO) to be simply related to $E_{1/2}$ for a reduction, the process must be reversible, *i.e.*, $E_{1/2} = E_0'$, where E_0' is the formal electrode potential (due allowance being made for the ratio of the square roots of the diffusion coefficients of the oxidized and reduced species), since then $E_{1/2}$ is simply related to the standard free energy change of the process, ΔG° . Any deviation of $E_{1/2}$ from E_0' can be expressed as $E_{1/2} = E_0' + \eta$ where η is the overpotential, *i.e.*, the extra energy required to cause the reaction to proceed at a reasonable rate, and, accordingly, is a measure of departure from reversibility. Comparison of the a.c. polarographic current for two closely related compounds, for which the electrochemical processes involve the same number of electrons in the rate-determining step, *i.e.*, n_a , and in any subsequent steps contributing to the overall a.c. current, should give a measure of the departure from reversibility for the two compounds, *i.e.*, the extent to which $E_{1/2}$ would be expected to differ from E_0' or the value obtained quantum mechanically from LEMO calculations.

If the a.c. reductions of adenine and cytosine are assumed to involve an identical number of electrons (either one or possibly two), adenine is reduced far more reversibly than cytosine, *i.e.*, the electrode reaction for adenine reduction is far more rapid; hence, the departure of $E_{1/2}$ from the order predicted by LEMO values would be less for adenine than for cytosine. Evaluation of the present semiquantitative experimental results in terms of the expected $E_{1/2}$ shift with pH is insufficiently precise to predict with assurance the difference in measured and reversible half-wave potentials.

Although adenine appears to be more strongly adsorbed than cytosine at potentials preceding the regions of faradaic electrochemical activity, the slight base current elevation immediately before these regions suggests that desorption occurs and that adsorption effects are not of major consequence in the energetics of the overall faradaic processes at mercury.

The likely result, therefore, is an overall shift of $E_{1/2}$ for adenine to a less negative potential than that of cytosine.

Account should also be taken of the contribution of solvation energy to $E_{1/2}$ in correlation of the latter with theoretically calculated LEMO energies.²³

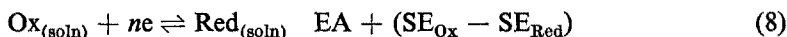
For a reduction process in solution,



it can be shown that, for a "reversible" polarographic process,

$$E_{1/2} = \frac{-\Delta H^\circ}{nF} + \frac{T\Delta S^\circ}{nF} - \frac{RT}{nF} \ln \frac{f_{\text{Red}}}{f_{\text{Ox}}} - \frac{RT}{nF} \ln \frac{D_{\text{Ox}}^{1/2}}{D_{\text{Red}}^{1/2}} \quad (6)$$

where the symbols have their usual thermodynamic and electrochemical significance. By considering the reactions



where EA is the electron affinity of the Ox species and SE is the solvation energy of the oxidized and reduced species as designated, and using the approach of Pysh and Yang,²³ ΔH° in equation (6) can be replaced to give

$$E_{1/2} = \text{EA} + (\text{SE}_{\text{Ox}} - \text{SE}_{\text{Red}}) + \frac{T\Delta S^\circ}{nF} - \frac{RT}{nF} \ln \frac{f_{\text{Red}} D_{\text{Ox}}^{1/2}}{f_{\text{Ox}} D_{\text{Red}}^{1/2}} \quad (9)$$

This expression is similar to equation (1) of Pysh and Yang,²³ although the latter refers to an oxidation process and contains minor error.

The theory of linear combination of atomic orbitals (LCAO) gives the energies of the molecular orbitals of the π -electrons in the form

$$E_i = \alpha + \beta k_i \quad (10)$$

where α is a coulomb integral, β a resonance integral and k_i the matrix eigen value. Rewriting equation (9) and neglecting the small entropy and logarithmic terms,

$$E_{1/2} = \beta k_i + (\text{SE}_{\text{Ox}} - \text{SE}_{\text{Red}}) + \text{constant} \quad (11)$$

Then, plots of $E_{1/2}$ vs. the LEMO energy (usually, k_i expressed in units of β) should be linear, provided that for the series of compounds studied the solvation energy terms

between members of the series are constant or vary in a regular fashion. It is unlikely that the latter would be true for the different purines and pyrimidines in aqueous solution because of the variation in nature, number and strength of the hydrogen-bonding donor and acceptor groups in the different compounds and in the reduction products derived from them.

However, it is not unlikely that the rate-controlling step for the reduction of each compound might involve only a single electron and that the difference in solvation energies of the reactant and initial product in the rate-controlling step for the first electron transfer for a given compound would be small, if not negligible.

Many of the purines and possibly pyrimidines appear to be reduced only in the protonated state, *e.g.*, adenine, whereas others, *e.g.*, purine and pyrimidine themselves, are reduced in an unprotonated form; this, of course, does not affect the initial or overall electron consumption, although significant solvation energy differences might be involved.

ANALYTICAL IMPLICATIONS AND APPLICABILITY

A particularly difficult problem in many *in vivo* studies of purines and pyrimidines is the availability of suitably selective and sensitive methods for their determination. No completely satisfactory method for the determination of even the oxypurines and pyrimidines has ever been developed;²⁴ for example, it is difficult to determine xanthine in the presence of hypoxanthine. Accordingly, the present study has been used to define the analytical applicability of cyclic voltammetry and alternating current polarography for the determination of individual purines and pyrimidines and the analysis of mixtures.

Cyclic voltammetry at HMDE. The concentration studies on the four purines and pyrimidines examined in detail indicates an accuracy of 2–3% relative. The time necessary for a determination is considerably less than for DME polarography.

The data on cytosine, in particular, showed that at 0.26 V/sec scan rate the peaks are much better formed and easier to measure than at lower scan rates; this suggests that, for compounds which give slow scan voltammetric peaks very close to background discharge, necessitating point-by-point background current subtraction, fast scan cyclic voltammetry could provide, on occasion, a rapid and convenient analytical method.

Cyclic voltammetry at PGE. Generally, current reproducibility at the PGE was poor, due, at least in part, to adsorption phenomena, since purine and adenine, which gave non-linear i_p - C curves, also showed very poor reproducibility; pyrimidine, which gave linear relations, showed far better reproducibility. It is likely that with each resurfacing of the electrode different numbers of active adsorption sites are exposed, which in turn would give rise to differing current values. Adsorption apart, the agreement between replicate determinations, even for pyrimidine where apparently adsorption is not pronounced, is somewhat inferior to that for the HMDE, especially at fast scan rates; again, the conclusion is that poor surface area reproducibility is probably responsible.

Of particular interest was the behaviour of purine for which even at pH values where the cathodic peak disappeared against the background discharge, an anodic peak could still be observed on the return scan; this indicates that it is possible, utilizing cyclic voltammetry, to determine purine and probably many other compounds

under conditions where they appear by single-sweep methods to be non-electroactive. Significantly, the anodic purine-product wave did not decrease appreciably in height even when the purine cathodic peak must have occurred several hundred mV beyond the onset of background discharge, *e.g.*, for 1mM purine, the current was constant in the pH range 9–12.

A.c. polarography. The analytical implications of the a.c. polarographic behaviour of purines and pyrimidines are obvious, *e.g.*, it would be a relatively simple matter to determine these compounds in the presence of other compounds which possess no degree of a.c. polarographic reversibility. The sensitivity is quite high; the lower limit is 0.1mM or lower, depending on the sensitivity of the current-measuring device employed.

CONCLUSIONS

Cyclic voltammetry at the HMDE gives results comparable to those obtained at DME, indicating that earlier proposed mechanistic routes for purine, adenine, pyrimidine and cytosine are generally correct. In contradiction to earlier studies, purine is polarographically reducible at pH values up to well above 9.

Electrocapillary curve measurement and a.c. polarography indicate that purine, adenine, adenosine, adenosine phosphates and cytosine are adsorbed at the DME. Since all of these compounds appear to be desorbed before faradaic reduction, it is unnecessary to consider adsorption in the overall energetics of the polarographic reduction. With the exception of cytosine, none of the pyrimidines examined is adsorbed at the DME.

A.c. polarography of the purines and pyrimidines studied indicates them to be reduced in only slightly reversible processes.

The very marked difference in height of the a.c. polarographic waves for adenine and cytosine indicates that, if solvation energy effects are neglected, the disagreement between quantum mechanical prediction and polarographic observation regarding the ease of reduction of these compounds is due to marked differences in electrochemical reversibility, *i.e.*, electrode kinetics favour the more ready reduction of adenine.

Purine and adenine or their electrochemical reduction products are probably strongly adsorbed at the graphite electrode, which explains the prolonged electrolysis time required for macroscale electro-oxidation of adenine.³

Cyclic voltammetry and a.c. polarography provide potentially useful approaches for the determination of purines and pyrimidines.

Reagents

Buffer solutions were prepared from analytical reagent grade chemicals. Nitrogen used for deoxygenating was purified and equilibrated by bubbling it successively through acidic vanadium(II) chloride solution kept over heavily amalgamated zinc, saturated calcium hydroxide solution and distilled water.

Apparatus

Triangular potential sweep voltammetry (cyclic voltammetry) was carried out with an apparatus essentially as described by Schwarz and Shain.²⁵ Cyclic voltammograms were recorded on a Moseley Model 135 X-Y recorder.

A.c. polarography was carried out by modifying the cyclic voltammetry unit as described by Smith.²⁶ The applied d.c. potential was obtained from a potentiometer employing a 2.7 V mercury battery. The a.c. signal was generated with a Hewlett-Packard Model 202A function generator. All a.c. polarograms were recorded manually; the alternating current (rms) was measured with Hewlett-Packard Model 400D vacuum tube voltmeter. In order to eliminate high-frequency noise generated in the operational amplifier circuits, the cell signal was filtered through a suitable low-pass filter²⁷ before measurement.

EXPERIMENTAL

A conventional three-electrode cell was employed. The pyrolytic graphite electrode (PGE) was prepared as described previously.²⁸ A normal hanging mercury drop electrode (HMDE) was prepared as described previously.²⁹

Because preliminary electrocapillary studies had indicated rather erratic variation of the DME drop-time at very negative potentials and often also in the presence of the electroactive compounds at less negative potentials, the capillary was silanated. The most successful procedure was to draw dichlorodimethylsilane vapour through a length of clean capillary for a few minutes, followed by air for a further 30 min; the top of the capillary was then broken off. Unfortunately, this silanation deteriorated and, after some days, a new capillary had to be prepared. Accordingly, in some of the later electrocapillary work, the capillary was silanated by immersing it for a few minutes in Beckmann Desicote with the mercury drops falling, allowing the excess Desicote to drain off and washing the top with acetone and water; the electrode then appeared to be suitable for use, *i.e.*, stable drop-times were obtained at least up to about -1.2 V.

Capillary characteristics of the DME used for a.c. polarography are presented where appropriate in the text.

General voltammetric procedures

Test solutions were prepared by diluting appropriate quantities of stock solutions (prepared by dissolving weighed quantities in distilled water and diluting to a known volume) with suitable buffer solution. Solutions were always deoxygenated for at least 10 min before examination. Buffer and background solutions were of the same types as used in reference 9. All test solutions had an ionic strength of 0.5M.

The PGE and HMDE were always left for about 30 sec in the test solution to allow equilibration before the voltammetric scan was begun.

For a.c. polarography, an applied a.c. frequency of 50 Hz and 4 mV (rms) amplitude was employed unless otherwise stated; readings were taken at every 10 mV. Although an Hg/Hg₂SO₄ reference electrode (MSE) was used at positive potentials *vs.* SCE, all potentials cited are referred to the SCE at 25°, unless otherwise specified.

Reproducibility and compatibility of data

Current measurements on cyclic voltammetry at the HMDE were reproducible to 2–3% relative. The poor precision on cyclic voltammetry at the PGE is to be associated with the specific compounds involved; satisfactory precision has been reported by the authors and others for different compounds.

Reproducibility of potential at the PGE was more satisfactory than that of current, being about ± 25 mV; it was ± 10 mV at the HMDE.

It is difficult to compare decomposition potentials at graphite and mercury electrodes because of the differing magnitude of the respective charging currents and the rapidity with which the current rises at the PGE as compared to the HMDE. An estimate of the decomposition potential, *i.e.*, the potential at which the current is 5 μ A above the extrapolated residual current, in pH 4.6 acetate solution at a scan rate of 0.10 V/sec would be -1.23 V at PGE (area 12 mm²) and -1.36 V at HMDE (area 5 mm²).

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Zusammenfassung—Um die große Diskrepanz zwischen der leichten polarographischen Reduzierbarkeit von Adenin und Cytosin im Experiment und der durch molecular orbital-Berechnung vorhergesagten näher zu untersuchen, wurde das elektrochemische Oxidations- und Reduktionsverhalten von Pyrimidin, Cytosin, Purin, Adenin und verwandten Verbindungen sowohl an Quecksilber- als auch an Graphitelektroden studiert. Es ergaben sich Informationen über die spezifische Adsorption von Reaktanten und Produkten an der Elektrode, die Reversibilität des energiekontrollierenden Elektronenübergangsschrittes und begleitende chemische Reaktionen. Dreiecksvoltammetrie, Wechselstrom- und Gleichstrompolarographie und Elektrokapillardaten wurden im einzelnen verwendet. Die ersten drei Verfahren wurden auf ihren eventuellen analytischen Nutzen hin kritisch geprüft. Die Ergebnisse wurden mit früher erhaltenen elektrochemischen Daten verglichen und die Abfolge der Schritte mit Elektronenübergang und der vielen ohne Elektronenübergang wurde besser festgelegt. Es wurde klar, daß zur gültigen Korrelation von quantenmechanisch berechneten Daten über die Energie, die man zur Zufügung oder

Wegnahme eines Elektrons zu oder von dem äußersten Elektronenzustand des Moleküls (in der Gasphase) braucht, mit elektrochemischen Redoxpotentialen (in Lösung) die Effekte der Adsorption, der Reversibilität des Elektronenübergangs und die Solvatationsenergie berücksichtigt werden müssen.

Résumé—Afin d'évaluer la différence remarquable entre la facilité expérimentale de réduction polarographique de l'adénine et de la cytosine et celle prédite par le calcul d'orbitale moléculaire, on a étudié le comportement à l'oxydation-réduction électrochimique des pyrimidine, cytosine, purine, adénine et composés apparentés sur électrodes de mercure et de graphite. On a obtenu des informations sur l'adsorption spécifique du réactant et de l'espèce sur l'électrode, sur la réversibilité du stade de transfert électronique régulateur d'énergie et sur les réactions chimiques concomitantes. On a utilisé en particulier la voltammétrie de balayage triangulaire, la polarographie à courants alternatif et continu et des données électrocapillaires. Les trois premières techniques ont été examinées de manière critique quant à leur utilité analytique potentielle. On a comparé les résultats à des données électrochimiques antérieurement obtenues et l'on a établi plus solidement la séquence des stades de transfert électronique et de divers transferts non électroniques. Il est devenu clair que, dans le but de relier de façon valable les données calculées par la mécanique quantique pour l'énergie requise pour ajouter un électron au niveau électronique externe de chaque molécule (en phase gazeuse) ou l'en soustraire, et les potentiels redox électrochimiques (en solution), il faut prendre en considération les influences de l'adsorption, de la réversibilité du transfert électronique et de l'énergie de solvatation.

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