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TEMPERATURE DEPENDENCE OF THE INTERFACIAL BEHAVIOR OF URACIL DERIVATIVES

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The effect of temperature on the interfacial behavior of uracil, thymine and 1,5-dimethyluracil has been used to characterize the thermodynamics of adsorption of these compounds at a mercury electrode-aqueous electrolyte interface. Between 4.5°C and 40°C all three compounds exhibit two adsorption regions. The first, a dilute layer where the molecules adsorb in a flat orientation on the electrode surface. The second, a compact layer where the molecules adsorb in a perpendicular orientation. The thermodynamic constants characterizing formation of these surface layers have been deduced.

1. Introduction

We recently reported on the adsorption of uracil, thymine and 1,5-dimethyluracil at a mercury electrode, from aqueous solutions at pH 8 [1]. Differential capacitance and electrocapillary measurements were used to obtain values of surface pressure (π) as a function of potential (E), and concentration (c). By fitting π-c-E data to the Frumkin equation and to several empirical adsorption isotherms, it has been possible to infer values of the surface excess quantity, (Γ, in mole cm^{-2}) at various concentrations and potentials and the thermodynamic constants characterizing the equilibrium adsorption of uracil, thymine and 1,5-dimethyluracil on mercury at 25°.

Our surface electrochemical results have made it possible to obtain a fairly detailed picture of the behavior of these compounds at mercury electrodes, both in the dilute concentration region and at relatively high concentrations. At low bulk solution concentrations the pyrimidines appear to adsorb in a flat orientation (i.e., with the plane of the pyrimidine ring parallel to the plane of the mercury surface). At higher concentrations, these compounds undergo surface reorientation to a more compact film, in which the planes of the rings are apparently aligned nearly perpendicular to the surface. We have provided substantial evidence which suggests that the points of attachment of the adsorbed molecules to the surface in the denser film are the N(3)-H (and perhaps the N(1)-H) groups responsible for base-pairing in nucleic acids; we believe that in this film considerable stability is contributed by stacking interactions between the parallel pyrimidine rings.

Although isothermal adsorption data are available for uracil, thymine, 1,5-dimethyluracil and other pyrimidines, virtually nothing has been reported about the temperature dependence of adsorption for these systems. Such data are potentially valuable in that they can provide information about the enthalpy and the entropy of adsorption. By combining results of a temperature study with data already available at 25° it should be possible to obtain a much clearer understanding of the forces and molecular interactions responsible for the adsorption of these important bases.

This report is concerned with the interfacial behavior of uracil, thymine and 1,5-dimethyluracil at a mercury electrode at pH 8 at temperatures from 4.5°C to 40°C.

2. Experimental

2.1. Chemicals

Uracil and 1,5-dimethyluracil were obtained from Sigma; thymine was obtained from Nutritional Biochemicals.

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All measurements were performed in 0.5 *M* sodium fluoride with 0.01 M Na_2HPO_4 buffer, pH 8.0. The appropriate compound was weighed directly into this supporting electrolyte and, after dissolution, deaerated for at least 10 minutes with water-saturated nitrogen before study.

2.2. Differential capacitance measurements

Differential capacitance measurements were obtained by the phase selective a.c. polarographic method described previously [1,2]. Measurements were obtained at 4.5°C, 25.0°C and 40.0°C.

The measured value of the differential capacitance per unit area at fixed frequency in the dilute adsorption region (vide infra) at different times in the drop life was found to be constant at times between < 1 to > 10 s over the entire range of potentials studied for all compounds at all concentrations studied; i.e., d.c. equilibrium was rapidly established.

Between 10–250 Hz, with a 10 mV peak-to-peak amplitude for the modulating voltage, the measured capacitance was independent of frequency over the whole potential range of interest. Because of the absence of any significant frequency dispersion, all capacity data were measured at 100 Hz and 10 mV peak-to-peak at a controlled droptime of 2.00 s.

The d.c. potential was scanned at a sweep rate of 0.005 V s^{-1}. Alternating current versus potential curves and alternating current versus time curves were recorded on a Texas Instrument Model 341 *X-Y* recorder or a Tektronix Model 5111 storage oscilloscope.

Capacitance measurements were taken with the test solution, working electrode (i.e., dropping mercury electrode (DME)) and counter electrode (Hg pool at bottom of the cell) maintained at the desired temperature. The saturated calomel reference electrode (SCE) was maintained at room temperature (25 ± 2°C). Electrolytic contact with the test solution was accomplished with a Luggin capillary positioned very close to the tip of the DME. All potentials are referred to the SCE at 25°C.

Temperatures were measured with a Bailey Instruments Model BAT-8 digital thermometer; the thermistor probe of the thermometer was inserted directly into the test solution in close proximity to the DME.

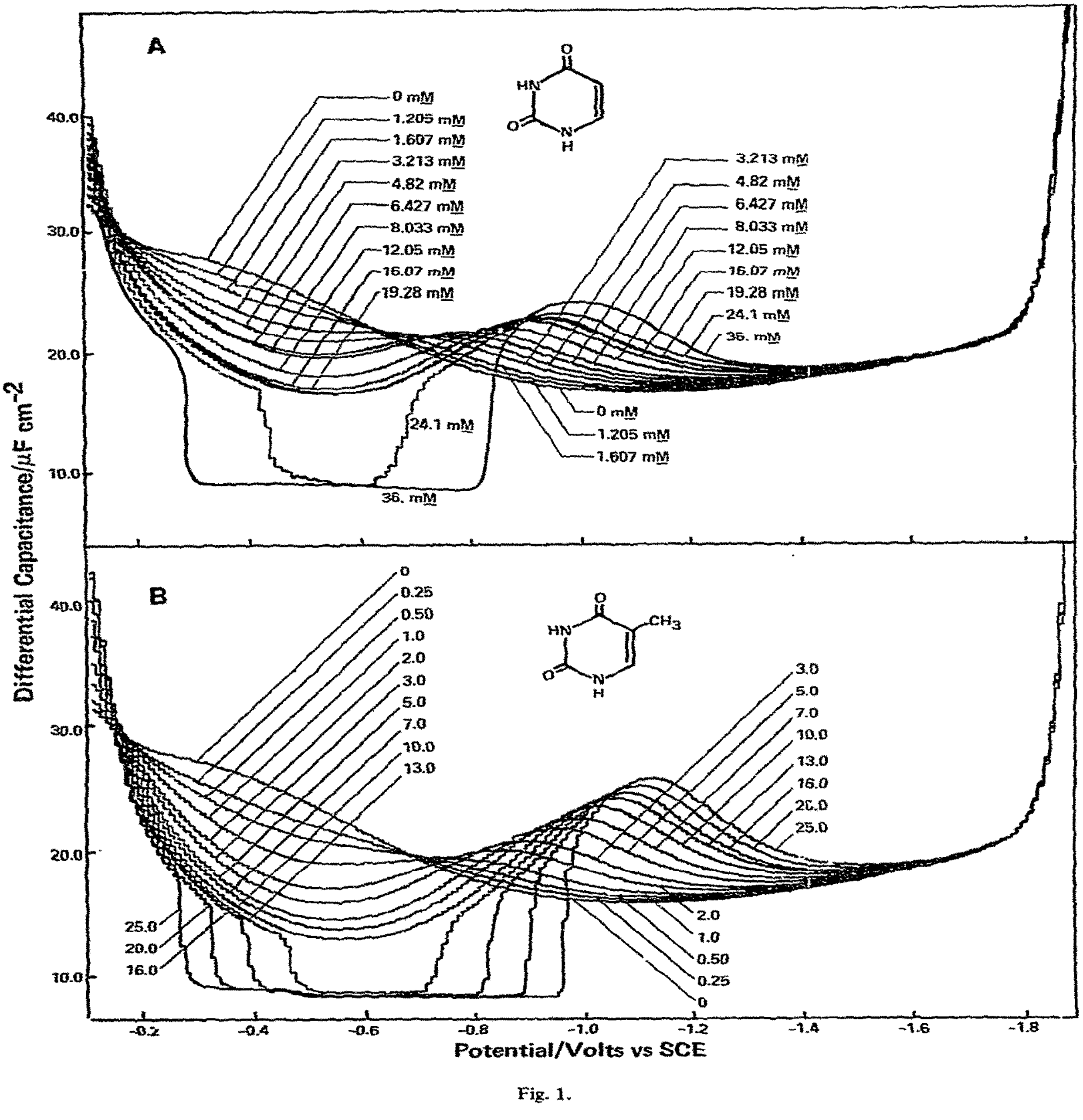
2.3. Direct interfacial tension measurements

In a previous report [1] the interfacial behavior of uracil, thymine and 1,5-dimethyluracil was studied by both indirect capacitance measurements and by direct measurements of interfacial tension using a capillary electrometer. Both techniques gave essentially identical results, hence in this study only differential capacitance measurements were utilized because of the experimental ease of taking the latter data. However, in order to analyze capacitance data it is necessary to know the electrocapillary maximum (ECM) potential, the value of the interfacial tension at the ECM potential and the decrease of the interfacial tension at other potentials for the electrode-solution interface for the pure supporting electrolyte solution. This information at 4.5, 25.0 and 40.0°C was obtained by use of a capillary electrometer using the J-shaped capillary described in an earlier report [1]. The ECM potential for the 0.5 *M* NaF + 0.01 *M* Na_2HPO_4 background pH 8.0 was −0.433 ± 0.005 V between 4.5 and 40.0°C. The procedures for calibration and use of the capillary electrometer have been described in detail elsewhere [3].

3. Results

3.1. Differential capacitance measurements

A typical set of capacitance versus potential (*C* versus *E*) curves for uracil, thymine and 1,5-dimethyluracil at pH 8.0 and 25°C between −0.1 V and −1.9 V are shown in fig. 1. This figure serves to illustrate the two adsorption regions for these molecules. At low concentrations the capacitance exhibits a general depression between about −0.2 V and −0.8 V with a broad low adsorption/desorption peak at more negative potentials. In the case of uracil, for example, this region of systematic depression of the capacitance between about −0.2 V and −0.8 V occurs up to concentrations of about 20 m*M*. At 21 m*M* concentration levels a small discontinuity may be noticed at about −0.55 V which with increasing concentration develops into the well defined pit shown in fig. 1A. Thymine and 1,5-dimethyluracil exhibit similar behavior except that the appearance of the capacitance pit is noted at lower bulk concentrations. The first or *dilute* adsorp-


A
O
HN
O
N
H
0 mM
1.205 mM
1.607 mM
3.213 mM
4.82 mM
6.427 mM
8.033 mM
12.05 mM
16.07 mM
19.28 mM
3.213 mM
4.82 mM
6.427 mM
8.033 mM
12.05 mM
16.07 mM
19.28 mM
24.1 mM
36. mM
0 mM
1.205 mM
1.607 mM
24.1 mM
36. mM
B
O
CH3
HN
O
N
H
0
0.25
0.50
1.0
2.0
3.0
5.0
7.0
10.0
13.0
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0.25
0
40.0
30.0
20.0
10.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
Differential Capacitance/μF cm^-2
Potential/Volts vs SCE

Fig. 1.

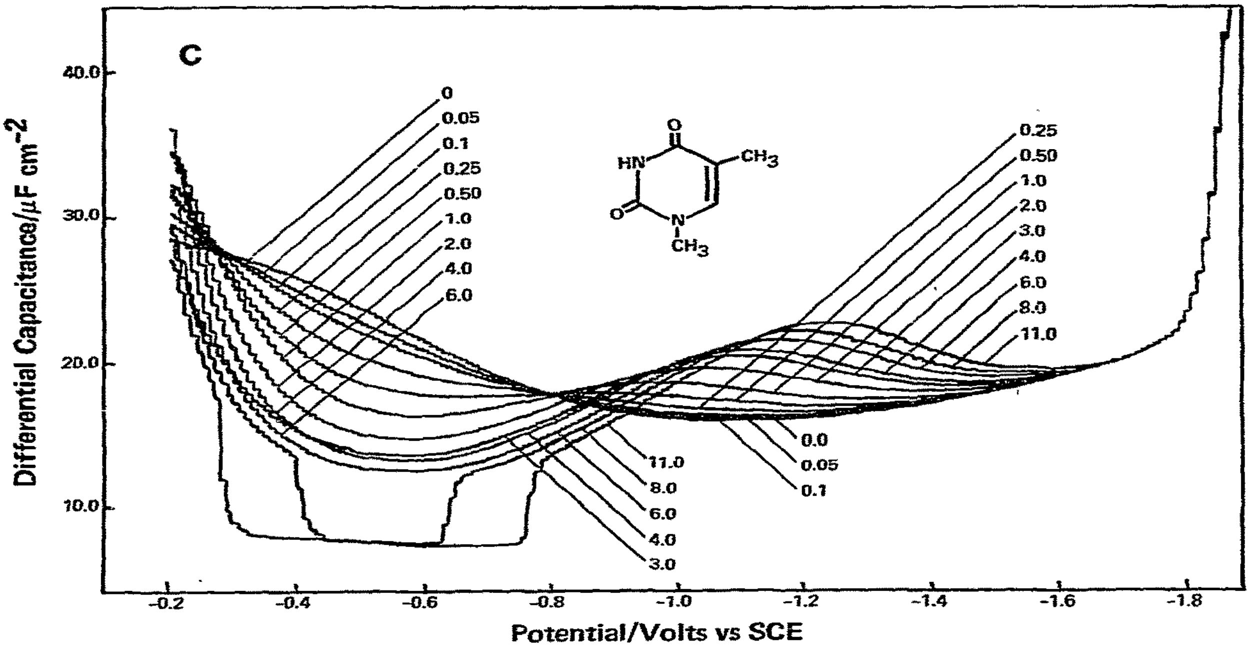

Fig. 1. Typical differential capacitance versus potential curves for (A) uracil, (B) thymine and (C) 1,5-dimethyluracil in 0.5 M NaF plus 0.01 M Na_2HPO_4 pH 8.0 at 25°C. Curves were obtained at a frequency of 100 Hz and an amplitude of 10 mV peak-to-peak. Concentrations are shown in the figure.

tion region corresponds to the range of concentrations where the broad general depression of capacitance occurs (i.e., up to ca. 20 mM in the case of uracil (fig. 1A), to ca. 10 mM for thymine (fig. 1B) and to ca. 6 mM in the case of 1,5-dimethyluracil (fig. 1C)). This region is where the uracil derivatives are adsorbed in a flat orientation on the electrode surface, i.e, with the plane of atoms of the pyrimidine ring parallel to the electrode surface. At potentials and bulk solution concentrations where the capacitance pit forms the adsorbed molecules reorient and adopt a perpendicular stance on the electrode. Our previous work strongly suggests that in the perpendicular mode these uracil derivatives are bound to the electrode primarily through their Watson–Crick hydrogen bonding sites (i.e., N(3)-H) or perhaps to some extent N(1)-H) [1].

Capacitance versus potential curves of the type shown in fig. 1 were also obtained at 4.5°C and 40.0°C. However, much lower bulk solution concentrations were required to reach monolayer surface saturation in the dilute region and to observe the formation of the capacitance pit corresponding to formation of the compact perpendicular oriented uracil derivatives at 4.5°C. Correspondingly, much higher bulk solution concentrations were required at 40°C.

Interpretation of differential capacitance data in the dilute adsorption region at each temperature was based on the approaches described in detail in previous reports [1,2,3]. Briefly, however, it was found that at all temperatures each compound gave C versus E curves which became coincident with the curve for pure background electrolyte solution at potentials of about −1.6 V and more negative (see for example fig. 1). Accordingly, the back-integration method of Grahame et al. [4] was used to calculate the charge of the mercury-electrolyte solution interface. Thus,

$$q - q^* = \int_{E^*}^{E} C\,\mathrm{d}E\,, \qquad (1)$$

where q is the charge relative to q^* the charge at the potential, E^*, where the integration is started (usually E^* was −1.8 V). At the ECM for the pure background electrolyte $q = 0$. Hence, the absolute charge for the latter solution at E^* may readily be calculated. At E^* all C versus E curves are coincident, hence q^* for all solutions at E^* must be the same [4]. Accordingly, values of $q - q^*$ in eq. (1) are readily converted to absolute charge values as a function of both E and the concentration of the adsorbate species. A further integration of charge was then carried out to obtain interfacial tension, i.e.,

$$\gamma = \gamma_0 - \int_0^E q \, \mathrm{d}E \,. \tag{2}$$

The value of γ_0, the interfacial tension for the pure background electrolyte solution at the ECM potential was obtained directly from capillary electrometer measurements at the appropriate temperature. Eq. (2) was used to obtain interfacial tension as a function of both E and concentration.

Activities of solute species are taken to be equal to concentrations in the very dilute solutions used in the present experiment (0 to 30 mM). Examination of thermodynamic and nmr spectral data for the pyrimidines indicates that deviations from the ideal dilute solution laws are quite small at concentrations less than 30 mM [6]. Moreover, we have applied a new method for inferring solute activities from spreading pressure-surface charge data [7] to several pyrimidine systems. The derived activity values vary linearly with measured concentrations within experimental error.

Analysis of γ data involved first calculation of the surface spreading pressure, π (dyne cm^{-1}), as a function of the adsorbate bulk solution activity, a, and electrode potential, E, using the equation

$$\pi = \gamma_w(E) - \gamma(E) \,, \tag{3}$$

where γ_w is the value of γ for the pure background electrolyte solution at $a = 0$.

Plots of surface spreading pressure, π, versus the logarithm of the activity of each uracil derivative over a wide range of potentials were readily superimposable by abscissa translation. Typical π versus $\ln a$ plots at 25°C for each of the compounds studied here have been presented elsewhere [1]. Similar results were obtained at 4.5°C and 40.0°C. Such π versus $\ln a$ plots were obtained by fitting π, a and E data to the empirical equation [3]

$$\pi = A[\ln(1 + Ba)]\left[1 + \frac{\alpha a}{(1 + Ba)^2} + \frac{\beta a^2}{(1 + Ba)^3} + \ldots\right] . \tag{4}$$

In this equation $A = \Gamma_m RT$ where Γ_m is the surface excess of the uracil derivative at $\theta = 1$; θ is the fractional surface coverage of the electrode. The parameter B is dependent on potential so that if data are to be fitted simultaneously at several different potentials, separate B values must be inferred at each potential. A non-linear least squares procedure is used to obtain optimum values of all parameters. A detailed description of the use of eq. (4) has been presented elsewhere [1,2].

Once a composite fit had been obtained for π and a data at several potentials for a particular uracil derivative at a given temperature the same functional form was used to fit data at fixed potentials typically between −0.4 V and −1.2 V. Because very good composite π versus $\ln a$ plots were obtained, it was assumed that at all potentials the value of $\Gamma_m RT$ (A in eq. (4)) was constant. This constant was used in fitting π and a data at individual potentials. Analytical differentiation of the π versus $\ln a$ fits at individual potentials was used to calculate ΓRT at various activities and concentrations using the Gibbs equation (eq. (5))

$$\Gamma RT = \mathrm{d}\pi / \mathrm{d} \ln a \,. \tag{5}$$

Tests of congruence of the electrosorption isotherms for each adsorbate molecule at 4.5, 25.0 and 40.0°C with respect to potential and charge were carried out as reported earlier [1]. These tests revealed that the electrosorption isotherms of uracil were congruent with respect to potential between −0.3 V and ⩾ −1.2 V; for thymine between −0.4 V and ⩾ −1.2 V and for 1,5-dimethyluracil between −0.5 V and ⩾ −1.2 V.

Since the adsorption isotherm for each uracil species is congruent with respect to potential, π, a and E data were fitted to the Frumkin isotherm. The generalized form of the Frumkin isotherm equation is shown in eq. (6).

$$\frac{\theta}{1 - \theta} = B_0 a \exp(2\alpha\theta) \exp(-\phi / \Gamma_m RT) \,. \tag{6}$$

In this equation Γ_m is the limiting surface excess of solute at full monolayer coverage in mole cm^{-2}, α is the lateral attraction coefficient and B_0 a constant related to the free energy of adsorption for the adsorbate at the ECM potential for the pure supporting electrolyte. The function

$$\phi = [\gamma_w(0) - \gamma_w(E)] + C'EE_N - \frac{C'E^2}{2} \quad (7)$$

is evaluated in terms of E, the potential relative to the ECM potential for pure supporting electrolyte solution, E_N the ECM potential for the mercury-solution interface at $\theta = 1$, C' the capacitance of the monolayer saturated surface (assumed to be independent of potential [5]) and γ_w the interfacial tension for the pure supporting electrolyte solution. The term $[\gamma_w(0) - \gamma_w(E)]$ was determined from electrocapillary measurements using the maximum bubble technique [3] for pure supporting electrolyte solution at the appropriate temperature.

Using a further non-linear least squares technique [1,2,3] C', E_N, α, B_0 and Γ_m in eqs. (6) and (7) were determined for each uracil derivative at three different temperatures. The adherence of the electrosorption of each of the uracil derivatives studied to the Frumkin model was tested by preparing reduced isotherms of ΓRT versus a/a' where a' is the bulk solution activity required to give an arbitrary value of ΓRT. The ΓRT values were obtained by analytical differentiation of π versus $\ln a$ curves at individual potentials. Such isotherms, which assume no adsorption model, were in excellent agreement with the fit of the π, a, E data to the Frumkin model. Examples of such reduced isotherms at 25°C have been presented previously [1]. Equally good agreement was obtained at 4.5°C and 40°C.

Analysis of π, E, a data in the dilute adsorption region using the Frumkin model gave the results shown in table 1. These results indicate a small but generally systematic decrease of the lateral attraction coefficient, α, with increasing temperature. The area occupied per molecule remains, within experimental error, constant with changes in temperature between 4.5°C and 40°C. In general, the area occupied by the uracil derivatives between 4.5°C and 40°C is between 60–70 Å^2.

3.2. Determination of enthalpies and entropies of adsorption

From the temperature dependence of adsorption isotherms in the dilute approach region, it is possible to infer values of the enthalpy and the entropy of adsorption. We consider first the thermodynamic constants for the reaction A (aqueous solution, ideal dilute solution at unit molarity) $\rightleftharpoons$ A (adsorbed film at surface concentration Γ), where A represents a molecule of adsorbate. It can be shown that for this reaction

$$\Delta H_a = -RT^2 \, \mathrm{d}\ln a/\mathrm{d}T, \quad (8)$$

Table 1
Parameters of the generalized Frumkin isotherm for uracil derivatives at various temperatures determined from capacitance measurements at pH 8.0 [a] in the dilute adsorption region.

Compound	Temp. (°C)	α [b]	$\Delta G°$ (cal)	Area per molecule (Å^2)	RMSD in π [c] (dyne cm^{-1})
Uracil	4.5	0.93 ± 0.04	−2987		0.097
	25.0	0.45 ± 0.08	−2996	67 ± 6	0.156
	40.0	0.31 ± 0.14	−2817		0.163
Thymine	4.5	0.97 ± 0.06	−3356		0.161
	25.0	0.21 ± 0.05	−3667	59 ± 4	0.088
	40.0	0.23 ± 0.05	−3488		0.108
1,5-dimethyluracil	4.5	0.85 ± 0.08	−3836		0.236
	25.0	0.20 ± 0.08	−4159	68 ± 9	0.105
	40.0	−0.15 ± 0.04	−4089		0.043

[a] 0.5 M NaF with 0.01 M Na_2HPO_4, pH 8.0.
[b] Lateral attraction coefficient of the Frumkin equation (eq. (6)).
[c] Root mean square deviation in π in fitting π, a and E data to eq. (6).

$$\Delta S_a = -RT\,\mathrm{d}\ln a/\mathrm{d}T - R\ln a\,, \qquad (9)$$

where ΔH_a and ΔS_a are the adsorption enthalpy and adsorption entropy at constant Γ, and where a is the solute activity in bulk solution in equilibrium with the film at surface concentration Γ [8]. In using adsorption data to calculate ΔH_a and ΔS_a, the following procedure was followed. First, plots of Γ versus a were constructed for data at each temperature, based on fits of $\pi\,(a, E)$ with the Frumkin equation, with eq. (4) and with an alternative empirical equation:

$$\pi = da + ea^2 + fa^3 + ga^4\,, \qquad (10)$$

where d, e, f and g are empirical constants determined by least squares analysis. The values of Γ at known values of activity were determined at potentials corresponding within ± 50 mV to the midpoint of the capacitance pit. In the case of uracil this potential is −0.5 V, while for thymine and 1,5-dimethyluracil a value of −0.6 V was employed. These potentials were selected to allow us to investigate the variation in thermodynamic parameters as the adsorbed uracil derivatives changed from the flat (dilute) to perpendicular (compact) surface layer. In general, values of Γ determined at known values of a agree within a few percent for the three methods, at least at values of Γ less than about 2×10^{-10} mole cm^{-2}. From the smoothed plots of Γ versus a, values of a corresponding to surface excess values of 0.3, 0.6, 0.9, 1.2, and 1.5×10^{-10} mole cm^{-2} were determined by interpolation. Table 2 summarizes values of a at these chosen values of Γ (at fixed potential) for the three systems at 4.5°, 25°, and 40°. Next, plots of $\ln a$ (at constant Γ) versus $1/T$ were constructed, and values of ΔH_a calculated from the slopes; entropies of adsorption were calculated from $\Delta S_a = \Delta H_a/T - R\ln a$ and free energies from $\Delta G_a = RT\ln a$. Table 3 lists values of ΔG_a, ΔS_a and ΔH_a at the selected values of surface excess.

An alternative heat of adsorption, based on use of the relation

$$\Delta H_\pi = -RT^2\,\mathrm{d}\ln a/\mathrm{d}T \qquad (11)$$

with values of a determined at fixed values of π has also been used to characterize adsorption results [9]. To obtain ΔH_π, it is only necessary to read values of a directly from smoothed plots of π versus a data at each temperature, and again make plots of $\ln a$ versus $1/T$, this time at constant values of π. The activity

Table 2
Activities corresponding to fixed values of Γ for uracil, thymine and 1,5-dimethyluracil

$10^{10}\,\Gamma$ (mole cm^{-2})	4.5°C $10^3\,a$ (mole ℓ^{-1})	25°C $10^3\,a$ (mole ℓ^{-1})	40°C $10^3\,a$ (mole ℓ^{-1})
Uracil, −0.5 V			
0.3	0.47	0.80	1.70
0.6	0.91	1.76	3.60
0.9	1.36	2.82	5.8
1.2	1.80	4.04	8.8
1.5	2.27	5.65	13.6
Thymine, −0.6 V			
0.3	0.22	0.28	0.55
0.6	0.43	0.63	1.22
0.9	0.62	1.03	2.07
1.2	0.80	1.55	3.20
1.5	0.99	2.22	4.97
1,5-Dimethyluracil, −0.6 V			
0.3	0.10	0.15	0.22
0.6	0.21	0.34	0.52
0.9	0.30	0.56	0.94
1.2	0.39	0.91	1.60
1.5	0.51	1.51	2.80

Table 3
Thermodynamic constants for the adsorption of uracil, thymine and 1,5-dimethyluracil at chosen values of Γ at 25°

$10^{10}\,\Gamma$ (mole cm^{-2})	ΔG_a (kcal)	ΔS_a (cal K^{-1})	ΔH_a (kcal)	ΔH_π (kcal)
Uracil, −0.5 V				
0.3	−4.22	−7.7	−6.5	−5.0
0.6	−3.76	−10.2	−6.8	−5.3
0.9	−3.48	−12.5	−7.2	−5.6
1.2	−3.27	−15.5	−7.9	−6.0
1.5	−3.07	−19.6	−8.9	−6.3
Thymine, −0.6 V				
0.3	−4.85	1.8	−4.3	−3.6
0.6	−4.37	−2.8	−5.2	−4.0
0.9	−4.07	−6.1	−5.9	−4.5
1.2	−3.83	−10.0	−6.8	−5.1
1.5	−3.62	−14.0	−7.8	−5.2
1,5-Dimethyluracil, −0.6 V				
0.3	−5.22	4.8	−3.8	−3.2
0.6	−4.73	1.1	−4.4	−3.2
0.9	−4.44	−4.2	−5.7	−3.6
1.2	−4.15	−9.2	−6.9	−4.1
1.5	−3.85	−14.6	−8.2	−5.0

Table 4
Activities corresponding to fixed values of π for uracil, thymine and 1,5-dimethyluracil

π (dyne cm^{-2})	4.5° 10^3 a (mole ℓ^{-1})	25° 10^3 a (mole ℓ^{-1})	40° 10^3 a (mole ℓ^{-1})
Uracil, −0.5 V			
0.74	0.55	0.80	1.50
1.52	1.08	1.76	3.15
2.40	1.65	2.82	5.10
3.34	2.27	4.04	7.4
4.49	3.06	5.65	10.7
Thymine, −0.6 V			
0.78	0.27	0.28	0.57
1.63	0.54	0.63	1.24
2.48	0.77	1.03	1.98
3.54	1.07	1.55	3.00
4.75	1.46	2.22	4.26
1,5-Dimethyluracil, −0.6 V			
0.74	0.11	0.15	0.21
1.66	0.26	0.34	0.49
2.58	0.40	0.56	0.82
3.90	0.60	0.91	1.38
5.58	0.80	1.51	2.27

values corresponding to given π values can be more reliably determined than the a values at constant Γ, because the Γ versus a curves are obtained by differentiating π versus a curves. There is very little variation among π values corresponding to a known a, whether these are obtained from the Frumkin equation, from either of the two empirical $\pi(a)$ equations, or graphically from plots of the raw data. Table 4 lists values of a at selected values of π, chosen to correspond to surface coverages in the same range as the $a(\Gamma)$ data in table 2. Values of ΔH_π, calculated from equation (11) are included in the final column of table 3 for comparison with the ΔH_a values.

Fig. 2 shows the dependence of ΔH_a and ΔH_π on Γ for all three systems. In each case, both adsorption heats become more negative as Γ increases, but this effect is greater for ΔH_a than for ΔH_π. The limiting values of ΔH_a and ΔH_π must approach a constant difference of RT in the limit as $\Gamma \to 0$. In the limiting low concentration region, ΔH_a will equal ΔH°, the standard enthalpy of association for the infinitely dilute surface film. By fitting the 25° data to the Frumkin equations, we previously obtained values of the standard free energy of adsorption, $\Delta G^\circ = -RT \ln B_0$, corresponding to the transfer of adsorbate from the ideal dilute solution at unit molarity to the ideal dilute film at $\theta/(1-\theta) = 1$. Table 5 summarizes values of ΔG°, ΔH°, and ΔS° [equal to $(\Delta H^\circ - \Delta G^\circ)/T$] for uracil, thymine and 1,5-dimethyluracil at 25°.

The limiting values of ΔH° are somewhat surprising, in that they show that the binding energies at $\theta = 0$ become *less* negative in the series uracil-thymine-1,5-dimethyluracil, whereas ΔG° values become more negative in this series. ΔS° also increases considerably as uracil is methylated. Examination of fig. 2 indicates that as θ increases, both ΔH_a and ΔS_a decrease much more rapidly for 1,5-dimethyluracil than for thymine and uracil.

The fact that changes in ΔH° in the series uracil—thymine—1,5-dimethyluracil are in the opposite direction to changes in ΔG° is not easy to rationalize. Traube's rule predicts that the free energy of adsorption will become consistently more negative as methylene groups are added in a homologous series of adsorbates [10]. This trend is observed in the adsorption of several aliphatic alcohols at the mercury electrode; ΔG° becomes more negative by about 700—800 cal per added CH_2 groups [11]. Similarly, the present results show that the change in ΔG° is about −600 cal for uracil → thymine and about −650 cal for thymine → 1,5-dimethyluracil. In the absence of unusual entropy effects, one would expect to observe a corresponding decrease in the enthalpy of adsorption in this series. However, it must be remembered that all of the standard thermodynamic functions for adsorption are actually differences between partial molar quantities for the adsorbate in the dilute surface film and in the dilute aqueous solution. There is evidence that dissolved organic molecules with nonpolar hydrocarbon groups tend to have relatively large negative entropies of transfer from the ideal gaseous state into aqueous solution [12,13]. Thus, the transfer of 1,5-dimethyluracil from aqueous solution to the surface film may lead to a more positive entropy change than the transfer of thymine or of uracil because 1,5-dimethyluracil has two substituent methyl groups, and thymine one methyl group, replacing H atoms of uracil. That is, the negative entropy contribution which is often attributed to a highly ordered water structure around hydro-

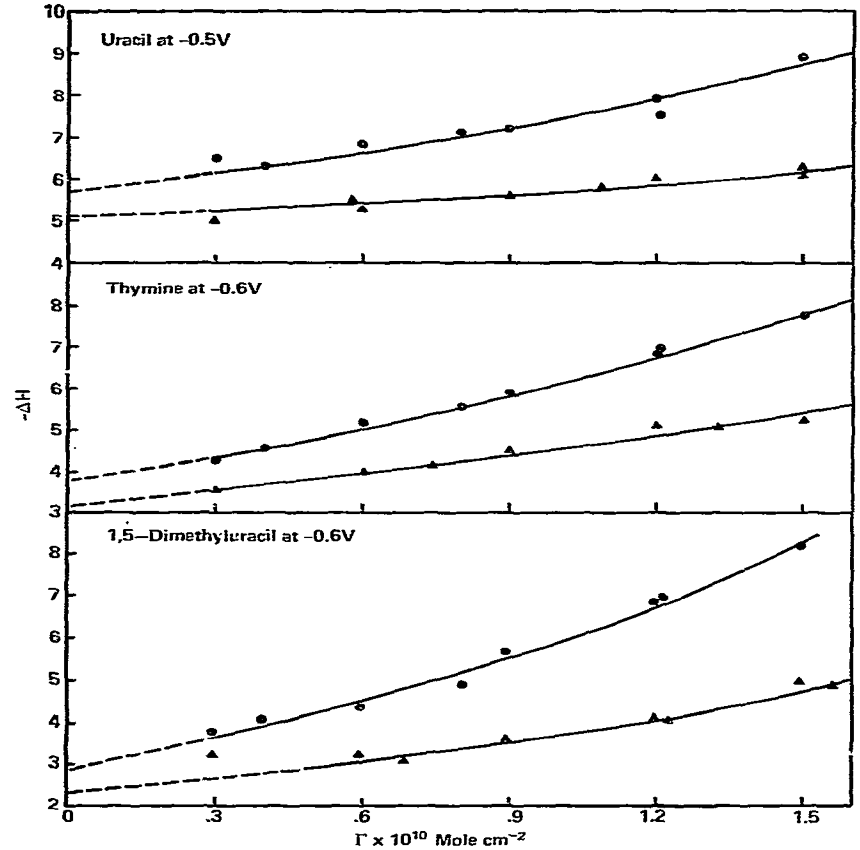

Fig. 2. ΔH_a and ΔH_π versus Γ plots for uracil at −0.5 V, thymine at −0.6 V and 1,5-dimethyluracil at −0.6 V. ΔH_a are represented by solid circles (●), ΔH_π by solid triangles (▲).

Table 5
Values of ΔG°, ΔH° and ΔS° for the ideally dilute films at 25°C [a]

System	$\Delta G^{\circ\prime}$ (kcal)	ΔH° (kcal)	ΔS° (cal K^{-1})
Uracil	−2.94	−5.7	−9.3
Thymine	−3.51	−3.7	−0.6
1,5-Dimethyluracil	−4.07	−2.9	+3.9

[a] $\Delta H^{\circ\prime}$ values obtained as limiting values of ΔH_a at $\Gamma = 0$ (see fig. 2); $\Delta G^{\circ\prime} = -RT \ln B_0$.

carbon groups in aqueous solution will tend to be lost as the methylated uracil derivatives are removed to the surface region.

Since the interaction of all three pyrimidines with the mercury surface probably occurs primarily through the π-electron orbitals in the dilute adsorption region, it is unlikely that the pyrimidine ring–mercury electrode binding energies change greatly in the series uracil–thymine–1,5-dimethyluracil. Thus, the trend in ΔH° probably reflects changes in the enthalpy of the pyrimidines in the dissolved state. In general, there is a more negative total energy of interaction between larger, as compared to smaller, organic molecules and the aqueous solvent. For example, energies of transfer of benzene, toluene, and m-xylene from the ideal gaseous state into the ideal dilute solute state in water are −7.0, −7.7, and −9 kcal/mole, respectively

[14,15]. It is probable that at least as great differences in transfer energy occur in the series uracil–thymine–1,5-dimethyluracil. Therefore, the adsorption of 1,5-dimethyluracil might be expected to be somewhat more endothermic than the adsorption of thymine and of uracil, since the surface interaction with mercury must occur with at least a partial loss of contact between the pyrimidine molecules and water. The observed changes in ΔH° (from −5700 cal for uracil to −3700 cal for thymine, to −2900 cal for 1,5-dimethyluracil are in fact similar in magnitude to the changes in transfer energies in the series benzene–toluene–m-xylene.

It is tempting to compare the binding of organic molecules at a mercury surface and the "hydrophobic bonding" which leads to clustering of hydrocarbon moieties in aqueous media. Hydrophobic bonding is often attributed to the relatively large positive entropy change which supposedly occurs when hydrocarbon groups contact each other, thus eliminating part of the water cage structure which may surround these groups in the dissolved state [13]. In the case of uracil, which has no methyl substituents attached to the ring, the major part of the driving force for adsorption is probably the energy of interaction with the surface. However, upon methylation (as in thymine and 1,5-dimethyluracil) the adsorption of pyrimidines may become more and more "entropy-driven" because of modification of the highly ordered H_2O structure around the hydrocarbon groups.

As we noted above, the tendency of ΔH_a and ΔS_a to become more negative as θ increases is considerably more pronounced for 1,5-dimethyluracil than for thymine and for uracil. This trend may reflect the increased ability of methylated bases to act as proton acceptors in hydrogen-bonding reactions, owing to electronic inductive effects [16,17]. It is likely that water molecules function as bridges between adsorbed pyrimidine molecules in films at relatively large values of θ. The enhanced basicity of the methylated bases might therefore lead to stronger hydrogen bonding and increased ordering within the films at high degrees of surface coverage. On the other hand, it must be recognized that methylation should decrease the acidity of the N-H protons and that 1,5-dimethyluracil has only one N-H group capable of donating a proton.

In order to investigate the thermodynamic constants for formation of the compact surface films, i.e., when the uracil derivatives adopt a perpendicular surface orientation, the following procedures were utilized. The bulk solution activity of the uracil derivative at each temperature was increased in ca. 0.1 *mM* increments until the first formation of the capacitance pit was noted. Alternatively, the bulk solution activity required to cause the capacitance to attain values intermediate between the minimum for the dilute film and the minimum for the compact film was measured. The former method was simpler from an experimental point of view. However, the bulk solution activities required to reach either of the above situations were very similar and gave nearly identical thermodynamic information (vide infra).

Table 6 lists the critical values of activity at which the denser film forms for each system, at potentials near the middle of the region of maximum stability of this film. Again, it is possible to calculate ΔH_a from the temperature dependence of these a values (using eq. (8)) and to infer ΔS_a from eq. (9). These constants and $\Delta G_a = RT \ln a$ are given in table 6. The ΔG_a values are necessarily more positive than ΔG_a for the dilute films (since they form at higher concentrations) but ΔH_a and ΔS_a are both quite negative for the compact film. Although we cannot give a convincing explanation for the trends in ΔH_a and ΔS_a in the series uracil–thymine–1,5-dimethyluracil, we do note that the magnitudes of these constants indicate the high degree of ordering and the large total binding energy of the films. We believe these large negative numbers reflect both strong interactions between the adsorbate

Table 6
Values of ΔG_a, ΔH° and ΔS° for the compact films at 25°C

System	Activity [a] (mole ℓ^{-1})	ΔG_a (kcal)	ΔH_a (kcal)	ΔS_a (cal K^{-1})
Uracil	2.1×10^{-2} [b]	−2.29	−9.1	−22.9
Thymine	1.1×10^{-2} [c]	−2.67	−12.4	−32.6
1,5-dimethyluracil	6.25×10^{-3} [d]	−3.01	−9.0	−20.1

[a] Bulk solution activity required to observe the first appearance of the capacitance pit.

[b] The activity value at 5.25°C was 7.0×10^{-3} *M*; at 40°C the value was 4.45×10^{-2} *M*.

[c] The activity value at 4.5°C was 2.47×10^{-3} *M*; at 40°C the value was 3.15×10^{-2} *M*.

[d] The activity value at 4.5°C was 2.10×10^{-3} *M*; at 40°C the value was 1.33×10^{-2} *M*.

and the mercury and the stacking forces operating between adsorbate molecules within the film. The fact that 1,5-dimethyluracil has only one N-H group may help to explain the observation that ΔH_a and ΔS_a are both less negative than the corresponding thermodynamic quantities for the compact films of thymine and uracil. Finally, we note that methylation of purines and pyrimidines ordinarily leads to more negative free energies of stacking in aqueous solution [6]. This trend is also obeyed by the ΔG_a values for the compact films of uracil, thymine and 1,5-dimethyluracil ($\Delta G_a = -2290$, -2670, and -3010 cal, respectively for these compounds).

The values of ΔH_a and ΔG_a and hence ΔS_a for the compact film could also be obtained by a third method which involved monitoring the capacitance at a DME as a function of solution temperature. This type of experiment may be illustrated with reference to fig. 3 which shows an experimental capacitance versus temperature curve for 3.92 m*M* thymine. This concentration of thymine is such that at 4.5°C it exhibits a pronounced capacitance pit centered at −0.6 V, corresponding to the existence of the compact layer at the electrode surface. Accordingly, a fixed potential of −0.6 V was applied to the DME and the capacitance monitored continuously as a function of the solution temperature. The temperature was allowed to rise from 4.5°C by natural heating of the solution; i.e., the circulatory bath was turned off when the solution was equilibrated at 4.5°C. The "melting" curve shown in fig. 3 represents the capacitance change as the surface layer at the electrode changes from the compact to dilute layer. The transition temperature is most conveniently measured as that corresponding to the midpoint of the *C* versus *T* curve. The melting curve shown in fig. 3 appears to exhibit a double inflection. This was noticeable on all such curves although often not quite as pronounced as shown in fig. 3. We are unable to explain the cause of the double inflection, although it may be speculated that a surface film intermediate in properties between the dilute and compact layers might exist at the electrode surface.

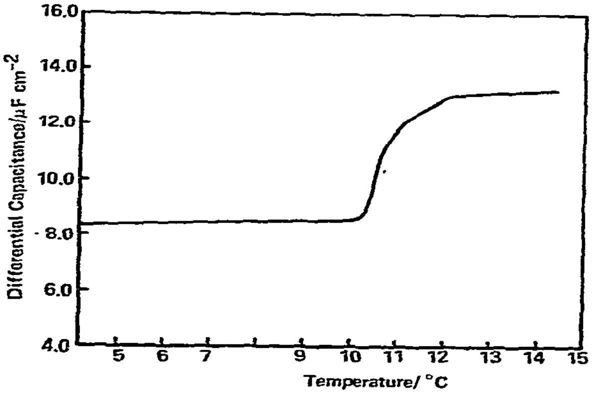

Fig. 3. Differential capacitance versus temperature curve for 3.92 m*M* thymine in 0.5 *M* NaF with 0.01 *M* Na_2HPO_4 buffer pH 8.0. Applied potential −0.6 V. Temperature of solution measured with thermistor probe placed close to DME. Controlled droptime of DME of 2.00 s.

However, by plotting bulk solution activity versus $1/T_m$, where T_m corresponds to the midpoint of a melting curve, virtually identical values of ΔH_a are calculated for the compact film as were obtained by use of the methods described earlier.

Conclusions

These studies of the effect of temperature on the interfacial behavior of uracil derivatives have revealed a number of significant findings. First, the general features of the interfacial behavior of uracil derivatives between 4.5°C and 40°C are, for all practical purposes, unchanged. Thus, the area occupied by each derivative in the dilute adsorption region is, within experimental error, the same at 4.5°C as it is at 40°C, and hence there is apparently no change in the flat orientation of the uracil molecules.

The thermodynamic measurements support the general view that with increasing methylation of uracil the adsorption becomes more and more entropy driven in the dilute adsorption region. The observation that the dilute films of the methylated uracil derivatives are formed less exothermically than the uracil films parallels the behavior noted in the hydrophobic bonding of hydrocarbon groups. Thus, when non-polar side chains are removed from an aqueous environment and buried within a large protein molecule, the process is accompanied by an increase in both enthalpy and entropy [13].

The magnitudes of ΔH_a and ΔS_a for the compact (or perpendicularly oriented) film (table 6) suggests a rather highly ordered structure of the adsorbed layer and a large total binding energy of the film. This binding energy includes both interactions with the mercury surface and lateral interactions between adsorbed molecules. The behavior of uracil derivatives at the mercury electrode-aqueous electrolyte interface is also interesting because many of the molecular properties of the species of profound structural importance in nucleic acids are reflected in their interfacial properties. Thus, in the dilute adsorption layer they adopt a flat surface orientation where, on the basis of the area they occupy at monolayer surface saturation, they are probably connected by a hydrogen bonding network involving bridging water molecules. At critical bulk solution concentrations over very precisely defined potential ranges, the uracil derivatives rearrange on the electrode surface and adopt a perpendicular orientation. In this compact layer the adsorbed molecules must undergo appreciable stacking interactions.

The hydrogen bonding phenomena in the dilute layer and the stacking interactions in the compact layer are not unlike the interactions observed between purine and pyrimidine bases found in nucleic acids.

It is tempting to speculate that the interfacial behavior of biologically important purines and pyrimidines observed at an electrode in this investigation might be paralleled in natural systems at a charged biomembrane or other biological interface.

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References

[1] V. Brabec, S.D. Christian and G. Dryhurst, Biophys. Chem. 7 (1978) 253.
[2] H. Kinoshita, S.D. Christian and G. Dryhurst, J. Electroanal. Chem. Interfac. Electrochem. 83 (1977) 151.
[3] V. Brabec, S.D. Christian and G. Dryhurst, J. Electroanal. Chem. Interfac. Electrochem. 85 (1977) 389.
[4] D.C. Grahame, E.M. Coffin, J.P. Cummings and M.A. Poth, J. Amer. Chem. Soc. 74 (1952) 1207.
[5] B.B. Damaskin, O.A. Petrii and V. Batrakov, Adsorption or organic compounds on electrodes (Plenum, New York, 1971) p. 112.
[6] P.O.P. Ts'o, in: Basic principles of nucleic acid chemistry, Vol. 1, ed. P.O.P. Ts'o (Academic Press, New York, 1974) p. 454.
[7] S.D. Christian, G. Dryhurst, V. Brabec and J.G. Baker, J. Colloid Interface Sci. 62 (1977) 454.
[8] J.J.F. Scholten and S. Kruyer, in: Physical and chemical aspects of adsorbents and catalysts. ed. B.G. Linsen (Academic Press, New York, 1970) pp. 148–149.
[9] D.H. Everett, Trans. Faraday Soc. 46 (1950) 453.
[10] I. Traube, Ann. Chem. 265 (1891) 27.
[11] B.B. Damaskin, A.A. Survilla and L.E. Rybalka, Elektrokhimiya 3 (1967) 146.
[12] H.S. Frank and M.W. Evans, J. Chem. Phys. 13 (1945) 507.
[13] W. Kauzmann, Advan. Protein Chem. 14 (1959) 1.
[14] R.L. Bohon and W.F. Claussen, J. Amer. Chem. Soc. 73 (1951) 1571.
[15] K.O. Yeo, Ph.D. Dissertation. The University of Oklahoma, 1973.
[16] E.E. Tucker and S.D. Christian, J. Amer. Chem. Soc. 97 (1975) 1269.
[17] H.S. Frank and W.Y. Wen, Discuss. Faraday Soc. 24 (1957) 133.