Comparing the observed $pK'$ changes to the theoretical expression enables us to determine the constants $c_1$ and $c_2$. Therefore we need two linear independent relations between $c_1$ and $c_2$.

We know that $ApK$ between $\omega = 0.01$ and $\omega = 1$ equals 1.43 $pH$ units (Table 2) and by extrapolating the linear logarithmic part of the curve $ApK = f(\omega)$ we get $ApK = 0$ for $\omega = 2.7$.

It follows that $c_2 = 1.7; c_1 = 0.43; c_3 = 0.73$.

The electrostatic potential of poly U is given by $-e\psi/kT = 0.73 - 1.7 \log C_{NaCl}$.

The theoretical values for $c_1$ and $c_2$, assuming a rod model with radius 10 Å and an average linear charge density of 3.4 Å\(^{-1}\) require that $c_1 = 0.95$ and $c_2 = 1.92$. The agreement between the theoretical values of $c_1$ and $c_2$ and the experimental values suggests that the model and the hypothesis assumed describe quite accurately the real situation.

Interactions of polynucleotides and their components

II. Interaction of uracil derivatives with alkali- and alkali earth chlorides

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(Z. Naturforsch. 23 b, 30–32 [1968]; eingegangen am 10. April 1967)

During spectrophotometric titrations of uracil in different alkali- or alkali earth chlorides, specific changes in the U.V. absorption of uracil at $pH$ 12.0 have been observed. The spectra of 4-methyl and 5-methyl uracil change in the same way whereas 1-methyl and 3-methyl uracil are not influenced even by strong divalent cations. From spectrophotometric and potentiometric titrations it could be concluded that the changes are not due to the dissociation of the $-HN(\text{H})-C(6)=0$ groups. From the probable site of interaction it seems impossible that uridine or uridylic acid may be involved in such interactions. From optical density measurements the $K_s$ value has been determined.

In a study on spectrophotometric titrations of several purine and pyrimidine bases, nucleosides and nucleotides, specific changes in the U.V. absorption spectrum of uracil have been observed when adding alkali- or alkali earth chlorides to basic solutions. The importance of an understanding of interactions between metal ions and nucleic acids incited us to examine the properties of these uracil-metal ion complexes.

Methods and materials

The spectrophotometric titrations have been performed as previously described\(^1\). The U.V. absorption spectra have been measured with a Cary model 14 recording spectrophotometer using 1 cm quartz cuvettes. The association constant $K_s$ has been determined according to the method used by PERRIN\(^2\).

Abbreviations. U, uridine; Up, uridine-3'(2')-phosphate; UpC, uridylyl-(3' → 5')-cytidine; UpU, uridylyl-(3' → 5')-uridine; CpUpC, cytidylyl-(3' → 5')-uridylyl-(3' → 5')-cytidine; poly U, polyuridylic acid.

2 D. D. PERRIN, J. Amer. chem. Soc. 82, 5642 [1960].
We have for a 1-1 complex
\[ K_s = \frac{(U^\circ \cdot Ca^{2\circ})}{(U^\circ \cdot (Ca^{2\circ})^\circ)} \]
\[ E = \varepsilon_0 \cdot (U^\circ) + \varepsilon \cdot (U^\circ \cdot Ca^{2\circ}), \]
\[ E_0 = \varepsilon_0 \cdot (U^\circ) = \varepsilon_0 \cdot (U^\circ) + \varepsilon \cdot (U^\circ \cdot Ca^{2\circ}), \]
\[ \frac{E - E_0}{(Ca^{2\circ})} = \varepsilon \cdot K_s \cdot (U^\circ) - K_s \cdot E. \]

By plotting \( E - E_0 \) against \( E \), the \( K_s \) value can be determined.

Uracil, uridine, uridylic acid, 4-methyl and 5-methyl uracil were products of Calbiochem; 1-methyl and 3-methyl uracil have been prepared by methylation of uracil with dimethyl sulfate and separated by paper chromatography. All other reagents were of an analytical grade. The pH of the solutions has been adjusted to pH 12.0 by adding sodium hydroxyde.

**Results and discussion**

Fig. 1 shows the U.V. spectra of uracil at pH 12.0 in different CaCl\(_2\) concentrations and in aqueous solutions at pH 7.0 and pH 12.0. NaCl and KCl exert the same effect on the uracil spectra at pH 12.0 but divalent Ca\(^{2\circ}\) ions have roughly the same effect as a thirtyfold higher concentration of the univalent K\(^{\circ}\) cations.

The spectral changes strongly suggest an interaction between the negatively charged uracil ion and Ca\(^{2\circ}\). In order to gain more information about the structure of the uracil ion that is involved in the complex formation, spectra of several uracil derivatives have been recorded with and without CaCl\(_2\) at pH 12.0. The spectra of 4-methyl- and 5-methyl uracil are qualitatively and quantitatively influenced in an identical way as uracil by CaCl\(_2\) at pH 12.0. On the contrary the spectra of 1-methyl and 3-methyl uracil do not change in the presence of CaCl\(_2\) at pH 12.0. The spectra of uridine and uridylic acid are slightly influenced by strong CaCl\(_2\) solutions at basic pH values. This suggests that N\(_{(d)}\) and N\(_{(3)}\) and accordingly also C\(_{(2)}\) and C\(_{(6)}\) may not carry a substituent nor that the charge of these atoms may be affected, in order to obtain the U\(^{\circ}\)-Ca\(^{2\circ}\) complex.

The pK values of the dissociable groups are lowered by strong CaCl\(_2\) concentrations\(^4\) so that the possibility may not be excluded that the spectral changes of uracil at pH 12.0 by adding CaCl\(_2\) may be caused by a partial dissociation of the \(-HN\(_{(1)}\)-C\(_{(6)}\) = 0 groups. The nature of the spectral changes does not agree however with this supposition\(^4\); the spectrophotometric titration curves of uracil solutions with strong CaCl\(_2\) or NaCl concentrations also indicate that only the dissociation of the \(-NH\(_{(3)}\)-C\(_{(2)}\) = 0 groups occurs by titrating up to pH 12.0 since they conform to the expression \( pH = pK + \log \frac{x}{1-x} \) and the isosbestic points do not change up to pH 12.0 (Fig. 2).

Potentiometric titrations have shown that the dissociation of the \(-HN\(_{(1)}\)-C\(_{(6)}\) = 0 groups has not to be considered when uracil solutions are titrated up to pH 12.0 in the presence of CaCl\(_2\) or NaCl since we need only 1 equivalent of base per uracil to obtain pH 12.0.

From these facts we concluded that a weak U\(^{\circ}\)-Ca\(^{2\circ}\) complex is formed probably by means of an ionic bond and that the most probable site for it is the \(-N\(_{(3)}\)-C\(_{(2)}\) = O (or \(-N\(_{(3)}\)-C\(_{(2)}\)-O\(^{\circ}\)) group. The charge distribution of the \(-HN\(_{(1)}\)-C\(_{(6)}\)

\(^3\) J. P. Scannell, A. M. Crestfield, and F. W. Allen, Biochim. biophysica Acta [Amsterdam] 32, 406 [1959].

\(^4\) D. Shugar and D. D. Fox, Biochim. biophysica Acta [Amsterdam] 9, 199 [1952].
Fig. 2. Spectrophotometric titration curves of uracil in aqueous solution (I) and in 0.16 M CaCl$_2$ (II). IA: uracil at pH 7.0 with or without CaCl$_2$, IB: uracil at pH 12.0 in aqueous solution, IPK: uracil at pH = $pK'$ in aqueous solution, II$_B$: uracil at pH 12.0 in 0.16 M CaCl$_2$, II$^R$: uracil at pH = $pK'$ in 0.16 M CaCl$_2$.

= O group however also influences the complex formation.

The association constant has been determined from optical density measurements of uracil solutions at pH 12.0 containing different CaCl$_2$ concentrations (Fig. 3 and Table 1).

The association constant $K_s$ deduced from the values of Table 1 amounted to $K_s = 16 \pm 3$ mol$^{-1}$. Calculations from extinction values at other wavelengths agreed within experimental limits.

From the above mentioned facts it can be deduced that the molar extinction coefficient of uracil, 4-methyl and 5-methyl uracil at pH 12.0 depends on the presence of univalent and divalent cations; this presence can thus interfere in quantitative estimations of uracil or 5-methyl uracil (thymine) from jant optical density measurements at pH 2.0, pH 7.0 and pH 12.0.

The probable site of the uracil-cations interaction suggests that this kind of interaction is very weak or impossible for uridine and uridylic acid or uridylic acid incorporated into a polynucleotide.

| (Ca$^{2+}$) | $E_{280}$ nm | $-E + E_0$ | $-E + E_0$  
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Table 1. Optical densities and corresponding $E - E_0$ values.