dium yielded spherical globules (Fig. 2[9]). Methanol, *n*-butanol, and *n*-hexane formed ellipsoidal globules. Globule formation seemed dependent on the polymer present in the mixture and on a precipitation medium temperature not above −24°. When these conditions were not met, coalescence but not stabilization occurred.

Release of Potassium Chloride—In the spheres containing polymethyl methacrylate as the polymer and diethylene glycol dimethacrylate as the monomer (Samples 2–7), the methanol precipitation medium increased potassium chloride release (Fig. 3). In nonmethanol precipitation media, release from relatively strongly hydrophilic beads (Samples 9 and 11) was faster than that from less hydrophilic matrixes.

The binary monomer spheres containing polyethylene glycol 600 in place of polymer showed a release rate independent of the methyl methacrylate–trimethylolpropane trimethacrylate proportions (Fig. 4). The polyethylene glycol 600 concentration strongly affected potassium chloride release (Fig. 5). Larger polyethylene glycol 600 proportions together with 1,4-butanediol in the final polymerization step yielded spheres with a markedly porous internal structure (Fig. 6[10]).

[9] Nikon F, Nippon Kogaku Co.
[10] Model JSM-U3, Japan Electron Optics Laboratory Co.

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UV Studies of Nucleic Acid Base Complexation with Isoproterenol in Different Solvents

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Abstract □ Charge transfer complex formation between nucleic acid bases and isoproterenol was demonstrated from UV absorption measurements. The solvent polarity effects on equilibrium constants were investigated. The solvent systems containing 0.1 *N* HCl were 20% aqueous ethanol, water, and water containing sodium chloride. The equilibrium constants, calculated from UV absorption data by the application of the Benesi–Hildebrand equation, were small and increased with increasing ionic strength. Equilibrium constant wavelength dependence was demonstrated in some cases.

Keyphrases □ Complexes, charge transfer—nucleic acid bases with isoproterenol, various solvents, UV absorption study □ Nucleic acid bases—charge transfer complex formation with isoproterenol, various solvents, UV absorption study □ Solvents, various—charge transfer complex formation, nucleic acid bases with isoproterenol, UV absorption study

Catechol forms the catecholamine aromatic nucleus and anchors these molecules to their receptors (1). The adrenergic sites and the biogenic amine storage granules are known to contain adenosine triphosphate, phosphate, and magnesium ions (2, 3). Complex formation between these ions and catecholamines has been demonstrated (4, 5). Evidence for charge transfer complex formation between catechol, epinephrine, and nucleic acid bases has been presented (6–8).

The present study was undertaken to trace the catecholamine action mechanism, to obtain more information about adrenergic receptors, and to investigate adenosine triphosphate-like action of some other storage granule nucleoside triphosphates. UV absorption was used to study the interactions between the nucleic acid bases and isoproterenol in the following solvents containing 0.1 *N* HCl: 20% aqueous ethanol, water, and water containing sodium chloride.

EXPERIMENTAL

Isoproterenol sulfate dihydrate[1], thymine[2], cytosine[2], standard hydrochloric acid solution[2], adenine[3], uracil[3], and sodium chloride[4] (BP) were the highest commercially available purity (>99%) and were used without further purification.

Solutions containing a fixed nucleic acid base concentration (0.02 *M*, except cytosine-containing solutions in 20% alcohol, fixed at 0.01 *M*) and different isoproterenol sulfate concentrations (0.3–0.8 *M*) were prepared by dilution from concentrated standard solutions.

The solvents used were 20% aqueous ethanol containing 0.1 *N* HCl, 0.1 *N* aqueous HCl, and water solutions containing 0.1 *N* HCl and different amounts of sodium chloride.

All solutions were freshly prepared, and their spectra were recorded within 6 hr in 1-cm path length rectangular cells with stoppers. Most experimental work was carried out in subdued light to avoid isoproterenol light oxidation and at room temperature (20–25°).

The spectrophotometers[5] were checked and calibrated with an oxide film[6]. Cell holders were thermostated to maintain the temperature at 25°. The baseline was recorded before the experiments with the same solvent in the reference and in the sample compartments.

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] BDH Chemical Co., Poole, England.
[3] Hopkin and Williams Chemical Co., Chadwell Heath, Essex, England.
[4] Evans Medical Ltd., Liverpool, England.
[5] Perkin-Elmer model 402 and Pye Unicam model SP 800 UV-visible.
[6] Holmium.

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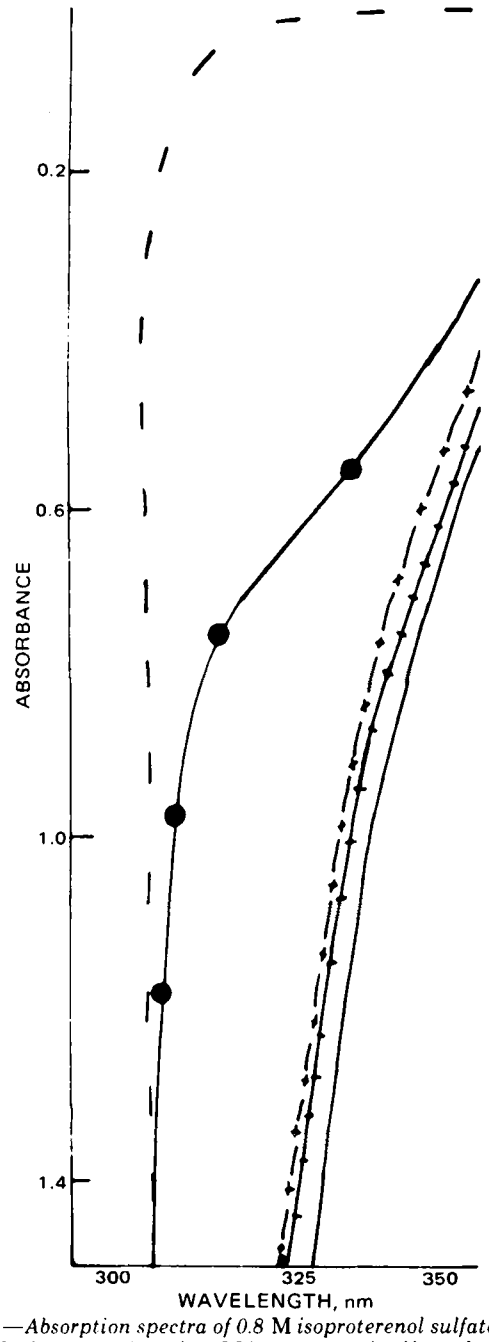

Figure 1—*Absorption spectra of 0.8 M isoproterenol sulfate (-●-), 2 × 10^{-2} M adenine (– –), and 0.8 M isoproterenol sulfate plus 2 × 10^{-2} M adenine (—) in aqueous solutions containing 0.1 N HCl at an ionic strength of 3.0; —+—+— and -×-×- represent the absorption spectra of the mixture at ionic strengths of 3.5 and 4.0, respectively. Measurements were at 25 ± 0.5°.*

RESULTS AND DISCUSSION

A representative nucleic acid base–isoproterenol UV absorption spectrum (Fig. 1) showed a shift toward longer wavelengths. The increase in absorption at longer wavelengths (Table I) was attributed to weakly bonded complex formation.

Isoproterenol and the nucleic acid base absorption at the specified wavelengths depended only on concentration. This result was to be expected if no self-association had taken place. The pH deviation (~0.8) from the initial working acidity, as well as changes in temperature and ionic strength, did not affect the pure component absorption spectra, further supporting the suggestion that the increased absorbance observed in the mixtures was due to complex formation.

Absorbances at different isoproterenol concentrations were estimated from the difference between the mixture absorbance and a sum of the absorbances of the individual substances having concentrations identical to those in the mixture. The absorbances at different wavelengths were calculated from molar absorptivities.

All UV binding interaction measurements were made at fixed wavelengths; for each mixture examined, measurements were made at several wavelengths. The complex absorbance was measured in regions where the free component absorptions were small and the complex absorption was appreciable. The accuracy of these measurements was ±0.01 unit of absorbance.

The complex equilibrium constants were calculated by assuming simple complex formation:

$$A + D = AD \qquad \text{(Eq. 1)}$$

and by application of the Benesi–Hildebrand equation:

$$\frac{[A_0]}{A_\lambda^{AD}} = \frac{1}{\epsilon_\lambda^{AD}} + \frac{1}{(K_c^{AD})(\epsilon_\lambda^{AD})[D_0]} \qquad \text{(Eq. 2)}$$

where K_c^{AD} is the equilibrium constant; $[A_0]$ and $[D_0]$ are the initial concentrations of the reactant species; and A_λ^{AD} and ϵ_λ^{AD} represent the absorbance and molar absorptivity of the complex, respectively, at the wavelength λ.

Equation 2 is based upon the assumption that $[D] \simeq [D_0]$ when designing an experiment such that $[D_0] \gg [A_0]$.

A plot of $[A_0]/A_\lambda^{AD}$ *versus* $1/[D_0]$ leads to an intercept of $1/\epsilon_\lambda^{AD}$ and a slope of $1/K_c^{AD}\epsilon_\lambda^{AD}$, whereby the molar absorptivity and the equilibrium constant can be calculated graphically.

The standard free energy changes associated with various interactions, $\Delta G°$, are calculated from the following well-known equation:

$$\Delta G° = -RT \ln K_c^{AD} \qquad \text{(Eq. 3)}$$

where R is the gas constant and T is the absolute temperature. Representative Benesi–Hildebrand plots are presented in Fig. 2; the thermo-

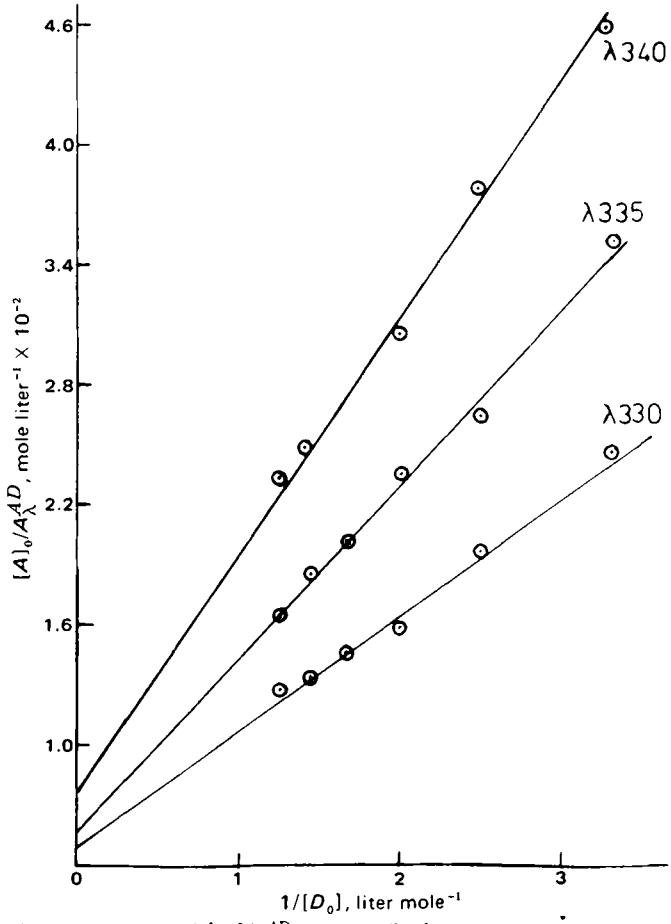

Figure 2—*Plots of* $[A_0]/A_\lambda^{AD}$ versus $1/[D_0]$ *of isoproterenol sulfate containing cytosine in 20% alcoholic solutions with 0.1 N HCl. The cytosine total concentration,* $[A_0]$, = 10^{-2} M, *and the isoproterenol total concentration,* $[D_0]$, = 0.3–0.8 M. *The* A_λ^{AD} *represents the absorbance of the complex at the wavelength. Measurements were at 25 ± 0.5°.*

Table I—Equilibrium Constants (K) and Standard Free Energy Changes ($\Delta G°$) Associated with Interactions of Nucleic Acid Bases with Isoproterenol Sulfate in Different Solvents Together with Molar Absorptivities (ϵ) at Different Wavelengths (λ) [a]

Nucleic Acid Base	λ, nm	ε	K, M^{-1} ± 0.01	$\Delta G°$, kcal/mole
	20% Alcoholic Solutions Containing 0.1 *N* HCl			
Adenine	325	71.4	2.12	−0.44
	330	42.3	1.77	−0.34
Cytosine	330	200	0.88	0.07
	335	178	0.65	0.25
	340	131	0.60	0.30
Thymine	310	50	1.31	−0.16
	315	31	1.15	−0.08
Uracil	310	27.2	5.10	−0.96
	Aqueous Solutions Containing 0.1 *N* HCl			
Adenine	323	89	1.70	−0.31
	325	78	1.44	−0.22
Cytosine	340	76	1.13	−0.07
	345	71	0.60	0.30
Thymine	312	100	0.59	0.31
	315	93	0.58	0.32
	Aqueous Solutions Containing 0.1 *N* HCl at Different Ionic Strengths (I)			
Adenine[b]	330	91	1.00	0.00
	335	78	1.60	−0.28
Cytosine[b]	340	100	0.71	0.20
	345	68	0.87	0.08
Adenine[c]	330	41	4.00	−0.82
	335	23	4.70	−0.92
Cytosine[c]	340	58	2.10	−0.44
	345	45	2.08	−0.43
Adenine[d]	330	36	5.90	−1.05

[a] Measurements were at 25 ± 0.5°. [b] At I = 3.0. [c] At I = 3.5. [d] At I = 4.0.

dynamic constants and molar absorptivities are summarized in Table I.

The molar absorptivities of adenine–isoproterenol and thymine–isoproterenol complexes were higher in water than in water–ethanol; the reverse was obtained with cytosine–isoproterenol. Furthermore, the adenine–isoproterenol and cytosine–isoproterenol complex molar absorptivities were ionic strength dependent.

In general, the equilibrium constants were sensitive to changes in the wavelengths at which the measurements were taken, except for thymine–isoproterenol in water. Almost all equilibrium constants were less than two, except for uracil–isoproterenol in 20% alcohol. Therefore, $\Delta G°$ values were small. The equilibrium constants of adenine–isoproterenol and thymine–isoproterenol (Table I) decreased as the solvent was changed from alcoholic to aqueous, while the cytosine–isoproterenol complex equilibrium constants increased.

Three- and fourfold increases in adenine–isoproterenol equilibrium constants at λ335 and λ330 nm, respectively, were produced by increasing the ionic strength of aqueous solutions from 3.0 to 3.5. A sixfold increase could be obtained at λ330 nm by increasing the ionic strength from 3.0 to 4.0. The same trend was observed for cytosine–isoproterenol equilibrium constants; 2.4- and threefold increases were produced by changing the ionic strength from 3.0 to 3.5 at λ345 and λ340 nm, respectively.

The increase in absorbance at longer wavelength was attributed to charge transfer complex formation where the nucleic acid bases were assumed to act as electron acceptors with catechol or epinephrine as electron donors (6–8). Similar donor and acceptor species may be hypothesized for isoproterenol–nucleic acid base complexes.

Exact complex geometry cannot be determined from UV absorption measurements, but parallel stacking interactions between nucleic acid bases and isoproterenol can be proposed. This mechanism was suggested previously for a similar system (9–12). The geometry is likely to be affected by the stoichiometry (13–17), but the present results are best interpreted by a 1:1 stoichiometry.

To account for the decrease in adenine–isoproterenol and thymine–isoproterenol equilibrium constants as the solvent polarity increases (Table I), the complex dipole moment was assumed to be larger in the excited state than in the ground state, resulting in the formation of a highly polar complex that was more solvated than the sum of the uncombined components. The reverse situation can be assumed for the cytosine–isoproterenol complex (18–20).

Little work has been done on the salt effect on the equilibrium constants. The large increase in equilibrium constants as salt concentrations were increased (Table I) can be explained by assuming ion-pair formation between the chloride ions and the protonated centers around the interacting molecules, resulting in an electrostatic repulsion decrease. Other weaker forces seem to dominate the repulsive forces. The same explanation accounted for the decrease in the equilibrium constants (21–25). Borazan (13, 17) showed that the equilibrium constants of nucleoside self-association and aqueous complex formation are similar to those obtained at higher salt concentrations.

The equilibrium constant wavelength dependence (Table I) could indicate the formation of more than one complex type; the wavelength independence was explained as due to the formation of one complex type in solution (26).

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