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STUDIES ON THE MECHANISM OF ADSORPTION OF PURINES IN SEPHADEX G-10 CHROMATOGRAPHY

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SUMMARY

The adsorption of purines to Sephadex G-10, as measured by the logarithms of corrected chromatographic elution volumes, V_e^0, shows correlations with the following physical properties of the purines: π-electron delocalization energy, electron donating ability, electron accepting ability, basic and acidic pK_a and water solubility. These correlations, together with equilibrium dialysis studies of adsorption of purines to dextran and Sephadex, indicate that purines interact primarily with the dextran portion of Sephadex in contrast to substituted benzene derivatives, which interact primarily with the ether cross-linkages of Sephadex.

INTRODUCTION

The Sephadex gels (Pharmacia) are composed of dextran, a linear $\alpha(1\rightarrow 6)$ glucose polymer with $\alpha(1\rightarrow 3)$ branching points, which has been cross-linked with epichlorohydrin to give glyceryl ether linkages of the type: dextran–O–CH_2–CH(OH)–CH_2–O–dextran[1]. Originally employed as supports for zone electrophoresis and as gel filtration columns for determination of molecular weights of macromolecules[1], Sephadex gels were shown by GELOTTE[2] to adsorb reversibly aromatic and heterocyclic compounds. The highly cross-linked Sephadex G-10 has been shown to be a useful adsorbent for column chromatography of purines and related compounds[3–9]. The effects of extent of gel cross-linkage[8,10,11] and eluent flow rate[7], ionic strength[7,11] and pH[6,7,11–13] have been studied in order to improve the separation of purines by Sephadex chromatography. However, the specific chemical groups of the purines and the Sephadex gels involved in adsorption and the types of bonds formed have not been determined. Investigations of the mechanism of adsorption of other heterocyclic and aromatic compounds to Sephadex indicated some relationship between substituent group properties such as increasing electron donating ability and increasing elution volumes on Sephadex columns[14,15]. BROOK

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and coworkers showed that the adsorption of substituted phenols[16] and substituted anilines and benzoic acids[17] correlated with the electron donating ability of the substituent groups as measured by their Hammett σ values and therefore that adsorption was due to the hydrogen bonding of the amino, carboxyl, or hydroxyl group to the gel cross-linkages. DETERMANN AND WALTER[18] showed by equilibrium dialysis that the adsorption of phenol to Sephadex gels was due to interaction with the ether cross-linking groups and not the dextran portion of the gel.

The availability of data for Sephadex G-10 chromatography at pH 7.0 of a variety of substituted purines and related compounds previously reported[7], together with the additional data given here, suggested that studies of the correlations of elution parameters with structural and physicochemical properties of the purines could give considerable information about the mechanism of adsorption of purines to Sephadex gels. Further studies with different dextran gels were performed to determine whether the ether linkages of Sephadex are involved in the adsorption of purines as they are in the adsorption of benzene derivatives.

METHODS

The selection of suitable conditions for Sephadex G-10 chromatography of purines and the methods used have been described[7]. All compounds were chromatographed on a 1.0 × 100 cm column of Sephadex G-10 eluted with 0.05 *M* sodium phosphate buffer, pH 7.0. In addition, some purines and heterocyclic compounds were chromatographed on a 1.0 × 50 cm column of Sephadex LH-20 (hydroxypropylated Sephadex G-25) eluted with the same buffer. The void volumes (V_0) were determined as the elution volumes (V_e) of dyed blue dextran and the internal volumes (V_i) were calculated from the elution volumes of acetone minus the void volumes. The corrected elution volumes (V_e^0) of the compounds were calculated from $V_e^0 = (V_e - V_0)/V_0$ (ref. 7).

Equilibrium dialysis experiments were performed with solutions of Dextran T 40 (mol. wt. 40,000) and suspensions of Sephadex G-10 (water regain 1.0, 40–120 μ), obtained from Pharmacia, at concentrations between 0 and 35% (w/w) in 0.05 *M* sodium phosphate, pH 7.0. 3 ml of these solutions or suspensions in dialysis tubing were dialyzed against 6 ml of the phosphate buffer, or 0.1 m*M* adenine or 0.1 mmol xanthine in the same buffer. Equilibrium was reached in 4–5 h. The change in absorbance at 260 nm between 0 and 6 h for the adenine and xanthine dialysis solutions, corrected for the change in absorbance of buffer dialysis solution, was used to calculate the per cent change in purine concentration as a function of per cent of Dextran or Sephadex in the dialysis tubing.

Regression equations and correlation coefficients were calculated using programs supplied with the Olivetti-Underwood Programma 101.

RESULTS

The advantage of using V_e^0 as a measure of adsorption instead of K_d, defined as $(V_e - V_0)/V_i$ (ref. 1), where V_i was calculated from the difference between the elution volumes of dextran and acetone, is shown in Table I. Columns were prepared from two lots of Sephadex G-10 and a standard solution of four purines was chroma-

TABLE I

COMPARISON OF ELUTION PARAMETERS FOR PURINES CHROMATOGRAPHED ON 1.0 × 100 cm COLUMNS FROM TWO LOTS OF SEPHADEX G-10

Compound	*Lot 1*			*Lot 2*		
	V_e (*ml*)	K_d	V_e^0	V_e (*ml*)	K_d	V_e^0
Dextran	32.5			31.2		
Acetone	61.0			56.1		
Hypoxanthine	113.0	2.82	2.48	104	2.92	2.34
Xanthine	156	4.33	3.59	143	4.49	3.59
Guanine	199	5.84	4.85	181	6.02	4.81
Adenine	251	7.67	6.38	231	8.02	6.40

TABLE II

ELUTION VOLUMES OF PURINES AND RELATED COMPOUNDS ON SEPHADEX G-10

Compound	V_e (*ml*)	V_e^0	*Log* V_e^0
Purines			
Guanine(-3-)N-oxide	91	1.70	+0.231
8-Methyltheophylline	105	2.24	+0.352
3-Methylxanthine	125	2.72	+0.436
1,3-Dimethyluric acid	131	3.03	+0.482
3-Methyladenine	138	3.25	+0.512
1,7-Dimethyluric acid	145	3.46	+0.540
7-Methyluric acid	147	3.52	+0.547
1-Methyluric acid	170	4.23	+0.627
8-Mercaptopurine	329	9.15	+0.963
6-Selenopurine	347	9.68	+0.986
8-Nitrotheophylline	437	12.01	+1.081
8-Chloroxanthine	601	17.05	+1.232
6-Piperidino(1)purine	606	17.31	+1.240
6-Benzylaminopurine	1413	42.48	+1.628
Ribosyl purines			
N^2-Dimethylguanosine	92	1.83	+0.263
Ribosyl 6-mercaptopurine	138	3.14	+0.497
Ribosyl 6-methylmercaptopurine	177	4.33	+0.637
Isopropylideneadenosine	199	4.99	+0.698
8-Azapurines			
8-Azatheophylline	188	4.80	+0.682
Pyrazolo[3,4-*d*]pyrimidines			
4-Amino-6-hydroxy-	175	4.29	+0.632
Pyrimidines			
5-Acetylamino-6-amino-1,3-dimethyluracil	55	0.70	−0.151
5,6-Diamino-1,3-dimethyluracil	80	1.46	+0.166
Miscellaneous			
NADP	34	0.037	−1.432
Pyridoxal phosphate	45	0.38	−0.414
Trigonelline	46	0.41	−0.387
Nicotinic acid	82	1.53	+0.168
N-Methyl-2-pyridone-5-carboxamide	88	1.71	+0.233
Urocanic acid	102	2.11	+0.325
Benzoic acid	111	2.34	+0.371
Nicotinamide	112	2.46	+0.391
Ribosyl-5-amino-4-imidazolecarboxamide	117	2.50	+0.398

tographed on each column. The mean of the differences in K_d for the two lots of Sephadex G-10 was 0.20 ± 0.05 (S.E.) which gave a p value of 0.045 when compared by Student's t-test. The mean of the differences for V_e^0 was only 0.04 ± 0.04 indicating no significant difference in V_e^0 for the two lots.

V_e, V_e^0 and log V_e^0 values for 91 compounds chromatographed on Sephadex G-10 columns have been published[7] and data for 32 additional compounds are presented in Table II. It should be noted that log V_e^0 for non-adsorbed compounds with $V_e = V_0 + V_i$ is —0.056. Substituent Δlog V_e^0 values are defined as the change in log V_e^0 due to the addition of one group on the purine ring, with a positive value indicating increased adsorption and a negative value indicating decreased adsorption[7]. Additional Δlog V_e^0 values for purine ring substituents are given in Table III. The additivity of Δlog V_e^0 values and their use in predicting elution volumes has been described[7].

Table IV lists substituent Δlog V_e^0 values for various ring structures related to the purine ring, with the ring positions numbered similarly. The change in adsorption for a given substituent is in the same direction for the three-ring systems, with the values similar for the purine and pyrazolo[3,4-*d*]pyrimidine rings but not

TABLE III

Δlog V_e^0 VALUES FOR SUBSTITUENTS ON THE PURINE RING

	Group	*Parent compound*	Δlog V_e^0
N_1	Methyl	3-Methylxanthine	—0.026
		7-Methyluric acid	+0.007
		Uric acid	+0.015
C_2	Hydroxyl	1-Methylhypoxanthine	+0.244
	Amino	1-Methylhypoxanthine	+0.323
		6,8-Dihydroxypurine	+0.330
N_3	Methyl	Xanthine	—0.119
		Adenine	—0.293[a]
		1-Methyluric acid	—0.145
	N-Oxide	Guanine	—0.455[a]
C_6	Hydroxyl	2-Hydroxypurine	+0.372
	Seleno	Purine	+0.986
	Benzylamino	Purine	+1.302
	Piperidino(1)	Purine	+1.240
N_7	Methyl	1-Methyluric acid	—0.087
		Uric acid	—0.135
		3-Methylxanthine	—0.182
C_8	Hydroxyl	Theophylline	+0.072
		1-Methylxanthine	+0.137
		7-Methylxanthine	+0.141
		1,7-Dimethylxanthine	+0.181
	Chloro	Xanthine	+0.677
	Nitro	Theophylline	+0.671
	Mercapto	Purine	+0.963
	Methyl	Theophylline	—0.058
N_9	Ribosyl	N^2-Dimethylguanine	—0.273
		6-Methylmercaptopurine	—0.309
		6-Mercaptopurine	—0.354
	Isopropylidene-ribosyl	Adenine	—0.107

[a] Introduces ⊕ in ring.

for the 8-azapurine ring. Also shown in Table IV is the major exception to the rule that substituent $\Delta\log V_e^0$ values are independent and additive. The value for a hydroxyl group at any position on the rings is positive except for the addition of a hydroxyl group at position 2 of a ring with a hydrogen or amino group at position 6. The ring $\Delta\log V_e^0$ values in Table IV indicate that with the exception of 2,6-diamino-8-azapurine, the ring $\Delta\log V_e^0$ values are largely independent of the substituent groups on the rings. Removal of the imidazole portion of the purine ring greatly reduces adsorption as shown by the large negative purine to pyrimidine ring $\Delta\log V_e^0$ of —0.298. Conversion of the imidazole ring of purine to a triazine ring (mean $\Delta\log V_e^0 = +0.229$) or to a pyrazolo ring (mean $\Delta\log V_e^0 = +0.179$) greatly increases adsorption. The ring $\Delta\log V_e^0$ value of +0.319 for the change from imidazole to pyrazole is 1.8 times the value for conversion of purine to pyrazolo[3,4-*d*]-pyrimidine, indicating that the pyrimidine ring of these compounds influences the effects of changes in the five-membered ring.

The effects of substituents on the purine ring on log V_e^0 values suggest that a high electron density in the ring favors adsorption to Sephadex. Uric acid and methylated uric acids which exist as anions at pH 7.0 are strongly adsorbed while those compounds that contain a positive charge due to quaternization of a ring

TABLE IV

$\Delta\log V_e^0$ VALUES FOR VARIOUS RING SYSTEMS NUMBERED ANALOGOUS TO THE PURINE RING

2-Amino group substituent $\Delta\log V_e^0$ values

Parent compound	*Purine*	*8-Azapurine*
6-Amino	+0.293	+0.021
6-Hydroxyl	+0.320	+0.163

2-Hydroxyl group substituent $\Delta\log V_e^0$ values

Parent compound	*Purine*	*8-Azapurine*	*Pyrazolo[3,4-d] pyrimidine*
6-Amino	—0.304	—	—0.380
6-Hydroxyl	+0.189	+0.232	+0.162

Ring $\Delta\log V_e^0$ values

Parent purine ring to	*Pyrimidine*	*8-Azapurine*	*Pyrazolo[3,4-d] pyrimidine*
2,6-Diaminopurine	—	+0.001	—
Guanine	—	+0.156	—
Theophylline	—	+0.272	—
Adenine	—	+0.273	+0.207
Hypoxanthine	—	+0.313	+0.203
Xanthine	—0.333	+0.357	+0.176
Isoguanine	—0.294	—	+0.131
Purine	—0.266	—	—
Mean value	—0.298	+0.228	+0.179
S.E.M.	0.019	0.053	0.017

nitrogen or an amino group have greatly reduced adsorptions. Also, the purines in the lactim form, which are more aromatic, are more strongly adsorbed than are the purines in the lactam form. This is shown by the log V_e^0 values of +0.366 for 6-hydroxypurine (lactam) compared to +0.556 for 6-methoxypurine (lactim) and also +0.851 for 6-mercaptopurine (thione) compared to +0.946 for 6-methylmercaptopurine (mercapto). This increased adsorption is not due to the methyl group on the sulfur or oxygen substituent since the log V_e^0 value for 6-methylaminopurine of +0.826 is only slightly higher than that of +0.805 for 6-aminopurine.

The ring nitrogens or the hydrogen groups on the ring nitrogens of purines in the lactam form are of major importance for adsorption to Sephadex as shown by the large decrease in adsorption when the hydrogen is replaced by a methyl group (Table V). Methyl substitution at the 1 position gives the smallest Δlog V_e^0 value and hence this position contributes little to the adsorption of the purine ring. The values for a methyl group at positions 3, 7 or 9 are comparable indicating that these positions contribute about equally to the adsorption. This decrease in adsorption upon methyl substitution on the ring nitrogens is not a simple steric effect since a methyl group at the 6 carbon has a small positive Δlog V_e^0 value while a methyl group at the 8 carbon has a small negative Δlog V_e^0 value. Assuming that the negative of the methyl Δlog V_e^0 values is a measure of the contribution of the ring nitrogens to adsorption of the purine ring, the negative of the sum of the mean Δlog V_e^0 values for positions 1, 3, 7 and 9, +0.404, when added to the value for a nonadsorbed compound (—0.056) gives +0.348. This value can account for the log V_e^0 value of +0.326 observed for purine.

To obtain further information about the mechanism of adsorption of purines to Sephadex G-10, correlations of elution parameters with the physical properties of the purines were investigated. The appropriate elution parameter is log V_e^0 which is proportional to the logarithm of an adsorption coefficient which in turn is proportional to the difference in standard chemical potential between the molecules in solution

TABLE V

Δlog V_e^0 VALUES FOR METHYL GROUPS ON THE PURINE RING

	Ring position						
	N_1	N_3	N_7	N_9	N^{+a}	C_6	C_8
Mean	—0.048	—0.150	—0.134	—0.172	—0.457	+0.084	—0.058
S.D.	0.044	0.020	0.026	—	0.195	—	—
S.E.M.	0.015	0.009	0.008	—	0.098	—	—
n	8	5	9	1	4	1	1

	Ring position	
	N_6 (*amino*)	N_2 (*amino*)
First methyl	+0.021	—0.041
Second methyl	+0.058	—0.109
Third methyl	—0.326[a]	—

[a] Methyl group causes quaternization of amino group or ring nitrogen.

and those adsorbed to Sephadex. Thus, the following equation relates log V_e^0 and chemical potential:

$$\log V_e^0 = \frac{\mu^0_{liq} - \mu^0_{gel}}{2.33\,RT} + C$$

where μ^0 refers to the standard chemical potentials, R is the gas constant, T is the absolute temperature, and C is a proportionality constant. Since the values of log V_e^0 were determined at the same temperature (room temperature), log V_e^0 increases as the standard chemical potential of the compound on the gel is reduced relative to that of the solution. Differences in the standard chemical potentials of purines are related to their physical properties and therefore correlations between physical properties and log V_e^0 values are expected.

The correlations of the physical properties of the purines with their log V_e^0 values to be described concern mainly methyl, amino, and hydroxyl substituted purines since it appears that these groups exert their effects primarily through changes in the properties of the rings. Purines containing sulfur, halogen, carboxyl and cyano groups consistently failed to show correlations between their physical properties and adsorption to Sephadex. This is probably due to direct interaction of these groups with the gel and therefore these compounds were excluded from the correlations except where noted. It is interesting that the molecular weights of the halogens correlate well with their Δlog V_e^0 values for the 6 position of the purine ring (Fig. 1), and a similar trend is found for the chalcogens. Polarizability of the halogens is proportional to their molecular weights while electronegativity is inversely proportional. Therefore, adsorption of the halogen groups to Sephadex may involve dipole-induced dipole and dispersion forces. BROOK AND MUNDAY[17] have noted that halogen-substituted phenols, anilines and benzoic acids are more strongly adsorbed to Sephadex than predicted by their Hammett σ relations, and that the order of increasing adsorption was F, Cl, Br, I.

The greater adsorption of purines with the lactim structure compared to those with the lactam structure suggested that increased aromatic character might correlate with adsorption to Sephadex. Although few experimental data are available on the electronic structure of purines, an extensive series of molecular orbital (MO) calculations which give approximate values for the electronic properties of purines have been published by the PULLMANS[19,20]. The values used for the correlations with log V_e^0 values were those calculated by the Hückel MO Linear Combination of Atomic Orbitals method, with the imidazole hydrogen assumed to be at the 9 position.

The delocalization energy, E, a measure of the aromatic character of the compounds, was found to give a fair correlation with log V_e^0 for a large number of heterocyclic compounds (Fig. 2). The equation of the regression line with halogen, sulfur, carboxyl and cyano purines included is:

$$\log V_e^0 = -0.544(\pm 0.183) + 0.344(\pm 0.056)E \qquad (r = 0.750;\ n = 31).$$

The correlation between log V_e^0 of purines only and their delocalization energies shown in Fig. 3 gave the following regression equation:

$$\log V_e^0 = -2.00(\pm 0.46) + 0.707(\pm 0.128)E \qquad (r = 0.879;\ n = 11).$$

Restriction of the correlation to purines with the lactim structure gave a very good correlation:

$$\log V_e^0 = -3.12(\pm 0.40) + 0.987(\pm 0.103)E \qquad (r = 0.984;\ n = 5).$$

Inclusion of purines with a single lactam structure had little effect on this correlation. These results indicate that aromatic character is important for adsorption of purines to Sephadex.

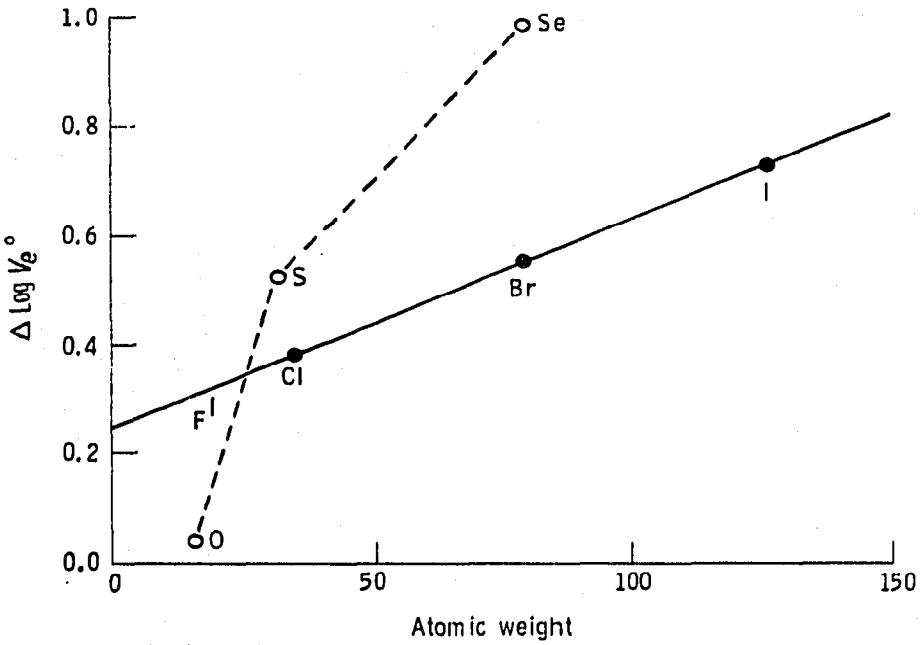

Fig. 1. Relationship between the atomic weights of the halogens (solid line) and chalcogens (dashed line) and their $\Delta \log V_e^0$ values for the 6 position of the purine ring. F^1 is a predicted value.

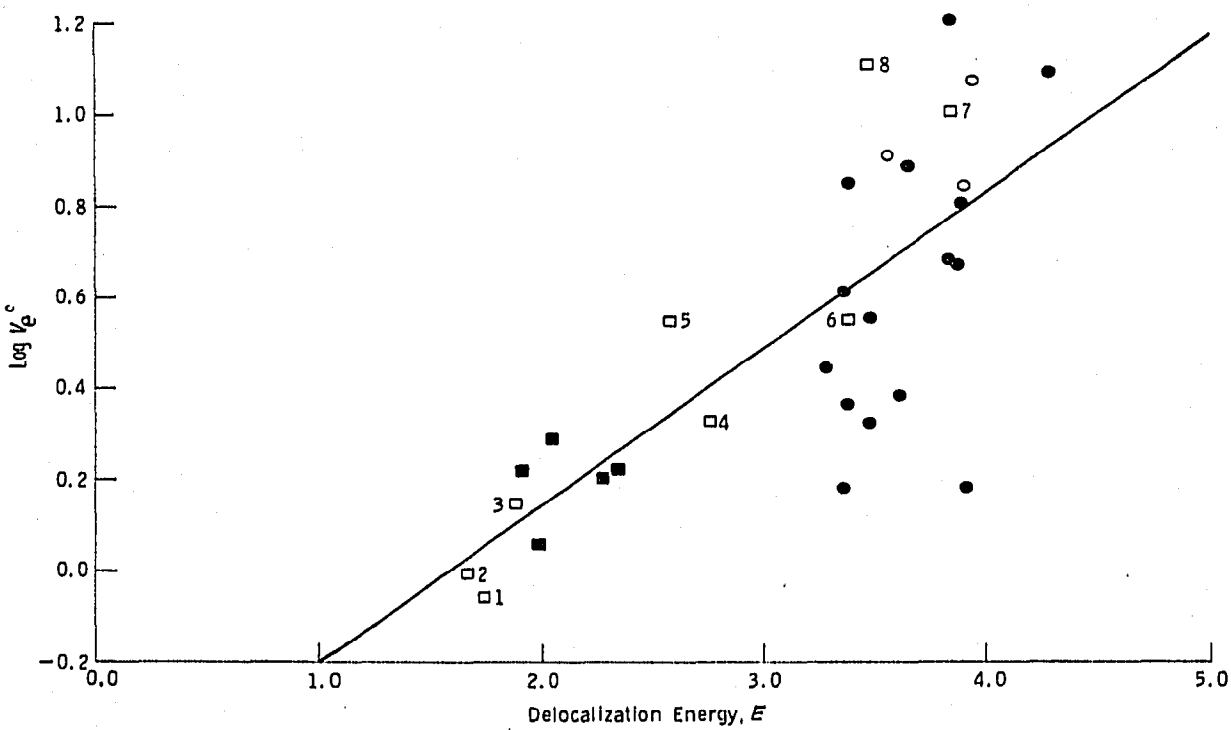

Fig. 2. Correlation between log V_e^0 and delocalization energy, E, for heterocyclic compounds. ●, purines; ○, 8-azapurines; ■, pyrimidines; □ other. 1 = creatinine; 2 = imidazole; 3 = allantoin; 4 = urocanic acid; 5 = 4-amino-5-imidazolecarboxamide; 6 = tryptophan; 7 = 4-aminopyrazolo[3,4-*d*]pyrimidine; 8 = benzimidazole.

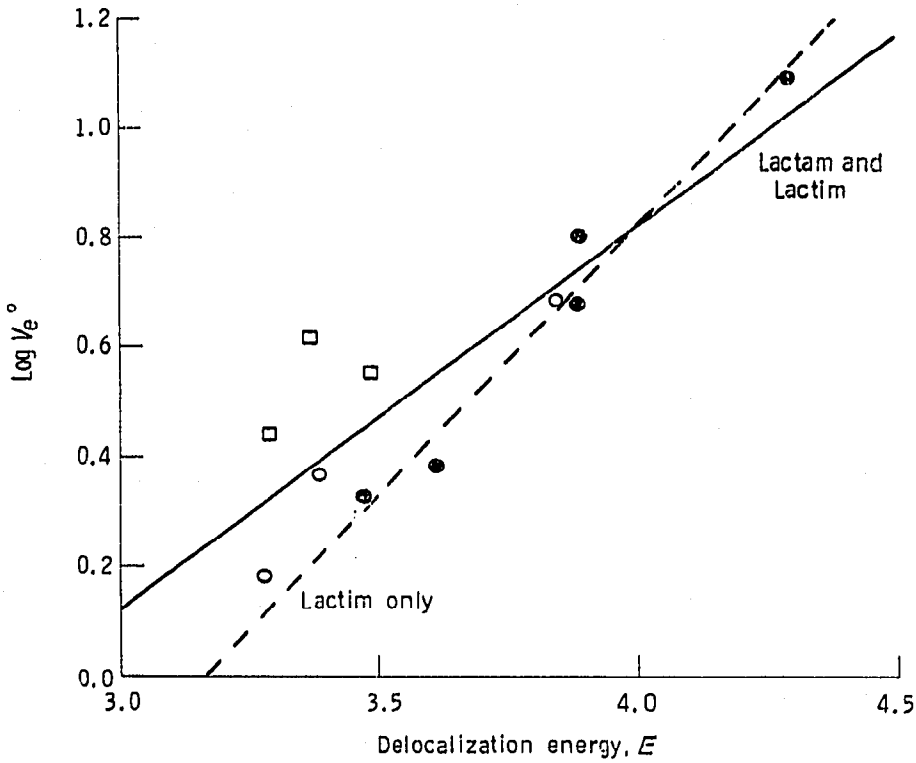

Fig. 3. Correlation between log V_e^0 and delocalization energy, E, for purines. Solid line: ●, lactim; ○, monolactam; □, di- and tri-lactam. Dashed line: ●, lactim only.

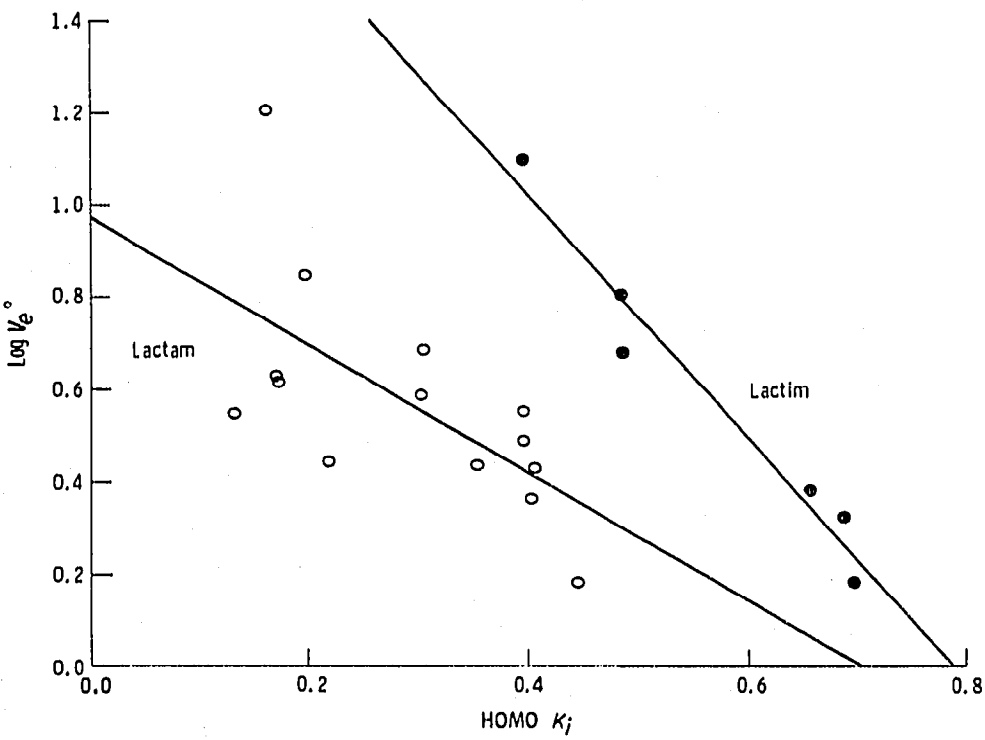

Fig. 4. Correlation between log V_e^0 and HOMO K_i for purines. π-electron donating ability decreases as HOMO K_i increases. ●, lactim; ○, lactam.

Another electronic property obtained from MO calculations is the energy coefficient of the highest occupied molecular orbital, HOMO K_i, which is proportional to the ionization potential of the molecule and inversely proportional to the π-electron donating ability. As shown in Fig. 4, an excellent negative correlation between log V_e^0 and HOMO K_i for purines with the lactim structure was found:

$$\log V_e^0 = 2.08(\pm 0.16) - 2.64(\pm 0.28)\text{HOMO } K_i \qquad (r = 0.977;\ n = 6).$$

However, for purines with one or more lactam structures, the correlation was poorer:

$$\log V_e^0 = 0.978(\pm 0.149) - 1.39(\pm 0.48)\text{HOMO } K_i \qquad (r = 0.642;\ n = 14).$$

In both cases, the slopes were negative, indicating that adsorption to Sephadex increases with increasing π-electron donating ability of the purines. In addition to donating electrons, purines are capable of accepting electrons. The energy coefficient of the lowest empty molecular orbital, LEMO K_i, is inversely proportional to the electron affinity of the molecule, *i.e.*, the electron accepting ability decreases as LEMO K_i becomes more negative. As shown in Fig. 5, increasing adsorption to Sephadex correlated with decreasing electron accepting ability. Again the purines fell into the classes, *viz.* lactim:

$$\log V_e^0 = -2.13(\pm 0.40) - 3.46(\pm 0.51)\text{LEMO } K_i \qquad (r = 0.959;\ n = 6),$$

and lactam:

$$\log V_e^0 = -0.355(\pm 0.240) - 0.837(\pm 0.239)\text{LEMO } K_i \qquad (r = 0.869;\ n = 6).$$

Purines with a lactim structure gave a higher correlation coefficient and a larger slope in the LEMO K_i and adsorption correlation than lactam purines. Thus, the

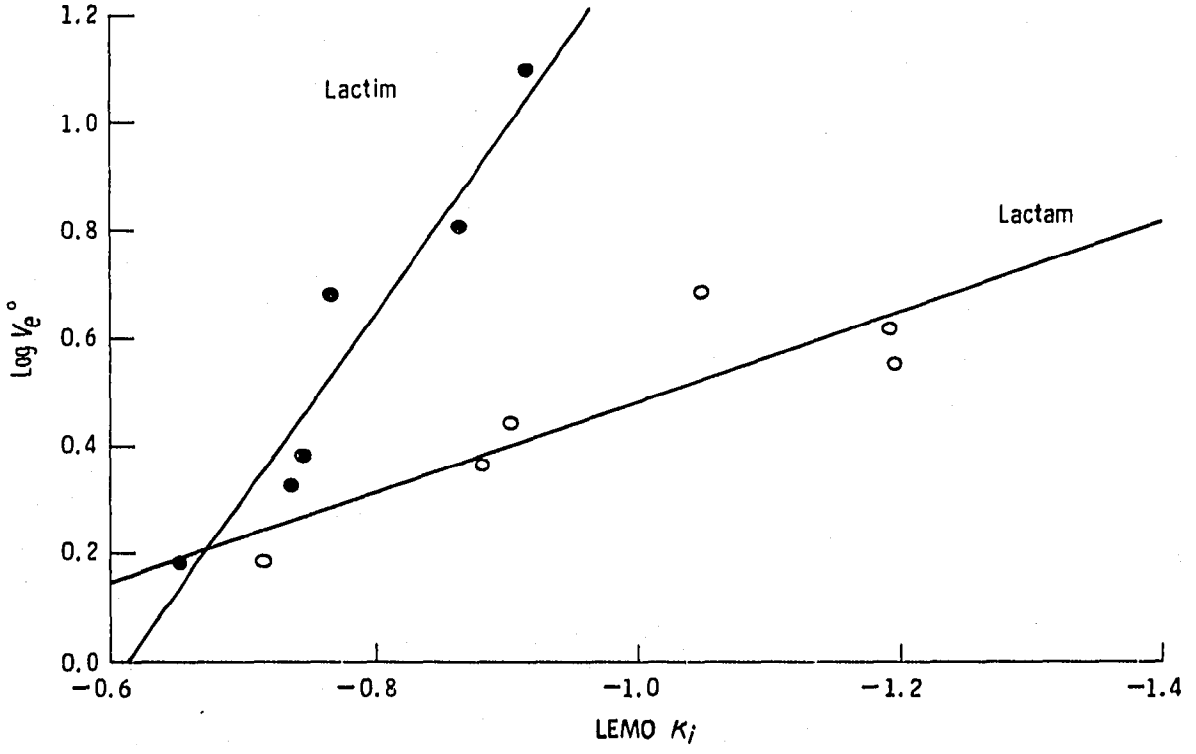

Fig. 5. Correlation between log V_e^0 and LEMO K_i for purines. π-electron accepting ability decreases as LEMO K_i becomes more negative. ●, lactim; ○, lactam.

correlations between the electronic properties of purines and their adsorption to Sephadex indicate that high aromatic character, high electron donating ability, and low electron accepting ability favor adsorption, particularly for purines with the lactim structure.

In addition to these general properties of the π-electrons of the purine rings, the charge density at specific positions of the ring may be of importance, as for example, the unshared electron pairs of the ring nitrogens. The large negative $\Delta \log V_e^0$ values for substitution of a methyl group on the ring nitrogens, especially at the 3,7, and 9 positions, indicate the importance of these nitrogens for adsorption. The basic pK_a values of the purines are proportional to the ease of protonization of the nitrogens and hence proportional to the electron density of the nitrogens. Basic and acidic pK_a values for the purines were obtained from the literature[22-24]. As shown in Fig. 6B, there is an excellent correlation between basic pK_a values and $\log V_e^0$

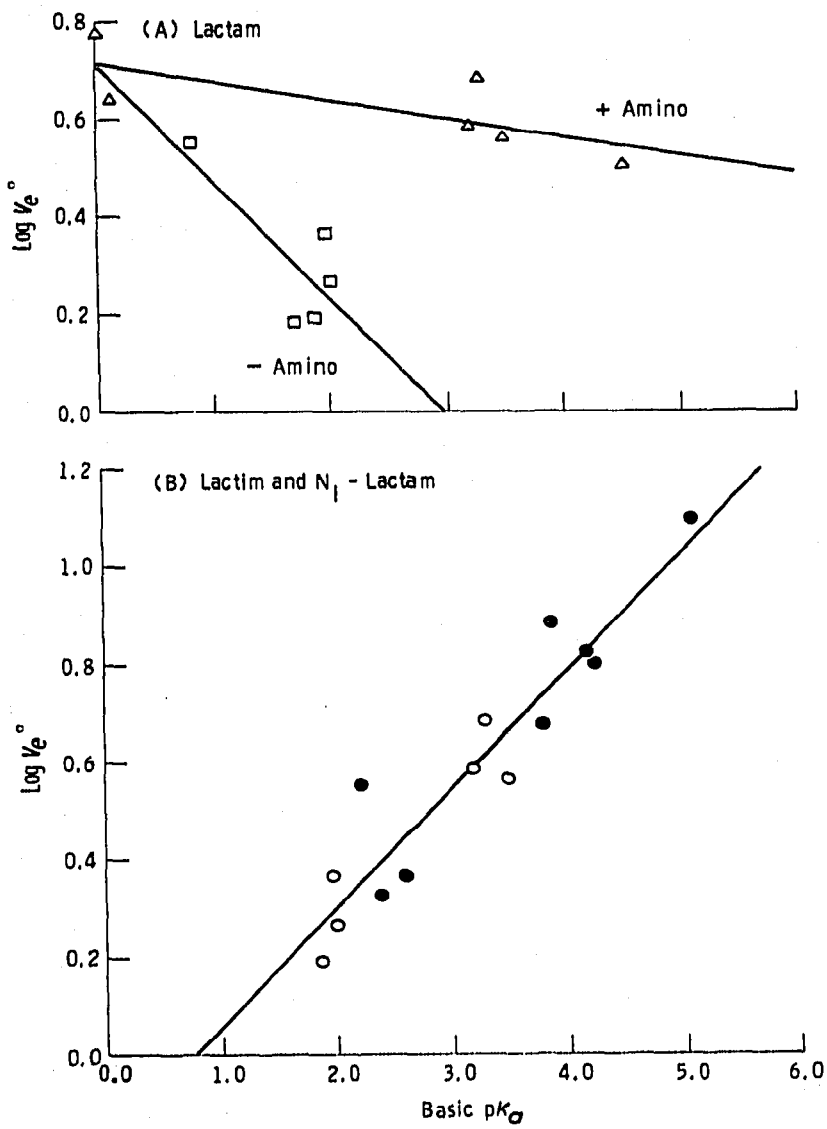

Fig. 6. Correlation between $\log V_e^0$ and basic pK_a for purines. (A) □, lactam without an amino group; △, lactam with an amino group. (B) ●, lactim; ○, N_1-lactam.

values for purines with the lactim structure and those with a single lactam structure involving the nitrogen at the 1 position:

$$\log V_e^0 = -0.189(\pm 0.084) + 0.246(\pm 0.025) \text{ basic } pK_a \quad (r = 0.941;\ n = 14).$$

The inclusion of purines with a lactam structure at the 1 position with the lactim purines is justified by the $\Delta\log V_e^0$ data for methyl substitution, which indicates that the 1 position contributes little to adsorption. However, the purines with one or more lactam structures show a negative correlation between $\log V_e^0$ and basic pK_a (Fig. 6A). For purines without an amino group:

$$\log V_e^0 = 0.705(\pm 0.198) - 0.235(\pm 0.115) \text{ basic } pK_a \quad (r = 0.764;\ n = 5),$$

and for purines with an amino group:

$$\log V_e^0 = 0.718(\pm 0.049) - 0.0378(\pm 0.0164) \text{ basic } pK_a \quad (r = 0.755;\ n = 6).$$

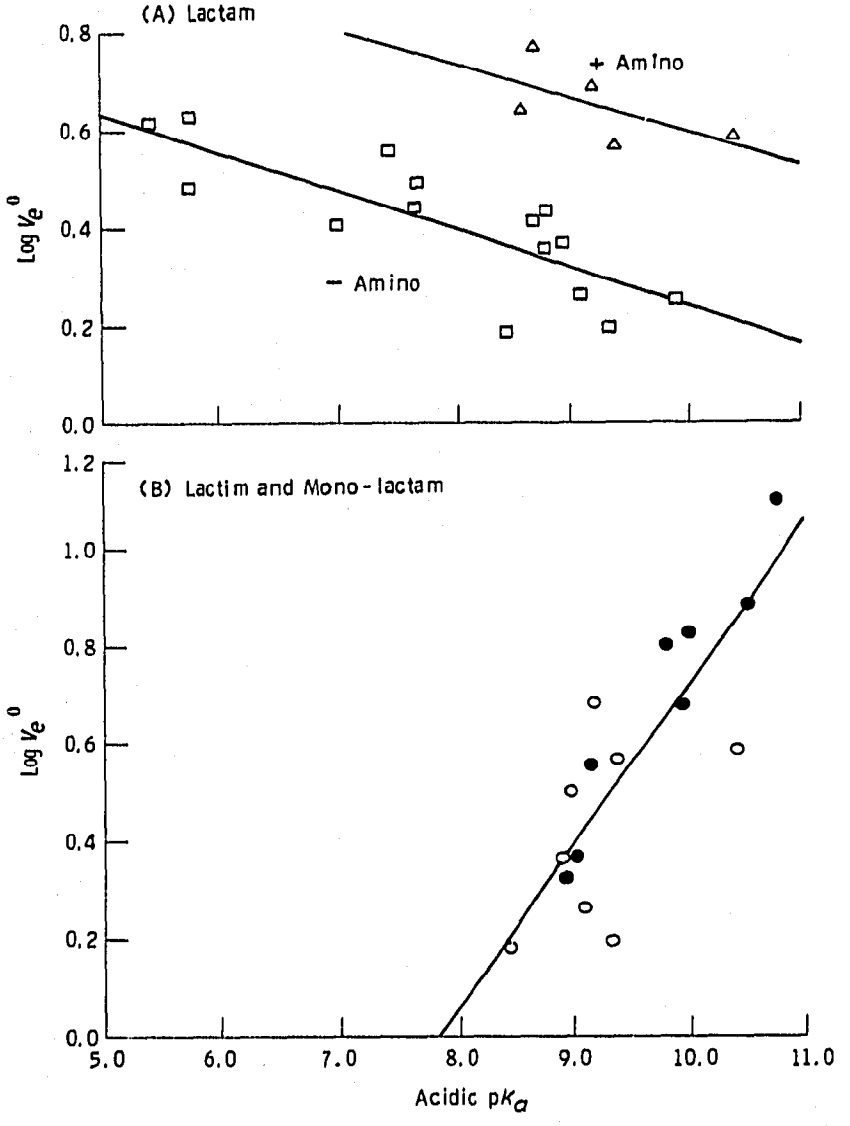

Fig. 7. Correlation between $\log V_e^0$ and acidic pK_a for purines. (A) □, lactam without an amino group; △, lactam with an amino group. (B) ●, lactim; ○, N_1- or N_3-lactam.

The log V_e^0 values were determined at pH 7.0, above the basic pK_a values of the purines, so that actual protonation of the purines is not a consideration in these correlations.

Another measure of the electron density of the ring nitrogens is the anionic pK_a, which is inversely proportional to the ease of removal of a proton from nitrogens with a hydrogen group, and proportional to the electron density of the nitrogens. Fig. 7B shows a good correlation between anionic pK_a and log V_e^0 for purines with the lactim structure and those with a single lactam structure:

$$\log V_e^0 = -2.61(\pm 0.56) + 0.333(\pm 0.059) \text{ anionic } pK_a \qquad (r = 0.834; n = 16).$$

The correlation with purines containing no lactam structure is even higher with $r = 0.959$ ($n = 8$). For purines with one or more lactam structures, there is a negative correlation between anionic pK_a and log V_e^0 (Fig. 7A). For purines without an amino group:

$$\log V_e^0 = 1.03(\pm 0.13) - 0.0794(\pm 0.0164) \text{ anionic } pK_a \qquad (r = 0.802; n = 15),$$

and for purines with an amino group:

$$\log V_e^0 = 1.30(\pm 0.48) - 0.0699(\pm 0.0516) \text{ anionic } pK_a \qquad (r = 0.616; n = 5).$$

Thus, increased electron density on the ring nitrogens, whether measured by increasing basic or acidic pK_a, correlates with increased adsorption to Sephadex for purines with the lactim structure and those with a single lactam structure, but correlates with decreased adsorption for purines with lactam structures. High electron density of purines with the lactim structure may contribute to adsorption by increasing the hydrogen bonding of the nitrogens with unshared electron pairs to the hydroxyl groups of Sephadex. However, for purines with lactam structures, high electron density could decrease adsorption by reducing the hydrogen bonding of lactam hydrogen groups to the oxygen groups of Sephadex.

The preceding correlations of the electronic properties of the purines and their log V_e^0 values reflect relationships between these electronic properties and the difference in standard chemical potentials of the adsorbed molecules and those in solution, *i.e.*, $\mu^0_{liq} - \mu^0_{gel}$. Assuming that μ^0_{liq} is comparable for most purines, adsorption would be related mainly to changes in μ^0_{gel}. However, this assumption may not be valid, and differences in chemical potential could be due to differences in μ^0_{liq} rather than μ^0_{gel}. The standard chemical potentials of purines in solution can be related to their water solubilities. The logarithm of the solubility is inversely proportional to the standard chemical potential difference between the solid and the solution. Assuming that the μ^0 of solid purines are similar, the logarithms of the solubilities are inversely proportional to μ^0_{liq}. Further assuming that μ^0_{gel} is comparable for purines, a negative correlation between log V_e^0 and log solubilities would be expected. For these correlations, the solubilities reported by ALBERT AND BROWN[21] were expressed as μg purine per g water (20°) and were not corrected for molecular weight differences. A good negative correlation between log solubility and log V_e^0 can be seen in Fig. 8, with the regression equation for lactim purines:

$$\log V_e^0 = 1.68(\pm 0.22) - 0.204(\pm 0.046) \log \text{solubility} \qquad (r = 0.840; n = 10),$$

and that for lactam purines:

$$\log V_e^0 = 0.941(\pm 0.114) - 0.156(\pm 0.036) \log \text{solubility} \qquad (r = 0.885; n = 7).$$

When the pyrimidines with lactam structures are included with the lactam purines the correlation coefficient becomes 0.913. The difference between the lines for lactim and lactam purines presumably reflects the lower μ^0_{gel} of the lactim structures with their greater aromatic character. The correlations with solubility suggest that the extent of adsorption of purines to Sephadex is greatly affected by competition between water molecules and the gel surface for the bonding sites of the purines.

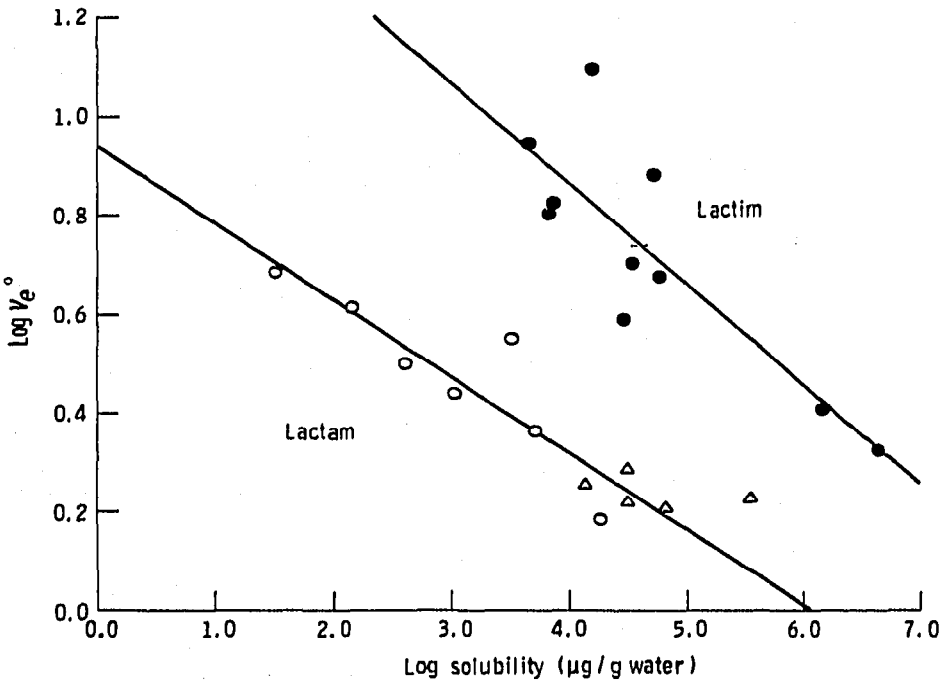

Fig. 8. Correlation between log V_e^0 and the logarithm of the solubility (μg/g water) of purines and pyrimidines. ●, lactim purines; ○, lactam purines; △, lactam pyrimidines.

Turning to the properties of Sephadex which contribute to adsorption of purines the correlations of electron density and adsorption of purines suggest that lactim purines may hydrogen bond to the hydroxyl groups and lactam purines may hydrogen bond to the ether and hydroxyl oxygens of Sephadex. To assess the importance of these groups of the Sephadex G-10 gel for adsorption, purines and heterocyclic compounds were chromatographed on Sephadex LH-20. This is Sephadex G-25 that has been reacted with propylene oxide so that about 60% of the hydroxyl groups have become hydroxypropyl ether groups[25]. Thus, the number of hydroxyl groups is unchanged but the number of ether groups has been greatly increased.

V_e^0 values cannot be used for comparing adsorption to Sephadex G-10 and LH-20 because the two gels have different densities and hence different adsorption capacities per unit column volume and also different internal volumes. Therefore, a new parameter, K_{ad}, was defined which expresses adsorption per unit volume of gel and is independent of column dimensions:

$$K_{ad} = \frac{V_e - (V_0 + V_i)}{V_g} = \frac{V_e - V_e^A}{V_t - V_e^A}$$

where V_g equals gel volume, V_t equals total column volume calculated from the dimensions of the column, and the elution volume of acetone, V_e^A, was used as

$(V_0 + V_1)$. K_{ad} equals zero for a nonadsorbed compound and increases with increasing adsorption. Another function used to compare the two gels was the affinity number, AN, of DETERMANN AND WALTER[18], which expresses adsorption per weight fraction of the dry gel in the gel bed (w):

$$AN = \frac{(V_e/V_e^G) - 1}{w}$$

where V_e^G is the elution volume of glucose. For the calculation of AN, the elution volume of acetone was used in place of that for glucose.

Table VI gives the column dimensions for Sephadex G-10 and LH-20 and the elution parameters for some purines and other heterocyclic compounds. If adsorption of lactim purines and pyridine is due to hydrogen bonding with the hydrogen of the hydroxyl groups of Sephadex G-10, there should be identical adsorption of these compounds to Sephadex G-10 and LH-20 since both gels have the same proportion of hydroxyl groups. Both K_{ad} and AN values indicate that there is no significant difference between adsorption of pyridine and lactim purines to the two gels (Table VI). If adsorption of lactam purines and pyrrole is primarily due to hydrogen bonding with ether oxygen groups, there should be increased adsorption of these compounds to Sephadex LH-20. But, as shown in Table VI, neither xanthine nor

TABLE VI

COMPARISON OF ADSORPTION OF PURINES AND OTHER HETEROCYCLIC COMPOUNDS TO SEPHADEX G-10 AND LH-20

Volume parameter	*Column volumes*			
	G-10		*LH-20*	
	ml	% *of column volume*	*ml*	% *of column volume*
V_t	78.5	100.0	39.3	100.0
$V_0 + V_i$ (V_e acetone)	61.0	77.7	35.4	90.1
V_0 (V_e dextran)	32.5	41.4	14.3	36.4
V_i	28.5	36.3	21.1	53.7
V_g	17.6	22.4	3.9	9.9
w	0.50		0.25	

Compound	V_e (*ml*)		K_{ad}		AN	
	G-10	*LH-20*	*G-10*	*LH-20*	*G-10*	*LH-20*
Lactim purines						
Purine	106	50.0	2.56	3.74	1.48	1.65
Adenine	251	88.4	10.80	13.59	6.23	5.99
Lactam purines						
Hypoxanthine	113	47.9	2.95	3.20	1.70	1.41
Guanine	199	64.8	7.84	7.54	4.52	3.32
Xanthine	156	55.5	5.40	5.15	3.12	2.27
Heterocyclic						
Pyridine	105	50.4	2.48	3.85	1.43	1.70
Pyrimidine	70	38.3	0.52	0.74	0.30	0.33
Pyrrole	173	59.4	6.38	6.15	3.68	2.71
Pyrazole	117	50.7	3.14	3.92	1.84	1.73
Imidazole	73	34.9	0.68	0.00	0.39	−0.06

pyrrole is more strongly adsorbed to Sephadex LH-20. These results suggest that, unlike substituted benzene derivatives, purines and heterocyclic compounds do not interact with the ether groups of Sephadex gels.

DETERMANN AND WALTER[18] have shown by equilibrium dialysis that phenol is adsorbed to polyethylene glycol but not to dextran and, therefore, that the adsorption of phenol to Sephadex involves the ether cross-links and not the dextran polymer. To study further the importance of ether cross-links of Sephadex for adsorption of purines, equilibrium dialysis binding of adenine and xanthine to

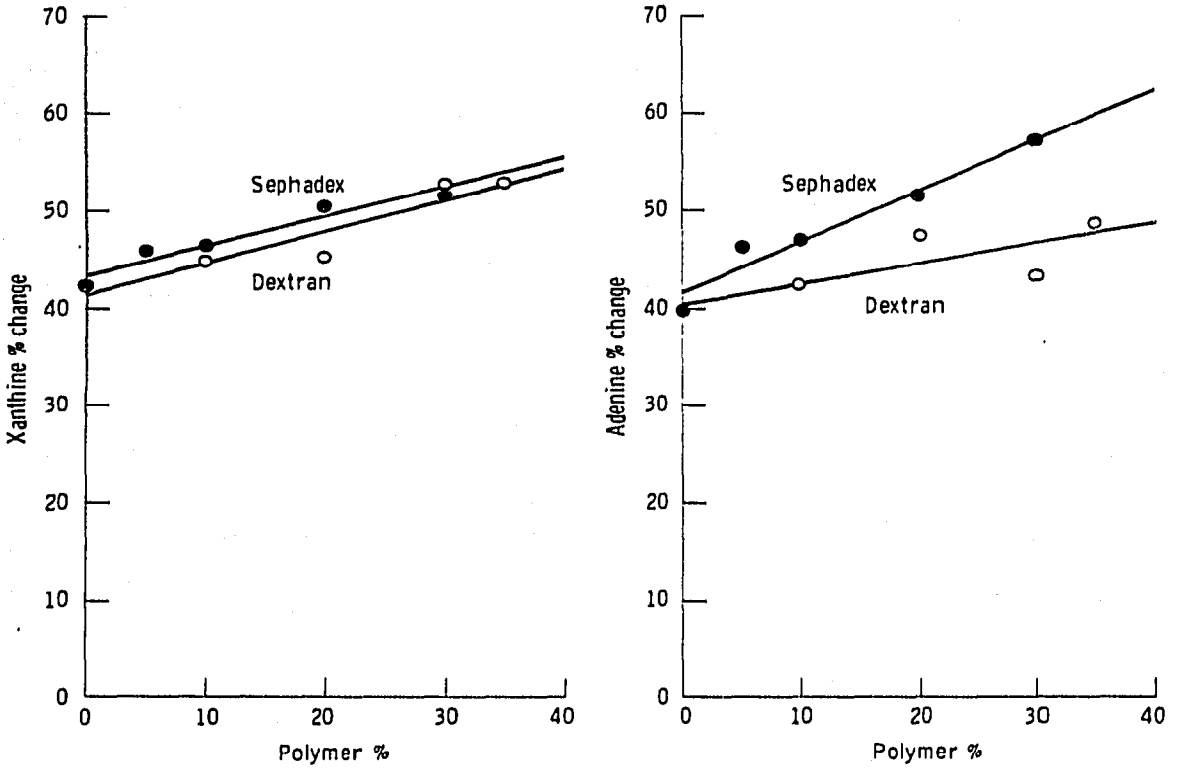

Fig. 9. Per cent change in concentration of xanthine (lactam) and adenine (lactim) after equilibrium dialysis against various concentrations (% w/w) of Dextran T-40 solutions (○) or Sephadex G-10 suspensions (●).

Dextran T-40 and Sephadex G-10 was determined as described in METHODS. Fig. 9 shows the per cent change in concentration of the purines in the dialysis solution as a function of the per cent of polymer in the dialysis tubing. The change in concentration of about 40% for dialysis against buffer is greater than the change of 33% expected from simple dilution, suggesting that the purines were slightly adsorbed to the dialysis tubing. This is consistent with the report of adsorption of uric acid to cellophane[26]. Xanthine was adsorbed equally to dextran and Sephadex, the slopes of the regression lines in Fig. 9 being 0.317 ± 0.066 (S.E.) and 0.297 ± 0.042, respectively. Adenine was adsorbed to dextran to a similar extent with a slope of 0.212 ± 0.091 but was much more strongly adsorbed to Sephadex with a slope of 0.521 ± 0.066. The ratio of the slopes of xanthine over adenine for dialysis against Sephadex G-10 was 0.57. This agreed well with the ratio of V_e^0 for xanthine to adenine of 0.56 from column

chromatography on Sephadex G-10, indicating that comparisons between dialysis and chromatographic results are valid.

These equilibrium dialysis results indicate that, unlike phenol, purines are adsorbed to the dextran portion of Sephadex. The equal adsorption of xanthine to dextran and Sephadex indicates that hydrogen bonding of lactam hydrogens to ether groups does not contribute to the adsorption to Sephadex. However, the stronger adsorption of adenine to Sephadex compared to dextrans suggests that the amino group may form hydrogen bonds with the ether cross-links of Sephadex as does the phenol group.

DISCUSSION

The following properties of purines correlated with increasing adsorption to Sephadex G-10: increasing π-electron delocalization energy, increasing π-electron donating ability, decreasing π-electron accepting ability, decreasing water solubility, and increasing electron density at the ring nitrogens as measured by basic and acidic pK_a for purines with lactim structures, but decreasing electron density at ring nitrogens for purines with lactam structures. These correlations suggest that the mechanism of adsorption of purines to Sephadex involves several types of molecular interactions such as weak hydrogen bonding between π-electrons and hydroxyl groups of Sephadex, hydrogen bonding of nitrogens with unshared electron pairs to hydroxyl groups of Sephadex, hydrogen bonding of purine lactam hydrogens to hydroxyl oxygens of Sephadex and hydrogen bonding of amino groups to ether oxygens of Sephadex. In addition, because of the polarizability of the π-electrons and the dipole moments of purines[27], dipole–dipole, dipole-induced dipole and dispersion forces may also play a role in the adsorption of purines to Sephadex. Furthermore, the correlations of elution volumes with decreasing water solubility indicate that competition between water molecules and the Sephadex gel for bonding sites of the purines plays an important role in chromatography of purines on Sephadex.

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