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THYROIDAL IODIDE TRANSPORT

IV. THE ROLE OF ION SIZE

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SUMMARY

1. The ability of various anions to react with the iodide-transport system of sheep-thyroid slices has been investigated. K_m and K_{ii} values ranged from $3–5 \cdot 10^{-7}$ M to $2 \cdot 10^{-2}$ M to give a series of increasing K values: $TcO_4^- \leqslant ClO_4^- < ReO_4^- < BF_4^- < SeCN^- \simeq SO_3F^- < SCN^- < I^- < NO_3^- \ll NO_2^- < OCN^- \simeq Br^-$.

2. These were compared with the partial molal ionic volumes at infinite dilution, Φ_0, which shows that this series also follows a decreasing order of Φ_0 (with the exceptions of ReO_4^-, SO_3F^- and $SeCN^-$). A linear relation exists between the pK values and the partial molal ionic volumes over the range of 25–46 ml/mole. Although the pK's decline with larger volumes, a clear-cut maximum was not observed. No similar correlation exists between the pK and other size parameters.

3. All of the anions (except TcO_4^-, which was not tested) were shown to be competitive inhibitors of iodide transport by double reciprocal plot analysis.

4. The importance of size, univalency and shape for anion transport in thyroid tissue are briefly discussed in relation to certain physical properties of the ions.

INTRODUCTION

The role of ionic size in anion transport by thyroid tissue has been suspected ever since it was found that the Br^- ion is concentrated to a lesser extent than iodide[1]. It was subsequently found that the halide, $^{211}_{85}At^-$, was well concentrated by thyroid glands[2], whereas the smaller ions Cl^- and F^- were not. The complex anions of periodic

group VIIA, TcO_4^- and ReO_4^-, are also concentrated by thyroid tissue[3,4]. Certain other complex anions had been found to inhibit iodide concentration by thyroid tissue *e.g.* SCN^-, ClO_4^-, NO_3^- (see ref. 5). It was postulated that this inhibitory potential was, in some manner, a function of the position of these ions in the Hofmeister or lyotropic series.

More recently ANBAR *et al.*[6] suggested that the inhibitory properties of the anions were related to their size. They showed that certain complex anions, BF_4^-, SO_3F^- and $PO_2F_2^-$, chosen for their size, could inhibit iodide transport. Furthermore, ClO_4^- and BF_4^- were shown to be concentrated by thyroid tissue[7]. Ion sizes were calculated according to HÜCKEL[8], apparently as multiples of the oxygen or fluorine volumes at the corners of the body-centered tetrahedra. Such size estimates were not, however, in agreement with our finding[4] that TcO_4^- and ReO_4^-, are more avidly concentrated by thyroid tissue than I^-, despite the fact that their volumes would be of approximately the same size ($4 \cdot 10^{-23}$ cm^3) in the above calculations.

In order to decide what sort of size parameter would best describe the size requirement of the anion-transporting system of the thyroid, it is necessary to have some quantitative measure of the anion effects on thyroid tissue. We have, therefore, measured "constants" of the Michaelis type, K_m or K_1, that describe half-saturation of the transport system in the steady state, for a number of univalent anions. These have been compared with certain size parameters taken from the literature or determined by us. By far the best correlation between K values and size exists with partial molal ionic volumes. A preliminary report of these findings has appeared[9].

MATERIALS AND METHODS

Analytical reagents were used unless otherwise indicated. KSeCN (Bios) was purified by acetone extraction at room temperature and crystallization from acetone. Hot acetone and evaporation under vacuum led to decomposition to free selenium in our hands. NH_4BF_4 was made according to BOOTH AND REHMAR[10]. Crude NH_4SO_3F was obtained from the Chemicals Procurement Laboratories, N.Y. It was purified by methanol extraction and crystallization from ethanol to yield a product with a melting point in agreement with the literature (245°). Elemental analysis of the ammonium salt yielded: found: N, 11.88%; F, 16.36%; S, 27.03%; theoretical: N, 11.96%; F, 16.22%; S, 27.38%.

Radioactive iodate was made by oxidation of 25–30 μC of $^{131}I^-$ in the absence or presence of carrier (15 μg KI) in H_2O with Cl_2 gas. This material was then chromatographed[11–13] on Whatman No. 3MM paper by the ascending technique. The position of the band was identified by brief exposure to X-ray film. The appropriate strip was cut out, eluted with H_2O, and concentrated. The yield was essentially quantitative. Control strips with stable iodate were stained with diphenylamine–H_3PO_4 (see refs. 11–13).

Partial molal ionic volumes were taken from the literature[14], or, when not available, they were derived from the densities of their solutions determined in Richardson pycnometers at 25.0°. Volumes at infinite dilution were determined graphically from a minimum of five points per curve according to the empirical equation of MASSON[15]:

$$\Phi = \Phi_0 + S\sqrt{c}$$

where Φ is the apparent molal volume; Φ_0 is the molal volume extrapolated to infinite dilution; S is the slope; and c is the concentration.

Cation volumes were subtracted according to the convention of FAJANS AND JOHNSON[14]. Potassium iodide, NaSCN and NH_4SCN were used to check the method against values in the literature. Iodide ion gave a value of 36.8 ml/mole (literature 36.7 ml/mole) and SCN^- ion was 41.0 ml/mole as compared to 40.6 ml/mole[14]. A detailed description of these data will be reported elsewhere[16].

K_m and K_i values were determined in sheep thyroid slices by methods previously described[4,17,18]. The volume of medium was increased to 5 ml to reduce concentration changes in the medium at the steady state. Equilibration time for iodide in the anion-inhibited slice was checked with SCN^- and ClO_4^- and was found to be well within the 90–100 min incubation period. K_i values were determined at three levels of iodide and five of inhibitor. All points were in duplicate so that a single K_i value was obtained graphically from 30 flasks on the same tissue. Generally, this procedure was followed on four different thyroid glands for each compound. It was assumed that the system behaves as a simple saturatable system[19]. Although this may be too simple a view[20], the K values so obtained are useful for comparative purposes. No corrections were made for extracellular (inulin) space as the gradients (T/M $[X^-]$) were usually quite large. A value of 0.75 was subtracted from the T/M $[X^-]$ for the anion that entered by "diffusion"[17]. K_i values were obtained from plots of the reciprocal of the steady state iodide concentration in the slice *vs.* the inhibitor concentration. The K_m values were obtained graphically from double reciprocal plots as previously described[4,17]. The methods were first applied to thyroidal transport problems by WOLLMAN[21].

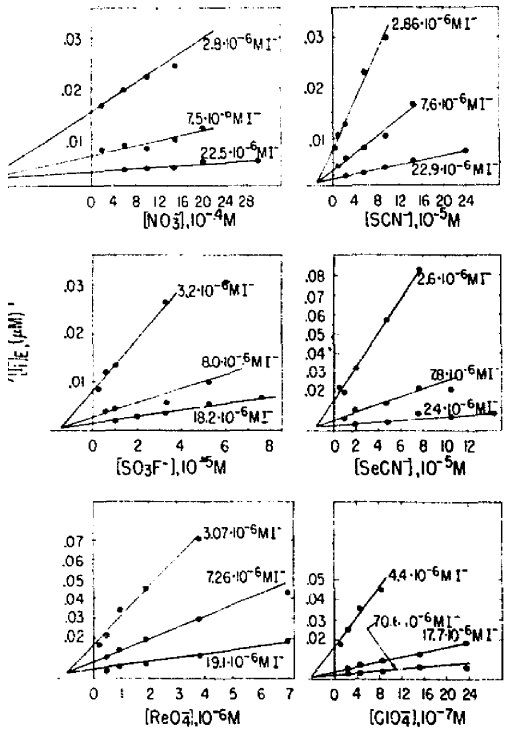
To check for protein binding of anions in the medium, slices were incubated under identical conditions but with a tissue concentration thirty-fold that usually employed (3 g slices/10 ml medium). After 90 min the medium was centrifuged to remove cells. The calculated protein concentration (on the basis of thyroglobulin) was 18.5 mg/ml. This solution was dialyzed against either $3 \cdot 10^{-5}$ M I^- or $4 \cdot 10^{-7}$ M TcO_4^- (in Ringer solution) at 4° with internal and external stirring. Similar experiments were carried out with a 5% solution of "pure" beef thyroglobulin (95% 19-S fraction and 5% 25-S fraction). With the membranes used, >99% equilibration had occurred in 2–3 h.

RESULTS AND DISCUSSION

The nature of the curves obtained by plotting the reciprocal of the iodide ion concentration present in the slices when the steady state is attained against the concentration of inhibitor used is shown in Fig. 1. Iodide values are calculated from the concentration of $^{127}I^-$ added and the fraction of radioactivity remaining in solution when equilibration has occurred (90–100 min). It was assumed that the iodide contributed by the washed slice was not important at the iodide concentrations used. Preliminary studies measuring the $^{127}I^-$ released into the medium[22] suggest this to be the case. In about a fourth of the experiments the data obtained did not yield lines satisfactory for reciprocal plot analysis. The deviations of the points did not show any consistent pattern, however, and the reason for this is, at present, unexplained. Such data were not used.

In Table I are listed the K_m values for I^-, TcO_4^- and ReO_4^-, taken from our

previous work[4,17], as well as K_I values for iodide transport of the several anions chosen for this study because of their size. It is apparent that the range of these "constants" extends over nearly five orders of magnitude, the smaller anions having larger constants than most of the large, tetrahedral ions. Of interest is the finding that for ReO_4^-, the K_m value, using ^{186}Re was identical to a K_I value determined against



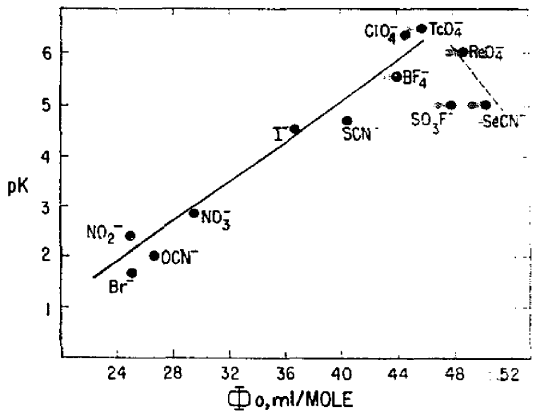


Fig. 2. The relation between the pK (—logK_m or K_I) for anion transport in sheep thyroid slices vs. the partial molal ionic volume at infinite dilution, Φ, of these anions. Iodide and TcO_4^- values are derived from the K_m values, ReO_4^- from both K_m or K_I, and all other anion pK values from the K_I values.

Fig. 1. The effect of various anions on the accumulation of intrathyroidal iodide. Plots are of the reciprocal of the corrected iodide concentration in the slice in the steady state $1/[I_i^-]_{SS}$ vs. the concentration of inhibiting anion. Curves for three different steady state concentrations of iodide in the medium are given. Their intersection determines the K_I value.

iodide ion (Table I). These values agree well with the *in vivo* findings: Br^- ion is poorly concentrated[1], NO_3^- is a poor inhibitor, whereas ClO_4^- has been found to be very potent[5]. They are in satisfactory agreement also with the concentrations required for 50% inhibition of iodide accumulation in the choroid plexus of rabbits[23,24]: ClO_4^-, $2\cdot10^{-6}$ M; BF_4^-, $5\cdot10^{-6}$ M; SCN^-, $3\cdot10^{-5}$ M and NO_3^-, $3\cdot10^{-3}$ M; and in mouse salivary glands: ClO_4^-, $2\cdot10^{-6}$ M; I^-, $3\cdot10^{-5}$ M; and SCN^-, $3\cdot10^{-5}$ M (see ref. 24). The SCN^- values should also be compared with a K_I of $6\cdot10^{-5}$ M obtained by WOLLMAN in the thyroids of intact mice[21]. The slight discrepancy can probably be explained, at least in part, by binding of SCN^- to serum proteins in the intact animals. When the K_m or K_I values are plotted (as the pK's) *vs.* the partial molal ionic volumes, Φ_0, of the anions, a highly significant correlation was obtained (Fig. 2). The maximum pK value was near $3\cdot10^{-7}$ M with TcO_4^-. Since technetium exists only in the radioactive form it was not practical to obtain a partial molal volume for TcO_4^-, and we have used the mean between the MnO_4^- and ReO_4^- volumes. Whether

TABLE I

THE RELATION OF K_m AND K_i TO ANION SIZE

Name	*Transport K_i or K_m* (M)*	*Partial molal volume Φ_v at 25.0° (ml/mole)*	*Volumes of* ANBAR *et al.*[6] *(10^{-23} cm³)*	*Relative hydrated size $K^+ = 1.00$*[25]	*Volumes of* COUTURE AND LAIDLER[26] *(ml/mole)*	*Structure*[6,22]
Bromide (Br^-)	$\sim 2 \cdot 10^{-2}$	25.1**	3.23	0.94	25.0	Spherical
Iodate (IO_3^-)	—	25.1**				Pyramidal
Cyanate (OCN^-)	$1\text{–}2 \cdot 10^{-2}$	26.7***				Linear
Nitrite (NO_2^-)	$4 \cdot 10^{-3}$	25**,20°		1.02	26.5	Angular
Nitrate (NO_3^-)	$1\text{–}2 \cdot 10^{-3}$	29.4**		1.03	29.3	Planar
Iodide (I^-)	*$3 \cdot 10^{-5}$*	36.7**	4.23	0.96	36.6	Spherical
Thiocyanate (SCN^-)	$2\text{–}3 \cdot 10^{-5}$	40.6**		1.11		Linear
Monofluorosulfonate (SO_3F^-)	$1\text{–}2 \cdot 10^{-5}$	47.8***	~ 4			Tetrahedral
Selenocyanate ($SeCN^-$)	$1 \cdot 10^{-5}$	50.3***				Linear
Tetrafluoroborate (BF_4^-)	$3 \cdot 10^{-6}$	44.0***	~ 4	1.12	46.9	Tetrahedral
Perrhenate (ReO_4^-)	*$1 \cdot 10^{-6}$*, $1 \cdot 10^{-6}$	48.7***	4.10	1.34		Tetrahedral
Perchlorate (ClO_4^-)	$4 \cdot 10^{-7}$	44.5**	3.94	1.09	46.4	Tetrahedral
Pertechnetate (TcO_4^-)	*$3\text{–}5 \cdot 10^{-7}$*	46.0§	(4.05)§			Tetrahedral

* K_m values in italics from WOLFF AND MAUREY[4,17].
** From FAJANS AND JOHNSON[14].
*** Determined for this study.
§ Taken as the mean of MnO_4^- and ReO_4^- (see ref. 14).

there is a decline in the pK as the partial molal ionic volumes exceed 46 ml/mole (as suggested by ReO_4^-, SO_3F^- and $SeCN^-$) could not be adequately tested for lack of anions of suitable volume. The discrepant results with SO_3F^- are unexplained at present. Similarly, a "minimum" size is difficult to obtain because the concentrations required are so large as to demand deletion of a large fraction of Cl^- from the medium to maintain osmolarity. Non-specific effects would thus be likely to occur.

Table I also lists various other size parameters. In addition to the partial molal ionic volumes, we have listed, for comparison, the volumes given by ANBAR *et al.*[6], relative hydrated sizes from conductance measurements[25], and the "effective" radii as calculated by COUTURE AND LAIDLER[26]. Neither the values calculated according to HÜCKEL nor those obtained from kinetic measurements show a comparable correlation with the K_m or K_i values. This is in contrast to anion permeability as exhibited, for example, in cat neurones[25] where correlation with kinetic radii is found. It is clear, however, that the empirical equation developed by COUTURE AND LAIDLER[26], which is based on the number of ligands and the "effective" radius (sum of the bond length and Van der Waals radius of oxygen), yields volumes that show close correlation with most of the partial molal ionic volumes determined experimentally and hence also with the K_m or K_i values.

It should be pointed out that the Φ_{20} values were measured at 25° whereas K_m or K_i values were obtained at 37–38°. As the anion volumes show considerable temperature dependence not directly related to the values of Φ_0[14], it might be supposed that the order of sizes differs at 37°. The $d\Phi_{20}/dT$ is in the order $ClO_4^- > NO_3^- > I^- > Br^-$ but amounts to only 0.5–1.5 ml/mole for this temperature difference, hence the order of Table I or Fig. 2 would not be influenced.

There are several uncertainties in these data that deal with (a) possible decomposition of the anions in our system, and (b) possible binding of ions by protein that has leaked out of the slices. Both of these would tend to lower the concentration of anion and hence give falsely high K values.

1. The possibility that BF_4^- or SO_3F^- were inhibitory on the basis of hydrolysis to F^- ion could not be entirely discounted. We therefore measured the effect of F^- on iodide transport in the sheep thyroid slice. There was no significant effect on iodide transport with medium F^- levels as high as $3 \cdot 10^{-3}$ M. Thus the inhibitory effects of these ions cannot be accounted for by their potential content of F^- even if complete hydrolysis occurred. It has been reported that the breakdown of $B^{18}F_4^-$ is negligible[7].

2. WYNGAARDEN *et al.*[5] reported that IO_3^- was an effective inhibitor of iodide accumulation and also stimulated discharge of iodide from the gland. We therefore tested the accumulation of $^{131}IO_3^-$ in sheep thyroid slices. While considerable radioactivity did accumulate in the slices giving an apparent, though somewhat skewed, saturation curve, it was found that all the radioactivity in the thyroid behaved chromatographically[11–13] like iodide. There was no iodate. In the medium different proportions of the radioactivity were in the form of iodate or iodide, depending on the initial concentration of iodate added. It seems likely, therefore, that iodate is reduced (as also shown by LEBLOND AND SUE[27]), and that the accumulation of radioactivity of IO_3^-, and probably its inhibitory effect as well, result from the iodide so formed.

3. Chlorate and bromate were inhibitory for I^- transport with 50% points in the range of 10^{-3}–10^{-4} M. We were unable to obtain reproducible results, and high

levels caused oxidation of the tissue pigments. From their oxidation potentials, which are higher than those of IO_3^- (see ref. 28), they would not be expected to persist; however, we were unable to prove that they were reduced because our chromatographic techniques were not sensitive enough[11–13]. The fact that other strong oxidizing agents, such as $Cr_2O_7^{2-}$ or CrO_4^{2-}, were strongly inhibitory suggests that the effect of ClO_3^- or BrO_3^- may be non-specific. We find it difficult, however, to explain the inhibition *in vivo* reported for ClO_3^- (see ref. 5).

4. A certain degree of uncertainty applies to NO_2^- and OCN^-. The former was slightly less active than NO_3^- but as there was obvious interaction with the pigment of the slices, it may be that the effective concentration differed from the stated one. Sodium cyanate inhibited 50% at $1–2 \cdot 10^{-2}$ M, but the odor of HCN was easily detected in this system, and no accuracy can be claimed for these determinations.

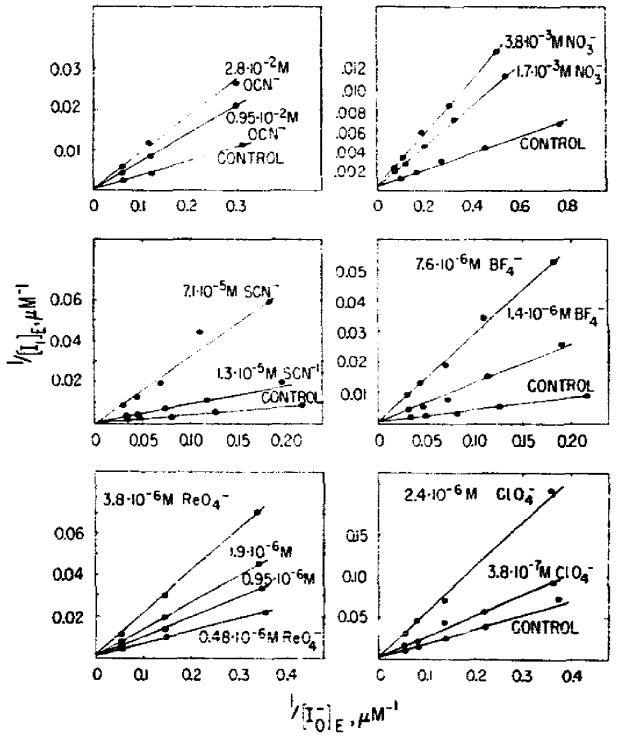


Fig. 3. The competitive nature of anion inhibition of iodide accumulation in sheep thyroid slices. Double reciprocal plots of the corrected steady state iodide concentration in the slice $1/[I_i^-]_E$ (ordinates) *vs.* that of the medium $1/[I_0^-]_E$ (abscissas). Control indicates the absence of inhibiting anions.

5. It is known that albumin, and probably other proteins, can bind these anions. The order of affinities seems to be similar to the present series[29]. It was therefore possible that enough thyroglobulin or other proteins leaked out of the slices to bind the anions and thus lower the concentration of free anions. Although the chloride concentration of the medium would tend to diminish this effect, experiments were carried out to test this directly. The largest gradient in favor of the protein compartment was 1.05 for iodide and 1.12 for TcO_4^-. Since the protein concentrations were

in excess (thirty-fold) of what is usually found in the medium under our conditions, it seems unlikely that the concentrations of free anions was significantly lowered by protein binding.

It seems probable from the evidence so far available that univalency is also a requirement of the system. Sulfate (16.4 ml/mole) and sulfite (9.5 ml/mole) caused no inhibition of iodide concentration at 20 mM. As these ions are of small partial molal ionic volume, decision between valency and size is not possible in this system. However, WO_4^{2-} (26.3 ml/mole), MoO_4^{2-} (29.5 ml/mole) and $S_2O_3^{2-}$ ($\sim$ 34 ml/mole), do fall within the size range of the smaller inhibitory univalent anions. None of these divalent ions inhibited iodide transport at a concentration of 20 mM.

It has been assumed by many authors that the ions studied here compete for the same site on a hypothetical carrier. In general, no evidence has been presented save that (a) SCN^- -goiter is prevented by an increased iodide intake[30] and (b) that I^-, TcO_4^- and ReO_4^- are mutually inhibitory, *i.e.* any one of them will inhibit the transport of any other into the thyroid[2,4]. The only rigorous demonstration for competitive inhibition prior to this study is that for SCN^- ion *in vivo*[21]. Similar criteria (no inhibition, *i.e.* the same maximum capacity, at infinite "substrate" concentration) have been applied in the present *in vitro* system. Fig. 3 shows that in double reciprocal plots the OCN^-, NO_3^-, SCN^-, BF_4^-, ReO_4^- and ClO_4^- ions inhibit iodide transport in a competitive manner. Not depicted, but also competitive inhibitors by this criterion, are the Br^-, NO_2^-, SO_3F^- and SCN^- ions.

COMMENTS

The concept of ionic size is an operational one which depends on the conditions under which it is measured[31]. Since the conditions at the site at which thyroid tissue concentrates anions are unknown, we have attempted to look at several types of ion "sizes" to find the best correlation with a biological transport parameter, in this case the K_m or K_i. Satisfactory correspondence between the transport parameter and size is obtained only when the latter is expressed as the partial molal volume, Φ_0, according to either MASSON[15] or COUTURE AND LAIDLER[26], but not with other expressions of anionic size. The anions studied here fit a series of increasing K values: $TcO_4^- \leqslant ClO_4^- < ReO_4^- < BF_4^- < SeCN^- \simeq SO_3F^- < SCN^- < I^- < NO_3^- < NO_2^- < OCN^- \simeq Br^-$. Such a series will also describe a number of chemical properties of these ions as far as they have been studied: (a) the partial molal ionic volumes at infinite dilution (except for SO_3F^-, ReO_4^- and $SeCN^-$ as discussed above); (b) the Hofmeister series; (c) the hydration energies, *i.e.* the Hofmeister series follows the inverse order of the hydration energies[32]. These can, in turn, be related to the ionic radii[33]; (d) the order of the selectivity coefficients of certain quarternary ammonium ion exchange resins, which can also be correlated with the partial molal ionic volumes of the exchanging anions[34].

It is realized that this size parameter includes effects on the solvent molecules, that this relationship may change during transport of the anion across the cell membrane (*e.g.* solvation by the membrane or carrier), and that interionic effects may not be negligible at a site of concentration. For example, MULLINS[35] had postulated that, for cations at least, a close fitting pore could replace the hydration of the ions, the

size determinant then becoming the crystal radius rather than the hydrated radius obtained from conductance data.

It must be emphasized that while univalency and proper size may be necessary, they are not sufficient conditions for inhibitors of iodide transport. In the series $SO_3NH_2^-$, SO_3F^-, $CH_3SO_3^-$ and $C_6H_5SO_3^-$, only SO_3F^- is significantly inhibitory for I^- transport, and it is out of line with the remaining pK–Φ_0 relationship. Sulfamate (41.4 ml/mole) and probably also methane sulfonate fulfill both the criteria of size and unit charge, but neither compound (nor phenylsulfonate, 106 ml/mole[35]) influenced iodide transport at concentrations as high as 30 mM. Furthermore, neither formate (26.3 ml/mole) nor acetate (40.5 ml/mole) were inhibitory at these concentrations. It is of interest that the correlation between Φ_0 and the selectivity coefficients on Dowex-2 for these two anions does not hold as it does for inorganic anions[34].

The above correlation between pK and Φ_0 neglects structural differences between the ions (Table I) which may be of considerable importance, and may explain the lack of concentration of some of them. Thus, it has been shown that the spherical halides Br^- (see ref. 1), I^- and At^- (see ref. 1), and the tetrahedral ions BF_4^- (see ref. 7), ClO_4^- (see ref. 7), TcO_4^- (see ref. 4), and ReO_4^- (see ref. 4) are concentrated by thyroid tissue as well as being inhibitors of iodide transport. On the other hand SCN^-, a linear anion, is not concentrated to any significant extent[26,27] even though it is a potent inhibitor of iodide transport. The determination of $SeCN^-$ concentration will be an important test of this concept of a different behavior for linear anions. Whether NO_2^- and NO_3^- will also fall into this group remains to be determined. It is of interest in this regard that the planar complexes of trivalent gold $Au(SCN)_4^-$ and $AuCl_4^-$, as well as the linear $Au(CN)_2^-$, are not concentrated by thyroid tissue[28]. The role of the shape of the anions studied here will therefore require further study.

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