THYROIDAL IODIDE TRANSPORT

I. CARDIAC GLYCOSIDES AND THE ROLE OF POTASSIUM

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SUMMARY

Iodide concentrating ability of sheep thyroid slices, measured at approximately 80–90 % equilibrium between intra- and extracellular iodide is depressed by various cardiac glycosides or their aglycones at concentrations of 10^{-7} *M* to 10^{-6} *M*. Quinidine is active at 10^{-4} *M* to 10^{-3} *M*. Beef, guinea pig and human thyroid glands are sensitive in the same concentration range, whereas certain other tissues are very much less sensitive.

Increased extra-cellular concentrations of potassium or rubidium ions can overcome the inhibition produced by ouabain in sheep thyroid slices, and K^+ can similarly reverse the depression in iodide concentrating ability resulting from the leaching of sheep thyroid slices in K^+-free Ringer solution. It is suggested that potassium is required for the establishment and maintenance of a concentration gradient for iodide in thyroid tissue.

INTRODUCTION

One of the important properties of thyroid tissue is its ability to concentrate iodide ion from the surrounding medium against a concentration gradient. Depending on the functional status of the tissue and on the species employed, tissue to medium iodide ratios may be as high as 300 or more. It has been assumed that this gradient is the result of "active" transport of iodide into the cell. The following findings, both *in vivo* and *in vitro*, taken together, may be considered as evidence supporting such an assumption: (a) despite intensive search, no bound form of iodide has been detected which would remove it from the equilibrium with extracellular iodide[1,2], (b) phosphate-bond energy seems to be required, as judged by the inhibition of iodide concentration produced by anaerobiosis and by agents that uncouple oxidative phosphorylation[3,4], (c) the concentrating mechanism exhibits saturation at extracellular iodide concentrations of approximately 10^{-3} *M*[2,5], (d) anions such as SCN^- or ClO_4^- can compete with iodide for the concentrating mechanism[6,7], (e) the thyroidal concentration gradient for iodide is abolished at low temperatures[3] (unpublished observations). Aside from the above factors, little information is available on the mechanism of iodide concentration in thyroid tissue.

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It has recently been possible, by the use of K^+-depleted erythrocytes, to show that K^+ influx can be inhibited by low concentrations of the cardiac glycosides[8-10]. This effect appears[10,11] to be the result of competition between glycoside and K^+. The transport of Cl^- ion in the colon is also inhibited by cardiac glycosides[12]. As a result of such observations we tested the effect of these agents on thyroidal iodide concentration and found recently[13] that glycosides such as digitoxin and strophanthin, or the aglycone strophanthidin, are able to inhibit this process. In the present paper these findings are extended and an attempt is made to show that the depression of thyroidal iodide concentration by the cardiac glycosides is linked to an interference with K^+ movement. The presence of an "adequate" level of K^+ ion is probably required for "active" entry of iodide into the thyroid cell.

MATERIALS AND METHODS

Sheep thyroid glands were obtained at the local abattoir and we should like to thank F. Ensten & Co., Totteridge, London, for their generous co-operation. The glands were kept on ice, trimmed free of extraneous tissue and sliced free-hand. Whenever possible, slices from the same animal were used. In large experiments this was not possible and slices were pooled. Under these conditions the inherent error is about 10 % and for this reason all points were determined in triplicate unless otherwise noted. Human thyroid tissue, obtained at surgery, was made available through the kindness of Drs. D. DONIACH and I. ROITT of the Middlesex Hospital, London. Rat and guinea pig thyroids were obtained from male adults pre-treated for 8–10 days with thiouracil, or for two days with one I.U. subcut. daily of thyrotropin in order to enhance the iodide concentrating efficiency of the small amounts of tissue available. Experiments on salivary tissue were carried out on slices of submaxillary glands obtained from male mice of the C3H or Parkes strains weighing 35–45 g. Conditions were otherwise those used for thyroid tissue. Growing plants of *Fucus vesiculosus* were collected on the chalk cliffs near Rottingdean, Sussex, brought to the laboratory and incubated in sea water containing 10^{-3} *M* methyl mercaptoimidazole but no added iodide at 20° according to KLEMPERER[14]. Otherwise conditions were the same as for animal tissues.

Slices approximately 0.3 mm thick were rinsed for 5 min in iodide-free Ringer solution and were then incubated in a Ringer-phosphate medium pH 7.4 containing: 0.148 *M* Na^+, $4 \cdot 10^{-3}$ *M* K^+, $1 \cdot 10^{-3}$ *M* Ca^{++}, $1.33 \cdot 10^{-3}$ *M* Mg^{++}, $1.33 \cdot 10^{-3}$ *M* $SO_4^=$, $8.6 \cdot 10^{-3}$ *M* phosphate, 0.154 *M* Cl^-, and $1 \cdot 10^{-6}$ *M* I^- (added). Carrier-free ^{131}I iodide was added at approx. 1–2 μC/100 ml. In order to block the further utilization of iodide for hormone biosynthesis, 1-methyl-2-mercaptoimidazole was added to a final concentration of $1 \cdot 10^{-3}$ *M*. Under these conditions > 99.4% of thyroidal radioiodine was iodide as judged by trichloracetic acid solubility, and no organic iodine was found by chromatography. Slices were incubated at 37° with air as the gas phase and shaken at a rate of about 90/min. Incubation was carried out for 75–90 min at which time about 80 % to 90 % of the equilibrium value had been attained (Fig. 1). With very active glands equilibration either did not occur even in 4 h or occurred very late, and experiments with slices of such glands were not used.

Except where noted, 100–150 mg of tissue (wet wt.) were used per 3 ml of medium. After incubation, the slices were blotted and re-weighed. As weight is lost during

the experiment, all results are expressed in terms of the final wet weight and are listed as the ratio of counts of ^{131}I/g of tissue to the counts/ml of medium—hereafter referred to as the T/M [I^-], whose values generally ranged from 20–40. At medium iodide concentrations of 10^{-6} *M* the iodide content of pre-rinsed slices constitutes only a small fraction of the total iodide pool, hence exchange may be neglected and the T/M [I^-], as measured by ^{131}I, represents a meaningful concentration gradient.

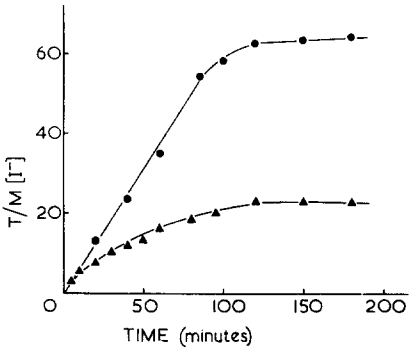

Fig. 1. The time course of iodide equilibration in sheep thyroid slices. T/M [I^-] represents the counts/min/g of slices: counts/min/ml medium. ● & ▲ are thyroids from different sheep.

When control values were less than 10–12, the experiment was discarded (except in the case of mouse submaxillary glands). Results in Tables I and II are expressed as the concentration of drug required to obtain a 50 % depression in the T/M [I^-] as determined from log-dose response of the type shown previously[13].

Ouabain, digitoxin, digitoxigenin, strophanthin K, strophanthidin and α-angelica-lactone were obtained commercially. Lanatoside B was generously supplied by Dr. R. A. ELLIS of Sandoz Products Ltd., London, and dihydrodigoxin through the kindness of Dr. G. H. ACHESON, University of Cincinnati. Except for ouabain, the glycosides were dissolved in ethanol diluted with water and added to the flasks in 0.1 of the final volume to yield a final ethanol concentration of 1–2 %. Control flasks contained equivalent quantities of ethanol. Because of its greater solubility in water ouabain was used in most of the studies. The molarity of ouabain was corrected for 20 % water of crystallisation. The other compounds were assumed to contain no water.

The veratrine alkaloids: veratridine, veratramine, cevadine and protoveratrine were generously supplied by Dr. O. KRAYER, Harvard University. They were dissolved in ethanol and HCl and neutralized to pH 7.4 with 0.1 *M* Tris buffer. Control flasks received equivalent additions. Desoxycorticosterone was the reference standard, eserine sulfate of B.P.C. grade and RbCl and CsCl of Lab. Reagent Grade. De-ionized water with a resistance of approx. 3 megohms/cm was used throughout.

RESULTS AND DISCUSSION

Several different cardiac glycosides are able to reduce the equilibrium T/M [I^-] at concentrations of 10^{-7} to 10^{-6} *M*. As can be seen from Table I, in which glycoside effects on iodide concentrating ability as determined in this study are compared with values from the literature on these effects on K^+ flux, these concentrations are

TABLE I

COMPARISON OF THE INHIBITORY LEVELS OF CARDIAC GLYCOSIDES ON THYROIDAL IODIDE CONCENTRATION AND ON POTASSIUM TRANSPORT IN ERYTHROCYTES

	I_{50} of T/M [I⁻]* sheep thyroid slices	I_{50} of K⁺ transport** human red cells from KAHN[15]	$K_{G\text{-}I}$ of K⁺ transport*** human red cells from SOLOMON et al.[10]
	M	*M*	*M*
Strophanthin K	$2\text{–}5 \cdot 10^{-7}$		
Strophanthidin	$2 \cdot 10^{-7}$	$1.2 \cdot 10^{-7}$	$9.9 \cdot 10^{-8}$
Digitoxin	$2 \cdot 10^{-7}$	$1.6 \cdot 10^{-6}$	
Digitoxigenin	$1\text{–}2 \cdot 10^{-6}$	$1.6 \cdot 10^{-6}$	$2.4 \cdot 10^{-7}$
Ouabain	$3 \cdot 10^{-7}$	$3\text{–}5 \cdot 10^{-8}$	$1.7 \cdot 10^{-8}$
Lanatoside B	$7 \cdot 10^{-7}$		$1.2 \cdot 10^{-6}$
Dihydrodigoxin	$3\text{–}4 \cdot 10^{-5}$	$9 \cdot 10^{-5}$	
α-Angelicalactone	$7\text{–}8 \cdot 10^{-4}$	$2 \cdot 10^{-2}$	

* Concentration required to reduce the 80–90 % equilibrium concentration gradient of $^{131}I^-$ (10^{-6} *M*) to half of the control value at $4 \cdot 10^{-3}$ *M* K^+.

** Concentration inhibiting K^+ loss from plasma into cold stored human erythrocytes to half of the control value.

*** K_{G-1} is the dissociation constant of glycoside from its binding site on the human erythrocyte and is analogous to the K_i used in enzyme inhibition.

TABLE II

THE EFFECT OF OUABAIN ON VARIOUS IODIDE CONCENTRATING TISSUES

Tissue	I_{50} of T/M [I⁻]*
Thyroid	*M*
Sheep	$3 \cdot 10^{-7}$
Beef	$2 \cdot 10^{-7}$
Guinea Pig**	$2 \cdot 10^{-6}$
Rat**	$2\text{–}3 \cdot 10^{-4}$
Human (Thyrotoxicosis)***	$9 \cdot 10^{-8}$
Human (Recurrent thyrotoxicosis)***	$1\text{–}2 \cdot 10^{-7}$
Other	
Submaxillary (Mouse)	$8 \cdot 10^{-4}$
Fronds of *Fucus vesiculosus*	$\gg 3{,}5 \cdot 10^{-3}$

* Concentration of ouabain required to reduce the T/M [I⁻] to one half of the control value.

** Curves on duplicate points. 10–15 mg of thyroid tissue/3 ml of Ringer solution.

*** Single experiment, points determined in triplicate.

comparable to those required to reduce K^+ influx to K^+-depleted erythrocytes*, but a more detailed inspection reveals that the spread in relative potencies is not nearly so great in the case of the thyroid cell as it is with the red cell. The aglycones,

* The values of KAHN[15] were calculated on the assumption that no change in K^+ concentration represents 100 % inhibition. If they had been corrected for reverse flux they would have to be increased by a factor of 1.7. Thus the 50 % inhibition point for ouabain becomes $6.8 \cdot 10^{-8}$ *M*. When the dissociation constants of SOLOMON *et al.*[10] are converted to 50 % inhibitory concentrations, these values will rise from a K_{G-1} of $1.7 \cdot 10^{-8}$ to a 50 % point of $5.6 \cdot 19^{-8}$ *M*. ^{131}I was measured as the equilibrium T/M [I⁻] in the present study, hence such corrections may not be directly applicable. Since it has not been possible to reduce the T/M [I⁻] to less than 1–2, such corrections would lower the values for 50 % inhibition by approx. 20 %.

strophanthidin and digitoxigenin, are also effective, although the latter is less potent by an order of magnitude.

Unsaturation in the α-angelicalactone ring appears to be of importance since dihydrodigoxin, in which the α-β double bond is reduced to yield the γ-butyrolactone derivative, is less potent by two orders of magnitude. GLYNN[11] has also shown that reduction of the lactone (a 2-pyridone derivative) of *Scillaren A* leads to considerable diminution in inhibitory activity on K^+ transport. Other deviations in the response of the T/M [I^-] are seen in the inhibitory effect of the steroid-free lactone, α-angelicalactone which is 25 times more active on the thyroid gland than in erythrocytes. However, the higher concentrations of this material tended to bleach the thyroid slices and the effect may well be of a different nature from that of the glycosides.

Since it has been postulated that quinidine acts by way of an interference in K^+ movement (K^+ efflux from the natrium[16] and K^+ influx in cold-stored erythrocytes[17]), it was of interest to test it in the present system. The concentration required for a 50% depression of the T/M [I^-] was $3 \cdot 10^{-4}$ *M*, an inhibitory potency of the same order of magnitude in the above systems.

Of the other compounds investigated some of the veratrine alkaloids were slightly active at the single high concentration tested ($2.6 \cdot 10^{-4}$ *M*): veratramine 28%, veratridine —9%, cevadine 10% and protroveratrine 17% of the control values. These findings are similar to those of KAHN AND ACHESON[8] who found these compounds to be inactive on cation transport in erythrocytes at somewhat lower concentrations ($1.5 \cdot 10^{-5}$ *M* to $2 \cdot 10^{-4}$ *M*). $2 \cdot 10^{-4}$ *M* deoxycorticosterone was inactive in agreement with the results of GLYNN on red cell K^+ transport[11] and $6 \cdot 10^{-4}$ *M* eserine led to a slight increase in the T/M [I^-].

A comparison was made of thyroids of various species as well as of certain other tissues known to concentrate iodide. As can be seen in Table II, thyroid from a number of species other than the sheep possess sensitivities to ouabain in the same range of concentration. The most sensitive gland was that from a 35-year old woman with thyrotoxicosis. Surprisingly, rat thyroid tissue was quite resistant to ouabain, a finding which raises doubts about the difference in the concentrating mechanism of a rat thyroid tumor recently postulated[4] from that of normal tissue because comparison was made with sheep tissue.

Since mouse salivary tissues can concentrate iodide[18] the marked resistance of mouse submaxillary slices to ouabain was unexpected. It was impossible to obtain slice medium ratios greater than 4 in mice of the C3H or Parkes strains, but it is doubtful that this can explain the lack of effect. Iodide concentrating ability in the brown alga, *Fucus vesiculosus*, was not inhibited by high concentrations of ouabain despite T/M [$^-$] value of 100 or greater. Because of the high K^+ levels (see below) in sea water, experiments were carried out with sea water diluted 1:2 with K^+-free Ringers solution—again ouabain was without effect. Whether or not the findings on non-thyroid iodide concentrating tissues imply a different concentrating mechanism remains a moot point. Preliminary experiments with ^{42}K indicate that ouabain produced roughly similar degrees of inhibition of entry of ^{42}K or ^{131}I into the various tissues tested. Thus, although net K^+ fluxes were much greater than iodide fluxes since the external K^+ concentration was about 4000 times that of the I^- concentration, 50% inhibition for ouabain occurred at about $3 \cdot 10^{-7}$ *M* for both ^{42}K and ^{131}I, and between 10^{-3} *M* and 10^{-4} *M* in mouse submaxillary slices for both isotopes.

^{42}K entry into *Fucus vesiculosus* was not influenced by $3.3 \cdot 10^{-3}$ *M* ouabain, corresponding to the lack of effect on ^{131}I transport. The results of Table II therefore suggest variations in tissue sensitivity to ouabain and conclusions about alternative iodide concentrating mechanisms are not warranted.

The relation of the iodide concentrating ability to K^+ transport

The above results emphasize the marked similarity between glycoside inhibition of erythrocyte K^+ transport and of thyroidal iodide concentration. Since GLYNN[11] and SOLOMON *et al.*[10] had shown that the glycosides probably act by competitive inhibition with K^+ on the transport site, attempts were made to see if K^+ ions could overcome the depression in the T/M $[I^-]$ produced by ouabain. Accordingly, sheep thyroid slices were incubated with progressive increments of K^+. As can be seen from Fig. 2, K^+ readily restored the T/M $[I^-]$ that had been depressed by ouabain. In view of the fact that K^+ increased iodide concentration to some extent in thyroid slices not treated with ouabain (Table IV) the corrected slope would be somewhat lower.

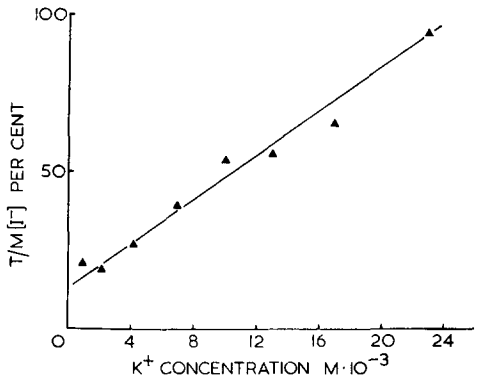

Fig. 2. The effect of extracellular K^+ concentration on the inhibition by $6.4 \cdot 10^{-7}$ *M* ouabain of iodide concentration in sheep thyroid slices. T/M $[I^-]$ per cent denotes the proportion of the control value (no ouabain) of the counts/min/g in sheep slices: counts/min/ml in the medium. Controls determined at $5 \cdot 10^{-3}$ *M* K^+. Incubation time: 90 min.

Direct "competition" between iodide and ouabain could not be demonstrated, in fact, the depression of the T/M $[I^-]$ appeared to increase slightly with increasing iodide load. Thus at 10^{-6} *M* added NaI, $3.2 \cdot 10^{-7}$ *M* ouabain led to a 53 % depression below the ouabain-free preparation and at $2 \cdot 10^{-5}$ *M* NaI to a 66 % depression in the T/M $[I^-]$. Attempts were made to determine whether or not other cations could likewise overcome ouabain inhibition in view of the known ability of Rb^+ and Cs^+ to replace K^+ in certain systems. It can be seen (Table III) that Rb^+ can replace K^+ in overcoming ouabain inhibition, that Cs^+ has a slight or questionable effect, but that none of the other cations added to the Ringer solution have any effect. This recalls the ability of these ions to replace K^+ in certain other systems where membrane phenomena are involved [19,20] and differs from the replacement of K^+ in enzyme systems[21–23] where the NH_4^+ ion usually has high activating activity.

While these findings suggest a competition between ouabain and K^+ for the depression of the T/M $[I^-]$, it must be remembered that K^+ also enters the cell by several processes, (*e.g.* diffusion), which are not sensitive to the cardiac glycosides[10–11].

TABLE III

EFFECT OF MONOVALENT CATIONS ON THE INHIBITORY ACTION OF OUABAIN ON THYROIDAL IODIDE ACCUMULATION

Sheep thyroid slices incubated in Ringer solution containing $1 \cdot 10^{-6}$ *M* NaI and $8 \cdot 10^{-7}$ *M* ouabain. All salts were added as the chlorides, to a concentration of 0.02 *M* of the added salt.

Additional cation	*Per cent of control T/M [I⁻]**
None (Control – no ouabain)	100
None	28
Li^+	27
NH_4^+	26
Na^+	37
K^+	96
Rb^+	90
Cs^+	41

* Ratio of ^{131}I in 1 g of thyroid tissue to the ^{131}I in 1 ml of medium.

TABLE IV

THE EFFECT OF HIGH EXTRACELLULAR K^+ LEVELS ON T/M $[I^-]^*$ DEPRESSION IN SHEEP THYROID SLICES

Additions	*T/M [I⁻]* at $3.6 \cdot 10^{-3}$ M K+*	*T/M [I⁻]* at $19.4 \cdot 10^{-3}$ M K+*
None	41.4	48.3
$3.1 \cdot 10^{-3}$ *M* DNP**	2.6	3.1
$3 \cdot 10^{-4}$ *M* ClO_4^-	1.1	1.1
$6.4 \cdot 10^{-7}$ *M* Ouabain	6.4	55.2
$8.3 \cdot 10^{-5}$ *M* DNP**	2.0	2.3
$1 \cdot 10^{-4}$ *M* ClO_4^-	1.2	1.2
$6.4 \cdot 10^{-7}$ *M* Ouabain	6.4	27.4

* ^{131}I/g of thyroid tissue: ^{131}I/ml of medium.
** 2,4-dinitrophenol.

The possibility thus remained that high extracellular K^+ concentrations could lead to enough K^+ diffusion to permit apparently normal I^- transport. If this were the case, then it should be possible to overcome T/M $[I^-]$ depression produced by other agents such as DNP but not that produced by agents that compete "directly" with iodide *e.g.* SCN^- or ClO_4^-. High extracellular K^+ levels were able to increase the DNP depressed T/M $[I^-]$ only slightly and did not influence the ClO_4^- effect (Table IV). In contrast, ouabain inhibition of the T/M $[I^-]$ was reversed by K^+. It is not likely therefore that simple diffusion contributes a large fraction to the K^+ reversal of ouabain inhibition of the T/M $[I^-]$.

It might be argued that the effect of the ouabain is merely one of depressed rates and that the T/M $[I^-]$ would approach the control value upon prolonged incubation. However, the depression in the T/M $[I^-]$ was identical at 75 and 180 min, suggesting that ouabain in some way alters the equilibrium between intra- and extra-cellular iodide.

Attempts to study the T/M $[I^-]$ in the absence of K^+ were successful in only about

half the cases. Incubation in K^+-free Ringer solution caused only a slight lowering in iodide-concentrating ability. It seemed possible that under these conditions enough K^+ leaked out of the thyroid slices to supply the "required" amount of K^+. Slices were therefore leached for four successive 15 min-periods in K^+-free Ringer solution at 20° and were then incubated at 37° in K^+-free Ringer solution to which varying amounts of K^+ had been added. It can be seen from Table V that this procedure reduced the T/M $[I^-]$ to about 60 % of the control value. While other materials besides K^+ were, no doubt, also leached out, increasing levels of added K^+ restored the T/M $[I^-]$ to the control value. It is not known why this procedure failed to demonstrate a K^+-dependence in half the experiments; it may possibly due be to failure to wash out sufficient intra-cellular K^+.

TABLE V

THE EFFECT OF LEACHING AND K^+ REPLACEMENT ON IODIDE CONCENTRATION IN SHEEP THYROID SLICES

*K^+ replacement**	*T/M $[I^-]$*
—	12.2
$1 \cdot 10^{-5}$ *M* K^+	15.2
$2.5 \cdot 10^{-4}$ *M* K^+	17.4
$5 \cdot 10^{-3}$ *M* K^+	21.6
$5 \cdot 10^{-3}$ *M* Control (Not leached)	19.0

* Slices were leached in K^+-free Ringer's solution for four 15-min periods at 20° and were then incubated at 37° for 90 min in Ringer's solution to which varying concentrations of K^+ had been added.

COMMENT

In the present study it has been found that K^+ ions are involved in the establishment and maintenance of a concentration gradient for iodide in thyroid tissue. This conclusion is based on the following evidence: (a) Known inhibitors of K^+ transport in other tissues (cardiac glycosides, quinidine) depress thyroidal iodide concentrating ability; the T/M $[I^-]$, at similar concentrations (Table I and II), (b) ouabain inhibits ^{131}I and $^{42}K^+$ entry into various tissues to roughly the same extent despite widely different sensitivities of these tissues to the inhibitor, (c) ouabain depression of the T/M $[I^-]$ can be overcome by increasing the K^+ concentration of the medium but not by increasing the I^- concentration (Fig. 1, Tables III and IV). (d) The depression in the T/M $[I^-]$ produced by leaching sheep thyroid slices in K^+-free Ringer solution is reversed by K^+ replacement (Table V). The latter two findings would, moreover, tend to rule out a similar glycoside effect on two otherwise independent processes.

If we assume that K^+ is intimately involved with iodide transport and the maintenance of the T/M $[I^-]$ a number of questions arise concerning its role in this process: (a) K^+ transport appears to be linked to an opposing Na^+ transport in a number of systems and low concentrations of extra-cellular K^+ may be required for Na^+ efflux[11,24,25]. It is clear therefore that in the present system, Na^+ efflux must be considered as a possible factor in the transport of iodide. It is also possible that the intra-cellular level, *per se*, of these ions is important. (b) High intra-cellular and low extracellular K^+ concentrations could establish a potential difference which would

lead to the transport of I^-. In the case of Cl^- transport across frog skin, USSING[26] has shown that the potential difference established by Na^+ is adequate to explain the observed movement of Cl^- ion. The mere operation of an electro-chemical potential cannot, however, be the only driving force for iodide transport since the smaller Br^- ion (Crystal radii: $Br^- = 1.95$ Å, $I^- = 2.16$ Å[28]; and the partial ionic molal volumes in dilute aqueous solution are: $Br^- = 25.1$ cc/mole and $I^- = 36.7$ cc/mole[29]). shows only very slight concentration in thyroid tissue[27]. An additional objection is that saturation of the iodide concentrating mechanism would not be expected at the relatively low absolute concentrations of iodide usually found. (d) The T/M $[I^-]$ has been used here as a convenient screening method for the inhibitory potencies of the cardiac glycosides. As it represents 80–90 % of the equilibrium state, conclusions about iodide flux are complicated by the non-linear iodide accumulation and can hence be compared only indirectly to the K^+ fluxes in erythrocytes. It is hoped that studies now in progress on the kinetics of the system, and on the stoichiometry between K^+ and I^- will allow direct comparison and shed light on the above problems.

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