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# THYROIDAL IODIDE TRANSPORT

## III. COMPARISON OF IODIDE WITH ANIONS OF PERIODIC GROUP VIIA

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SUMMARY

The present study offers evidence that the $TcO_4^-$ and $ReO_4^-$ ions, like iodide, are concentrated in sheep thyroid slices by a mechanism that requires cellular integrity, metabolic energy, and $K^+$ ion. The $K_m$ values are smaller than for iodide, being about $3 \cdot 10^{-7}$ *M* for $TcO^{4-}$ and about $1 \cdot 10^{-6}$ *M* for $ReO_4^-$ as compared to about $3 \cdot 10^{-5}$ *M* for $I^-$. The ions are concentrated unchanged, whereas the lower homologue $MnO_4^-$ was reduced in this system. $Mn^{2+}$ ion is concentrated but does not appear to require cellular integrity, metabolic energy or $K^+$, and appears thus to be concentrated by a quite different system.

The transport mechanism for $TcO_4^-$ and $ReO_4^-$ is similar to $I^-$ not only in the requirements for cellular integrity, $K^+$ and metabolic energy but also in that it is inhibited to the same extent by "competing" ions such as $ClO_4^-$, $SCN^-$ and $BF_4^-$. Furthermore, $I^-$, $TcO_4^-$ and $ReO_4^-$, are mutually inhibitory for transport in the thyroid slices. It is concluded that most, if not all, of the $I^-$, $TcO_4^-$ or $ReO_4^-$ concentrated in thyroid tissue is transported by the same mechanism.

### INTRODUCTION

Although iodide is probably the only halide metabolized by the intact mammalian thyroid cell, several reports have appeared that some other members of the VIIth group of the periodic table also can be concentrated by thyroid tissue. The two halides adjacent to iodide, *i.e.*, bromide and astatide, have been found to accumulate in thyroid tissue[1–7]. In contrast, there appears to be little chloride concentration, the thyroidal level being a function of the amount of colloid (extracellular space) present[8–10]. The non-halogen members of the VIIth periodic group (Group VIIA), Mn, Tc, and Re are, however, concentrated in thyroid tissue—the latter two presumably as the per-oxyanions pertechnetate and perrhenate, $TcO_4^-$ and $ReO_4^-$ (see refs. 11–15). Because of our interest in the parameters that describe the iodide-concentrating mechanism, "Michaelis constants" ($K_m$) of these ions and their behavior toward various inhibitors have been studied in thyroid and salivary slices.

## MATERIALS AND METHODS

Slices and media were prepared as described previously[16]. Pilot experiments revealed that >90% of the steady-state value had been attained for $I^-$, $TcO_4^-$ and $ReO_4^-$, if incubation was carried out for 100 min, and this procedure was followed throughout. Tissue concentrations for reciprocal plot analysis were obtained from the relation

$$[X_t^-]_E = (T/M\,[I^-] - D)\,[X_o^-]_E$$

where $[X_t^-]_E$ is the anion concentration in the tissue at the steady state, $[X_o^-]_E$ is the steady state medium concentration, and $D$ is the correction for "diffused" anion taken as 0.75 for thyroid slices and 0.68 for salivary slices.

The $^{99}TcO_4^-$ concentration was assumed to be that stated by the supplier, Oak Ridge National Laboratories. $^{186}ReO_4^-$ was obtained from the same source at specific activities > 13000 mC/g. We occasionally encountered poor $ReO_4^-$-concentrating ability with older samples of this ion. We attribute this to the high $NO_3^-$ content (1–3 $M$ $HNO_3$) with which this material is supplied[17]. With the dilution possible in samples that had not decayed extensively this effect was not troublesome. Stable $KReO_4$ and $NaReO_4$ of high purity were obtained from A.D. MacKay, Inc., N.Y. Carrier-free $^{54}Mn^{2+}$ as $MnCl_2$ was obtained from the Nuclear Science and Engineering Corp., Pittsburgh, Pa.

Tissue homogenates were prepared with distilled water and dialyzed in stretched Visking Sausage casing (18/32) for 20 h at 4–5°. Other conditions are listed with the respective tables.

## RESULTS AND DISCUSSION

One of the difficulties that has beset comparisons of the concentrating ability of thyroid tissue for these anions has been the specific activity of the radioactive available materials. This is particularly true of $^{82}Br^-$ ion where the specific activity tends to be low. We therefore attempted to compare $I^-$, $TcO_4^-$ and $ReO_4^-$ at comparable concentrations. It soon became apparent that the T/M ratios of $TcO_4^-$ and $ReO_4^-$ ($T/M[TcO_4^-]$ and $T/M[ReO_4^-]$ respectively) were generally greater than that for iodide when the specific activities were high, but decreased as a function of concentration before the $T/M[I^-]$. This suggested that the ratios were not being measured at identical positions on the T/M *vs.* concentration curves. In other words, the constants, $K_m$* for $TcO_4^-$ and $ReO_4^-$ differed from that for iodide. It can be seen from Table I that the apparent $K_m$ values for $TcO_4^-$ and $ReO_4^-$, obtained from reciprocal plots after correction for the diffused ion as shown previously[16], are considerably lower than for iodide in both sheep thyroid and mouse submaxillary slices.

Perrhenate and pertechnetate are also concentrated in slices of mammary tissue of lactating rats and in slices of a rat thyroid tumor. Equivalent constants were not measured in these tissues on the assumption that they are similar to those of sheep thyroid and mouse submaxillary tissue, as is the case with $I^-$ (see ref. 16). Perrhenate concentration has also been demonstrated in the stomach[13] and ovocytes[15] and pertechnetate in the gastrointestinal tract[14].

Initial studies with unlabeled permangenate ion showed that this lower homologue

* The justification for the use of this concept in "active" transport has been described previously[16,18].

TABLE I

APPARENT $K_m$ VALUES FOR $I^-$, $TcO_4^-$ AND $ReO_4^-$ OF THYROID AND SUBMAXILLARY TISSUE SLICES

| *Tissue* | *$K_m$ of $I^-$* | *$K_m$ of $TcO_4^-$* | *$K_m$ of $ReO_4^-$* |
|---|---|---|---|
| | *M* | *M* | *M* |
| Thyroid (sheep) | $3 \cdot 10^{-5}$ | $3-5 \cdot 10^{-7}$ | $1 : 10^{-6}$ |
| Submaxillary (mouse) | $3 \cdot 10^{-5}$ | $3 \cdot 10^{-7}$ | $0.8 \cdot 10^{-6}$ |

of the above ions of periodic group VIIA was rapidly reduced in Ringer medium, which contained, among other substances, glucose and mercaptoimidazole. Pertechnetate and perrhenate ions differed in that they were not reduced as determined by chromatography in butanol saturated with 1 *N* HCl, butanol saturated with 2 *N* acetic acid, butanol saturated with 2 *N* $NH_4OH$, and in isopropanol – 4 *N* $NH_4OH$ (4:1). This is, no doubt, a reflection of differences in the redox potential[19], *e.g.*:

| *in acid* | *E°* (V) |
|---|---|
| $MnO_2 + 2H_2O = MnO_4^- + 4H^+ + 3e^-$ | —1.695 |
| $TcO_2 + 2H_2O = TcO_4^- + 4H^+ + 3e^-$ | —0.7 |
| $ReO_2 + 2H_2O = ReO_4^- + 4H^+ + 3e^-$ | —0.51 |
| *in base* | |
| $MnO_2 + 4OH^- = MnO_4^- + 2H_2O + 3e^-$ | —0.588 |
| $ReO_2 + 4OH^- = ReO_4^- + 2H_2O + 3e^-$ | +0.594 |

The specificity of the concentration of $TcO_4^-$ and $ReO_4^-$ is demonstrated in Table II. Destruction of cellular integrity essentially abolished the concentration of these anions in thyroid tissue. The fact that there is concentration of $TcO_4^-$ and $ReO_4^-$ in rat tissue slices, as well as values slightly greater than 1.0 in some of the homogenates, may indicate a second order of binding. This is presently being investigated. The manganous ion has previously been shown to be concentrated in thyroid tissue[11,12,14,15]. This finding is confirmed here (Table II), but we find in addition that, in contrast to the iodide, pertechnetate and perrhenate ions, destruction of cellular integrity did not abolish $Mn^{2+}$ concentration. This suggests that the concentration is not the result of an "active" process. The lack of specificity of this $Mn^{2+}$ binding is shown also by the concentration of $Mn^{2+}$ in slices and homogenates of various other tissues. It is, furthermore, hardly to be expected on chemical grounds that this cation would be handled in the same manner as the above anions.

In an effort to determine whether the "sites" involved in the concentration of $TcO_4^-$ and $ReO_4^-$ were similar to those that are responsible for iodide transport, certain inhibitors were tested with the three ions and were also compared to $Mn^{2+}$ binding. It is clear from Table III that the three anions behaved similarly with all inhibitors, whereas $Mn^{2+}$ concentration, although showing rather wide and unexplained variations, was not affected in the same manner. The uncoupling agents, dinitrophenol and dicumarol, are non-specific inhibitors of numerous energy requiring processes and show only that $Mn^{2+}$ concentration is not dependent on such processes. Ouabain and other cardiac glycosides were shown to be potent inhibitors of iodide transport[20,21],

and ouabain inhibits the concentration of the two per-oxyanions in a like manner. The reversal by elevated $K^+$ levels attests to the specificity of the glycoside inhibition since DNP inhibition is not so reversed[21]. It also suggests a $K^+$ requirement for the transport of all three anions. Further similarity in the transport mechanisms of $I^-$, $TcO_4^-$ and $ReO_4^-$, is shown by their nearly identical behavior toward the anions listed in Table III which are believed to inhibit the transport mechanism competitively. This has actually been shown only in the case of $SCN^-$ (see ref. 18).

Perhaps the strongest evidence for a common mechanism involved in the transport of these anions comes from the fact that they are mutually inhibitory (Table IV). Concentrations of the competing anions were chosen as functions of their $K_m$ values. The degree of inhibition is found to be approximately that expected from the $K_m$

TABLE II

COMPARISON OF ION CONCENTRATION RATIOS IN TISSUE SLICES AND IN HOMOGENATES OF THYROID AND OTHER TISSUES

Tissues incubated in each medium indicated at 37° for 100 min. 50% water homogenates dialyzed at 4–5° for 20 h.

| System | Tissue | Medium or dialysate ion concentration | | | |
|---|---|---|---|---|---|
| | | $3 \cdot 10^{-6}$ M $I^-$ | $3 \cdot 10^{-7}$ M $TcO_4^-$ | $1 \cdot 10^{-6}$ M $ReO_4^-$ | $3 \cdot 10^{-6}$ M $Mn^{2+}$ |
| | | T/M [$I^-$] | T/M [$TcO_4^-$] | T/M [$ReO_4^-$] | T/M [$Mn^{2+}$] |
| Tissue slices in Krebs–Ringer phosphate buffer (pH 7.4) | Sheep thyroid | 18.8 | 72.0 | 34.0 | 14.9 |
| | Rat kidney | 0.83 | 2.68 | 1.21 | 14.2 |
| | Rat liver | 0.78 | 4.81 | 1.34 | 19.6 |
| | Rat heart | 0.93 | 1.69 | 1.13 | 21.8 |
| | | H/D* [$I^-$] | H/D* [$TcO_4^-$] | H/D* [$ReO_4^-$] | H/D* [$Mn^{2+}$] |
| Homogenates in $1 \cdot 10^{-3}$ M sodium phosphate buffer (pH 7.4) | Sheep thyroid | 0.89 | 0.73 | 1.12 | 39.9 |
| | Rat kidney | 1.09 | 1.01 | 1.10 | 56.7 |
| | Rat liver | 0.96 | 1.20 | 0.97 | 87.0 |
| | Rat heart | 1.26 | 0.91 | 1.12 | 42.9 |

* H/D refers to ratio of radioactivity per millilitre of homogenate to radioactivity per millilitre of dialysate (not corrected for concentration of water).

TABLE III

THE EFFECT OF VARIOUS INHIBITORS ON THE CONCENTRATION GRADIENT OF CERTAIN IONS IN SHEEP THYROID SLICES

| | T/M [$I^-$]* | T/M [$TcO_4^-$]* | T/M [$ReO_4^-$]* | T/M [$Mn^{2+}$] |
|---|---|---|---|---|
| | Percent of control | Percent of control | Percent of control | Percent of control |
| Control | 100 | 100 | 100 | 100 |
| 2,4-Dinitrophenol, $3 \cdot 10^{-5}$ M | 47 | 31 | 32 | 73 |
| $1 \cdot 10^{-4}$ M | 8 | 4 | 3 | 115 |
| Dicumarol, $3 \cdot 10^{-5}$ M | 9 | 4 | 4 | 158 |
| Ouabain, $7 \cdot 10^{-7}$ M | 10 | 15 | 5 | 122 |
| Ouabain, $7 \cdot 10^{-7}$ M + $K^+$ (25 mequiv./l) | 75 | 75 | 92 | 118 |
| $ClO_4^-$, $1 \cdot 10^{-4}$ M | 7 | 9 | 2 | 91 |
| $SCN^-$, $1 \cdot 10^{-4}$ M | 21 | 30 | 20 | 124 |
| $BF_4^-$, $1 \cdot 10^{-4}$ M | 9 | 8 | 3 | 94 |

* The initial concentration of anions was 0.1 of the $K_m$. $^{54}Mn^{2+}$ was carrier free.

values (Table IV) and can be altered by varying the proportion between "substrate" and inhibitor (not depicted). Although glands from different sheep may exhibit different control T/M values the inhibitory effects are of the same degree and hold the same relation to the $K_m$ values. Mutual inhibition has also been observed in *in vivo* experiments[13,14]. The failure, in one case[13], to demonstrate inhibition by $ReO_4^-$ may, perhaps, be due to the fact that the further utilization of iodide was not blocked. As with the other inhibitors listed in Table III, there was little effect on $Mn^{2+}$ ion, again attesting to the difference in the behavior of this ion.

TABLE IV

MUTUAL INHIBITION OF THYROIDAL CONCENTRATION OF $I^-$, $TcO_4^-$ AND $ReO_4^-$

| *Inhibitor* | *T/M [I⁻] in medium of $3 \cdot 10^{-4}$ M I⁻* | *T/M [TcO₄⁻] in medium of $3 \cdot 10^{-4}$ M TcO₄⁻* | *T/M [ReO₄⁻] in medium of $1 \cdot 10^{-4}$ M ReO₄⁻* |
|---|---|---|---|
| None (control) | 24.1 | 82.9 | 43.9 |
| $I^-$, $3 \cdot 10^{-4}$ *M* | — | 21.7 | 7.3 |
| $TcO_4^-$, $3 \cdot 10^{-4}$ *M* | 3.7 | — | 7.0 |
| $ReO_4^-$, $1 \cdot 10^{-4}$ *M* | 4.3 | 19.5 | — |
| $Mn^{2+}$, $3 \cdot 10^{-4}$ *M* | 18.0 | 71.7 | 43.3 |

As the properties of the site(s) for $I^-$, $TcO_4^-$ and $ReO_4^-$ are quite similar, their number, *i.e.*, the total capacities at saturation, might be expected to be the same. Thus on double reciprocal plots of $1/[X_i^-]_E$ *vs.* $1/[X_0^-]_E$ the ordinate intercepts, corresponding to $1/V_{max}$ of standard enzyme notation, should be the same. In experiments using rather large concentrations of the three anions this has not proved to be the case. Experiments to investigate these differences are in progress.

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