# Iodide transport in isolated cells of mouse submaxillary gland

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**Abstract**. A method has been developed to isolate cells from the submaxillary gland of mouse by treatment with pronase. Three fractions of cells have been isolated having almost equal iodide concentrating activity. The isolated cells show time dependent uphill transport of iodide. The transport is substrate-saturable, having a $K_m$ value of 0·3 $\mu$M for iodide. The transport is sensitive to antithyroid drugs, metabolic inhibitors and to some extent to ouabain. Pseudohalide such as thiocyanate competes with the transport of iodide. Thyroid hormones or thyroid stimulating hormone have no significant effect on the iodide transport in these cells.

**Keywords**. Iodide transport; iodide concentration; isolated cells of submaxillary gland.

## Introduction

It is known for a long time that apart from the thyroid gland, iodide ion is concentrated in the salivary gland of mouse (Fletcher *et al*., 1959; Banerjee and Datta, 1982), in saliva of a number of other species (Cohen and Myant, 1959; Honour *et al*., 1952), in mammary tissue and in milk (Brown-Grant 1961; Freinkel and Ingbar, 1956) and also in the stomach and its secretion (Banerjee and Datta, 1982; Honour *et al*., 1952). However, studying the mechanism of iodide transport in these extrathyroidal tissues by *in vivo* experiments, leads to faulty interpretation because of the presence of the thyroid gland which is the master organ for accumulation of iodide. Hence, an *in vitro* system is always desirable to overcome this problem. Iodide concentrating activity of mouse submaxillary gland was demonstrated earlier in the slice experiments *in vitro* (Fletcher *et al*., 1956). The similarities and dissimilarities of the iodide transport system of the submaxillary gland with that of the thyroid gland has also been reported in tissue slices (Wolff and Maurey, 1961). An extensive work has been done on dispersed thyroid cells after Tong *et al*. (1962) described a continuous flow trypsinization method for isolation of thyroid cells capable of concentrating iodide with subsequent formation of thyroid hormones. We have recently developed a method for the isolation of single cell from mouse submaxillary gland and have observed that these cells are

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Abbreviations used: MMI, Mercaptomethylimidazole; TSH, thyroid stimulating hormone: KRB, Krebs-Ringer bicarbonate; C/M ratio, cell/medium ratio.

capable of concentrating iodide in in vitro experiments. Various properties of this transport system in these isolated cells are being reported in the present communication.

## Materials and methods

### Chemicals

Protease from *Streptomyces griseus* (Pronase, 90,000 PUK units/mg) was purchased from Serva Feinbiochemica, Heidelberg, Federal Republic of Germany. Mercaptomethylimidazole (MMI), thiouracil, thyroid stimulating hormone (TSH), thyroid hormones, sodium azide, ouabain and N-ethylmaleimide were obtained from Sigma Chemical Co., St. Louis, Missouri, USA. $Na^{131}I$ was supplied by Bhabha Atomic Research Centre, Bombay. All other chemicals used were of analytical grade.

### Preparation of cells and assay of iodide transport

Male Swiss mice (20–25 g) of the Institute inbred strain were used throughout the experiments. Submaxillary glands from 8 mice were finely minced, placed in a fluted conical flask containing 12 ml of Krebs-Ringer bicarbonate (KRB) buffer having 1·3 mM $CaCl_2$ and 3 ml of 100 mM glucose. The content was stirred for 1 h in the presence of 0·5 mg pronase. The supernatant solution was decanted through glasswool and centrifuged at 2000 *g* for 10 min to collect fraction 1 cells. Rest of the tissue was resuspended with the above medium and treated with 0·5 mg pronase for another 1 h to collect fraction 2 cells. Finally fraction 3 cells were collected similarly from the rest of the tissues after further treatment with 0·5 mg of pronase for 2 h. The adhering pronase in each cell fraction was washed out with 4 ml of KRB buffer and the cells of each fraction were finally suspended in 0·5 ml of the KRB buffer. The combined cell volume when estimated was found to be around 100 $\mu$l. At least 80% of the cells were viable as observed by exclusion of trypan blue. All these fractions were finally combined to a volume of 4 ml of KRB buffer before use. The assay system for the transport of iodide contained in a final volume of 1 ml of KRB medium: 0·5 ml of the cell suspension (cell volume 12 $\mu$l), 10 mM glucose, 10% mouse serum and 1 $\mu$M KI containing 5–10 $\mu$Ci Na $^{131}$I. It was incubated at 37°C for 2 h and the viability of the cells was checked again as before. No significant cell death was observed during this period of incubation. The cell suspension was centrifuged at 1000 *g* for 10 min, to separate the cells from the medium. The cell washing is not necessary as the amount of radioactivity trapped in the small volume of the cells used was found to be insignificant in comparison to the radioactivity inside the cells. Both the cells (C) and the medium (M) were counted separately in a gamma ray spectrometer and iodide transport (iodide concentration) was expressed as C/M ratio which is calculated from cpm per ml of cell divided by cpm per ml of the medium.
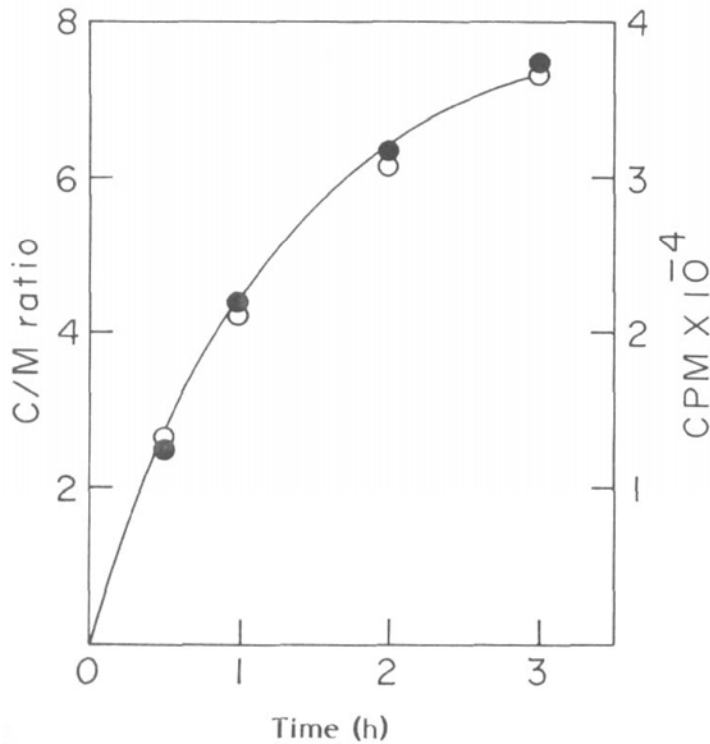
## Results

With a view to getting a population of cells rich in iodide concentrating activity, each fraction of cells was first assayed for the ability to concentrate iodide as shown

in table 1. The results show that the cells in each fraction were equally active in iodide concentrating activity. Hence a pooled cell fraction was used to study the mechanism of iodide transport throughout the subsequent experiments. Figure 1 shows the time course study of the iodide concentration process. The transport of

**Table 1**. Iodide concentrating activity in different fractions of isolated cells.
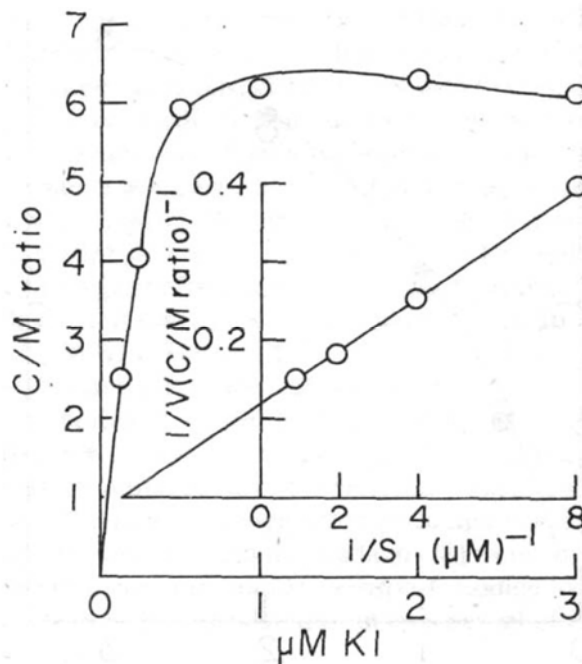
| | Iodide concentration (C/M ratio) | | | |
| --- | --- | --- | --- | --- |
| | Expt. 1 | Expt. 2 | Expt. 3 | Expt. 4 |
| Fraction 1 | 5·4 | 5·0 | 7·2 | 5·9 |
| Fraction 2 | 7·2 | 6·0 | 4·8 | 6·0 |
| Fraction 3 | 6·6 | 5·0 | 9·6 | 7·0 |

The method of isolation of individual cell fraction and assay of C/M ratio has been described in text. Each experiment was carried out using different cell preparation.



**Figure 1**. Kinetics of iodide transport. The cells were incubated with all the components at 37°C for different periods of time as indicated and C/M ratio (O) was calculated as described in the text; (●), indicates the radioactivity in the supernatant obtained after treating the cell homogenate with trichloroacetic acid.

iodide was almost linear for 2 h after which the rate decreased. The figure 1 shows that at 2 h, the concentrations of iodide inside the cells was 6 fold higher than that of the medium. The solid circle indicates the absolute amount of radioactivity present in the cytosol as recovered in the supernatant of the trichloroacetic acid treated cell homogenate. Chromatography and autoradiography of this supernatant indicates the presence of radioactivity with the iodide spot only. This transport of iodide was found to be linear when 0·2–2 mg of the cell protein was used in the assay system. Figure 2 indicates the effect of different concentrations of iodide in the medium on iodide concentrating activity. The activity was found to be linear up to a concentration of 0·5 $\mu$M KI above which the activity tends to saturate. The Lineweaver-Burk plot given as the inset of figure 2 shows that the transport system has the $K_m$ of 0·3 $\mu$M for iodide with a $V_{max}$ (C/M ratio) of 8·3. Table 2 demonstrates the effect of some inhibitors on the iodide transport activity. The cells usually exhibit a C/M ratio of 5-8. This activity is completely lost when the cells were heated at 100°C for 2 min (data not shown). Antithyroid drugs such as MMI and thiouracil strongly inhibit the iodide transport in these cells. Metabolic inhibitors such as azide and cyanide also inhibit the transport system. Ouabain, the inhibitor of Na$^+$–K$^+$ ATPase, causes only 30–40% inhibition. N-Ethylmaleimide, a sulphydryl reagent, has a slight stimulatory effect on the transport of iodide. Of the pseudohalides such as thiocyanate and perchlorate, only the former significantly



**Figure 2.** Effect of different concentrations of iodide on iodide transport. The cells were incubated with all the components containing different concentrations of KI in the presence of Na$^{131}$I (keeping the specific radioactivity constant). The C/M ratio was calculated as described in the text. (C/M)$^{-1}$ was plotted against ($\mu$M KI)$^{-1}$ to determine the $K_m$ as shown in the Lineweaver-Burk plot in the inset.

**Table 2**. Effect of some reagents on iodide concentrating activity

|  | Iodide concentration (C/M ratio) | | |
|---|---|---|---|
|  | Expt. 1 | Expt. 2 | Expt. 3 |
| Control Cells | 6·0 | 5·7 | 7·4 |
| +MMI[a] | 2·7 | 1·5 | 2·8 |
| +Thiouracil[a] | 2·7 | 1·9 | 2·5 |
| +Azide[a] | 2·9 | 1·9 | 1·5 |
| +Cyanide[a] | 2·6 | 2·0 | 3·0 |
| +Ouabain[a] | 4·0 | 4·0 | 5·0 |
| +N-ethylmaleimide[a] | 8·3 | 9·5 | 7·9 |
| +Thiocyanate[a] | 1·5 | 2·0 | 2·8 |
| +Perchlorate[a] | 4·1 | 5·0 | 7·9 |

[a] 1 MM.

The cells were preincubated with indicated concentrations of the reagents at 37°C for 10 min in the presence of all the components before the addition of serum. KI and $^{131}I$. Three different cell preparations were used in the 3 experiments.

competes with the transport of iodide. It is known that iodide transport in the thyroid gland is stimulated by thyroid stimulating hormone. However, neither thyroid hormone nor the thyroid stimulating hormone has any significant effect on the iodide concentrating activity of the submaxillary cells as shown in table 3.

**Table 3.** Effect of thyroid hormones and thyroid stimulating hormone on iodide concentrating activity.

|  | Iodide concentration (C/M ratio) | |
|---|---|---|
|  | Expt. 1 | Expt. 2 |
| Control cells | 6·0 | 7·3 |
| +Thyroxine 3 $\mu$g | 5·2 | 7·0 |
| +Triiodothyronine 3 $\mu$g | 5·0 | 7·6 |
| +Thyroid stimulating hormone 2 mu | 6·0 | 9·6 |

The cells were preincubated at 370 C for 10 min in the presence or absence of the indicated amount of thyroid hormones or thyroid stimulating hormone before starting the transport with radioiodide. Expt. 1 and 2 represent similar experiments using two different preparation.

## Discussion

Although iodide concentrating activity has been demonstrated *in vitro* in slices of mouse salivary gland (Fletcher *et al.*, 1956; Wolff and Maurey, 1961), this is perhaps the first report to demonstrate that the isolated cell preparation from mouse submaxillary gland can also concentrate iodide in *in vitro* experiments. The C/M ratio of 5-8 of the submaxillary cells is comparable to the value reported earlier in *in vivo* experiments (Banerjee and Datta, 1982) as well as in tissue slices *in vitro* (Wolff and Maurey, 1961). However, the lower value is not unusual in the isolated cells because of the damage of the cell membrane during isolation procedure.

   One of the most important criterion for a transport system is the substrate saturability. The transport of iodide in the isolated submaxillary cells shows saturation above 1 $\mu$M KI having a $K_m$ value of 0·3 $\mu$M. This value is at least 3 times less than the value reported earlier in slice experiments (Wolff and Maurey, 1961). The reason for this increased affinity in the dispersed cells is not clear. However, the possibility of the proteolytic modification of the transport system by pronase during cell preparation may not be excluded. The second important criterion is the sensitivity to some inhibitors and competitors. Iodide transport or concentration in any tissue is measured after blocking the iodide organification, if any, with antithyroid drugs such as mercaptomethylimidazole or thiouracil (Wolff and Maurey, 1961; Tong *et al.*, 1962). Previous workers have reported that mouse submaxillary gland does not have the ability to catalyze the organification of iodide to form protein-bound iodine (Fletcher *et al.*, 1956; Tong *et al.*, 1962). However, our studies indicate that dispersed cells from mouse submaxillary glands can catalyze protein-bound iodotyrosine formation with the help of the perioxidase present in this gland, although the amount of organification may be as little as 10% of the iodide concentrated (Banerjee *et al.*, 1985). Chromatography and autoradiography of the cell homogenate after incubation of the submaxillary cells with radioiodide have indicated that about 90% of the iodide concentrated remains as free iodide. Since a very small amount of iodide is metabolised in the organification by the endogenous peroxidase, we have not included antithyroid drugs such as MMI during transport studies similar to that reported earlier in the slice experiments (Wolff and Maurey, 1961). Furthermore, our *in vitro* studies indicate that iodide transport in the dispersed cells is strongly inhibited in the presence of these antithyroid drugs, an effect which limits the inclusion of antithyroid drugs during the assay of iodide transport in these cells. We cannot afford any explanation as to how these antithyroid drugs inhibit the transport in the isolated cells. In this connection, it may be mentioned that the cells also isolated either by trypsin or by collagenase treatment also showed similar inhibition with the antithyroid drugs indicating that the effect is not attributable to the use of pronase only. The inhibition of the transport with cyanide and azide is expected as they act metabolic inhibitors of the cells. It has been reported earlier that iodide transport in salivary gland slices is inhibited with ouabain which is a specific inhibitor of $Na^+$–$K^+$–ATPase (Wolff and Maurey, 1961). The authors concluded that iodide transport is dependent on $K^+$-concentration and ouabain perhaps inhibits the iodide transport by blocking $K^+$ entry inside the cell (Wolff and Maurey, 1961). The isolated submaxillary cells, however, show only 30–40% inhibition of the

transport of iodide with ouabain, which is lower than the inhibition reported in the slice experiment (Wolff and Maurey, 1961). This partial loss of ouabain sensitivity may be due to damage of the transport system during isolation of the cells by proteolytic treatment either by pronase or trypsin. However, sensitivity to ouabain indicates that the energy for uphill transport of iodide may be provided by the hydrolysis of ATP catalyzed by the $Na^+$–$K^+$ —ATPase. The reason for slight Stimulation of iodide transport by N-ethylmaleimide is not clear. However, the possibility of the presence of any sulphydryl group containing inhibitory protein controlling the iodide transport in these cells cannot be excluded. One of the most important properties of the iodide transport system is the competition by pseudohalides such as thiocyanate and Perchlorate. Iodide transport in the salivary gland slices has been shown to be strongly inhibited in the presence of thiocyanate or Perchlorate (Fletcher *et al.*, 1956). Thiocyanate also acts as a strong inhibitor of iodide entry in the dispersed submaxillary cells and appears to be more effective than Perchlorate. It is known that iodide transport in the thyroid gland is stimulated by TSH and this effect has also been demonstrated in isolated thyroid cells (Knopp *et al.*, 1970). However, TSH has been reported to exert no effect on the iodide transport in the salivary gland slices (Wolff and Maurey, 1961). Our results also show that neither TSH nor thyroid hormones have any significant effect on the iodide concentrating activity indicating the absence of specific receptors for TSH in the cells. This is in agreement with the previous findings that iodide accumulation in the extrathyroidal tissues *in vivo* is insensitive to TSH (Taurog *et al.*, 1959; Banerjee and Datta, 1982). It thus appears from the results presented that the submaxillary cells contain iodide carrier which actively transport iodide inside the cells.

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