*Biochimica et Biophysica Acta*, 318 (1973) 235–251


BBA 76397

# ROLE OF SODIUM ION IN ACTIVE TRANSPORT OF IODIDE BY CULTURED THYROID CELLS*

NANDALAL BAGCHI** and DAVID M. FAWCETT***

*Department of Medicine, University of Alberta, Edmonton, Alberta T6G 2G3 (Canada)*

(Received January 24th, 1973)
(Revised manuscript received May 2nd, 1973)

## SUMMARY

1. Bovine thyroid cells, grown in monolayer culture, were found to actively accumulate $I^-$. The presence of thyroid-stimulating hormone in the growth medium markedly enhanced this ability. Net accumulation of $I^-$ by the cultured cells was diminished by known specific inhibitors of $I^-$ transport ($ClO_4^-$, $CNS^-$), by inhibitors of energy metabolism ($CN^-$, 2,4-dinitrophenol) and by ouabain.

2. The rate of influx of $I^-$ into thyroid cells was decreased by $ClO_4^-$ and $CNS^-$, but not by $CN^-$, 2,4-dinitrophenol or ouabain. $I^-$ influx showed a specific and absolute requirement for extracellular $Na^+$. The influx rate was proportional to the concentration of $Na^+$ in the extracellular medium over the range 0–150 mM. The rate of influx followed saturation kinetics with respect to the concentration of $I^-$ in the medium. Reduction of the $Na^+$ concentration of the medium resulted in an increase in the apparent Michaelis constant ($K_m$) of $I^-$ influx with no change in the maximal velocity ($V$).

3. The rate of tracer efflux from preloaded thyroid cells was inhibited in the presence of $Na^+$ in the incubation medium. Addition of $I^-$, $ClO_4^-$ or $CNS^-$ to $Na^+$-containing media promptly and completely abolished the inhibitory effect of $Na^+$ on $I^-$ efflux. Addition of ouabain, $CN^-$ or 2,4-dinitrophenol to $Na^+$-containing media also stimulated the rate of efflux, but only after a lag period of 20–25 min. None of these reagents stimulated the rate of efflux when added to a $Na^+$-free medium.

4. A new model of thyroidal $I^-$ transport involving a sequential interaction of $Na^+$ and $I^-$ with a mobile carrier molecule in the cell membrane has been formulated to explain the above observations.

## INTRODUCTION

The active transport of solutes, such as glucose and several amino acids, into animal cells has been postulated to involve co-transport of the solute with $Na^+$, and

* Preliminary reports of this study were presented at the 15th annual meeting of the Canadian Federation of Biological Societies, June, 1972 at Quebec City, Canada, and at the IVth International Congress of Endocrinology, June, 1972 at Washington, D.C., U.S.A.

** Present address: Hutzel Hospital Medical Unit, Wayne State University, Detroit, Michigan, U.S.A.

*** To whom requests for reprints should be sent.

to depend upon the maintenance of a gradient for $Na^+$ across the cell membrane[1]. A few previous studies have suggested that the active transport of $I^-$ by the thyroid also may be $Na^+$-dependent. For example, the accumulation of $I^-$ by thyroid slices was inhibited[2], and the loss of $I^-$ from thyroid slices was stimulated[3] on incubation of the tissue in a media with low concentrations of $Na^+$. These data, however, were not sufficient to elucidate the nature of the interaction between $Na^+$ and the active $I^-$-transport process. In the present study, the influence of extracellular $Na^+$ on the influx of $I^-$ into thyroid cells and on the efflux of $I^-$ from thyroid cells has been investigated in separate experiments.

Intact thyroid tissue was rejected as a suitable experimental model because, in such a system, movements of $I^-$ would be transcellular across two cell membranes (apical and basal), and would involve three compartments (extracellular, cellular and colloid). Such structural complexities would make interpretation of experimental data difficult. In contrast, in a monolayer culture system movements of $I^-$ would occur essentially across a single membrane and the difficulties inherent in the use of whole tissue would be avoided.

Short term incubations were carried out with thyroid cell cultures under various conditions to determine the effect of $Na^+$ and other agents on the processes of influx and efflux of $I^-$. A new model of $I^-$ transport has been proposed to explain the results of such studies.

## MATERIALS AND METHODS

### *Culture of cells*

Isolated thyroid cells were obtained from approximately 50 g of fresh bovine thyroid glands by the continuous trypsinization process described by Tong *et al.*[4]. The cells were suspended at a concentration of $1 \cdot 10^6$ cells/ml in Eagle's minimum essential medium supplemented with fetal calf serum (20%), 20 mM glutamine, penicillin G (100 I.U./ml), streptomycin sulphate (100 $\mu$g/ml) and mycostatin (20 units/ml). When required, thyroid-stimulating hormone (Nordic Biochemicals) was added to the culture medium at a concentration of 70 I.U./l. Aliquots consisting of 15 ml and 1.5 ml of the cell suspension were transferred, respectively, to 250-ml plastic culture bottles and to short Leighton tubes containing 9 mm $\times$ 35 mm coverslips. The vessels were tightly capped and then incubated at 36 °C for one week. The culture medium was changed completely after 16 h and 96 h of incubation.

HeLa cells were cultured under the same conditions described for thyroid cells beginning with a stock HeLa cell culture at a concentration of $1 \cdot 10^5$ cells/ml.

Both thyroid and HeLa cells formed extensive monolayers which covered the entire available surface after 4–5 days of culture. Addition of thyroid-stimulating hormone to the growth medium shortened this period in the case of thyroid cells; however, follicle formation was not observed in the thyroid cultures under any condition. Cultures which were 5–6 days old were used for the experiments described below. Whenever possible, cells which had been grown from the same inoculum were utilized for comparative studies in order to minimize the variations in activity associated with different batches of cells.

*Incubation media*

Three different media were used for short term incubations: (i) Krebs–Ringer phosphate buffer, used for all experiments unless otherwise indicated. (ii) An "all-$K^+$" medium obtained by totally replacing the sodium salts of Krebs–Ringer phosphate buffer by the corresponding potassium salts. (iii) Tris media containing 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM $KH_2PO_4$, 15 mM Tris and 130 mM of either choline chloride (Tris–choline medium) or NaCl (Tris–$Na^+$ medium). All media had a pH of 7.4. Substitutions for $Na^+$ and $K^+$ in these media were made, when required, by using isoosmolar amounts of substituent solutes.

*Incubation of cells with $^{131}I^-$*

All incubations were carried out at 37 °C with gentle shaking in a Dubnoff metabolic incubator in a medium containing 0.1–1.0 $\mu$Ci/ml of carrier-free $^{131}I^-$ and 0.01 mM NaI. Methimazole, an agent known to block the metabolism of intracellular $I^-$, was added to the medium at a final concentration of 2 mM when transport function alone was to be studied.

Cells that had been grown on coverslips were incubated with $^{131}I^-$ by transferring each coverslip with attached cells to a 50-ml beaker containing 5 ml of the incubation medium. After incubation, the coverslip was removed from the beaker and washed free of the incubation medium by dipping it quickly and consecutively in 0.9% NaCl (70 ml) contained in each of the four beakers, the method of Tsan and Berlin[5]. In the case of cultures grown in flasks the cells were incubated with 10 ml of the medium after they had been washed with Krebs–Ringer phosphate buffer solution. Again the cells were rapidly rinsed after incubation with four portions (15 ml) of 0.9% NaCl solution. The adequacy of washing in both types of experiments was checked by measuring the radioactivity of the fourth wash fluid to insure that it was close to background levels.

*Measurement of iodoprotein formation*

Cells incubated for 1 h in culture flasks with $^{131}I^-$ were washed, harvested, and then homogenized in 0.9% saline. The protein fraction of the cells was isolated from the homogenate either by precipitation with 10% trichloroacetic acid[6] or by filtration through a column of coarse G-25 Sephadex. In each case the $^{131}I$ content of the proteins was determined.

In other experiments, a Pronase hydrolysate of the homogenate was analyzed for organic iodinated compounds using paper chromatography as described by Tong[7].

*Measurement of cell/medium (C/M) ratios for $I^-$*

C/M ratios, defined as below, were determined by the method of Tong[7] using cells freshly harvested from culture flasks. The cells were incubated with $^{131}I^-$ for 1 h, the cell suspension was then centrifuged and the radioactivity of both the cell pellet and an aliquot of the supernatant was measured. The results were expressed as C/M ratios where

$$C/M = \frac{^{131}I^-(\text{cpm})/\text{ml of packed cells}}{^{131}I^-(\text{cpm})/\text{ml of medium}}$$

*Accumulation of $I^-$ by cultured cells*

Coverslips bearing thyroid or HeLa cells that had been incubated with $^{131}I^-$ were transferred to counting tubes for measurement of radioactivity. The protein content of the cells on the coverslips was then estimated by a colorimetric method[8] using bromosulphthalein following digestion of the cells with 1–2 ml of M NaOH for 60 min at 37 °C. The accumulation of $I^-$ by the cells, expressed as moles $I^-$/mg of cell protein, was calculated from the known specific activity of $^{131}I^-$ in the medium.

*Measurement of tracer efflux*

The rate of efflux of $^{131}I^-$ from thyroid-stimulating hormone-grown thyroid cells (thyroid cells cultured in the presence of thyroid-stimulating hormone) which had been preloaded with $^{131}I^-$ was measured by determining the rate of loss of tracer from the cells into a tracer-free external medium. The volume of the medium was kept relatively large so that the concentration of $^{131}I^-$ in the medium would be very low throughout the expermiment. Because of this, recycling of the tracer into the cells would be negligible and the rate of loss of tracer from the cells would be nearly equivalent to the rate of tracer efflux.

Thyroid cells, which had been grown on coverslips, were preloaded with $^{131}I^-$ by incubation at 37 °C with 0.05 mM NaI and 10 $\mu$Ci/ml $^{131}I^-$ in a Tris–$Na^+$ medium. After 30 min incubation the cells were washed with 0.9% NaCl as described previously. The coverslip containing the cells was then placed in a 50-ml beaker and incubated at 37 °C with 10 ml of an appropriate medium. The beaker was shaken gently in a Dubnoff metabolic incubator. To measure the rate of efflux of $^{131}I^-$ from the cells, 1.0-ml aliquots of the medium were removed every 5–10 min and assayed for radioactivity. At the end of the experiment the coverslip was rinsed with 0.9% NaCl and the residual radioactivity of the cells measured. From these results the amount of tracer present in the cells at the beginning of the experiment and at each time interval was calculated. The rate of efflux of $^{131}I^-$ was derived from these data as described below.

In some cases the experiment was carried out as above for an initial period of 15–20 min to obtain a baseline rate of tracer efflux in a control medium. 1 ml of the reagent to be tested was then added to the beaker at an appropriate concentration and the experiment continued to determine the rate of efflux in the presence of the added reagent.

In still other cases, the initial measurements were made for 20 min using one medium after which the coverslip was drained and transferred to a second beaker containing 10 ml of a different medium. The measurement of efflux of $^{131}I^-$ from the cells into the new medium was continued as before. The data from each experiment were plotted on one graph to show consecutively the rates of efflux in the two media.

There was no appreciable desquamation of cells from the coverslip during the experiment. This was determined by centrifuging the residual medium obtained at the end of the experiment and examining it microscopically for sediment. No cells or cellular debris were seen in such preparations. Microscopic examination also showed the monolayer to be intact.

If efflux of $^{131}I^-$ from thyroid cells obeyed first-order kinetics, it can be shown that:

$$\ln [I^*/I_0^*] = -kt$$

where I* and $I_0$* are the amounts of tracer in the cells at times $t$ and zero, respectively, and $k$ is the rate constant for efflux. The efflux of $^{131}I^-$ from thyroid cells was found to follow first-order kinetics (*vide infra*) and plots of $-\ln [I^*/I_0^*]$ *vs* $t$ were used to estimate the rate constant $k$ for tracer efflux.

*Materials*

The components of the culture media were purchased from Baltimore Biological Laboratories. Penicillin and streptomycin were supplied together as a lyophilized mixture. The culture flasks were obtained from Falcon Plastics, and the Leighton tubes and coverslips from Bellco Glass, Inc. Carrier-free $^{131}I^-$ was purchased from Charles Frosst and Co.

## RESULTS

*Iodine metabolism in thyroid cells*

The cell to medium $I^-$ ratios (C/M) for cultured thyroid cells were well above unity (Table I) indicating that such cells retained the ability to concentrate $I^-$ from the medium. Thyroid-stimulating hormone-grown thyroid cells were more active than control cells (cultured in the absence of thyroid-stimulating hormone). HeLa cells did not concentrate $I^-$. The accumulation of $I^-$ by control and thyroid-stimulating hormone-grown cells is summarized in Table II. Again the stimulatory effect of thyroid-stimulating hormone was apparent. In contrast HeLa cells accumulated, under similar conditions, approximately 0.03 nmole $I^-$/mg cell protein per h. $I^-$ accumulation by thyroid cells was reduced markedly by factors such as incubation at 4 °C, or the presence of $ClO_4^-$, $CNS^-$, $CN^-$, 2,4-dinitrophenol or ouabain. The inhibitory effect of ouabain could be abolished by increasing the concentration of KCl in the medium to 20 mM.

Cultured thyroid cells, unlike freshly isolated cells, showed no ability to form iodoproteins. The iodoprotein content of homogenates prepared from thyroid cells which had been incubated for 60 min with $^{131}I^-$ was low (approximately 1.2 pmoles iodine/mg cell protein) and comparable to that seen in HeLa cells under similar con-

TABLE I

CELL/MEDIUM (C/M) $I^-$ CONCENTRATION RATIOS FOR CULTURED CELLS INCUBATED WITH $^{131}I^-$

Cell cultures were incubated with $^{131}I^-$ for 60 min at 37 °C. The cells were then removed from the surface by gentle scraping and suspended in the incubation medium. The suspension was centrifuged and the volume of the packed cells measured. The C/M $^{131}I^-$ concentration ratio was calculated from the radioactivity of the cells and of 1.0 ml of the supernatant medium.

| *Cell type* | *C/M ratio** |
|---|---|
| Control thyroid cells | 3.15 ± 0.19 |
| Thyroid-stimulating hormone-grown cells | 11.0 ± 1.1 |
| HeLa cells | 0.90 ± 0.04 |

* Mean of 4 determinations ± S.E.

TABLE II

THE EFFECT OF VARIOUS CONDITIONS ON THE ACCUMULATION OF $I^-$ BY CULTURED THYROID CELLS

Cell cultures were incubated with $^{131}I^-$ of known specific activity for 60 min at 37 °C. The cells were then rinsed and assayed for radioactivity and protein content as described in the text. The data in Columns A and B were obtained with two different cell populations. Final concentrations are given. $I^-$ accumulation was calculated from the known specific activity of $^{131}I^-$ in the medium.

| *Conditions* | *$I^-$ accumulation per mg cell protein (moles × $10^{10}$)* | |
|---|---|---|
| | *A. Control thyroid cells* | *B. Thyroid-stimulating hormone-grown cells* |
| Control | 2.05 ± 0.24 | 31.3 ± 3.0 |
| 4 °C | 0.31 ± 0.04 | 0.33 ± 0.03 |
| Addition of 0.1 mM $ClO_4^-$ | 0.28 ± 0.02 | 0.34 ± 0.02 |
| Addition of 0.1 mM $CNS^-$ | 0.30 ± 0.01 | 0.33 ± 0.02 |
| Addition of 10 mM NaCN | 0.42 ± 0.01 | 1.57 ± 0.17 |
| Addition of 0.1 mM 2,4-dinitrophenol | 0.43 ± 0.02 | 2.25 ± 0.29 |
| Addition of 0.05 mM ouabain | 0.40 ± 0.02 | 1.88 ± 0.20 |
| Addition of 0.05 mM ouabain in presence of 20 mM KCl | 1.96 ± 0.20 | 28.6 ± 3.1 |

ditions. When the hydrolysates of such homogenates were chromatographed no iodinated organic compounds could be detected.

*$I^-$ influx into thyroid cells*

Fig. 1 shows the uptake of $^{131}I^-$ by thyroid cells at various intervals of time. The concentration of $I^-$ in the medium was essentially constant in such experiments since the thyroid cells took up only a negligible fraction of the total $I^-$ present. The initial linear part of the curve was assumed to correspond closely to the influx of $I^-$ into the cells because the magnitude of efflux would be small at this stage. The uptake of $I^-$ by the cells during the first 5 min was therefore used to calculate the velocity of the influx process. Such influx values may include a contribution from exchange diffusion. However, in experiments not reported here preloading of the thyroid cells with different concentrations of $I^-$ prior to incubation did not change the influx values significantly. Exchange diffusion, if present, was thus regarded as minimal under the present conditions.

Table III shows the effect of various inhibitors on $I^-$ influx. Marked inhibition occurred in the presence of $ClO_4^-$ and $CNS^-$ which are known competitive inhibitors of $I^-$ transport. Inhibition of energy metabolism (by $CN^-$ and 2,4-dinitrophenol) or of $(Na^+-K^+)$-ATPase (by ouabain) was without effect on $I^-$ influx.

In other experiments the rate of influx of $I^-$ into thyroid cells was determined in the presence of 0.1 mM $KClO_4$ at various concentrations of extracellular $I^-$. Under these conditions the small amount of $I^-$ influx that did occur was assumed to be the result of processes other than active transport because, on the basis of the above data, $ClO_4^-$ would almost completely abolish the active process. In all subsequent ex-

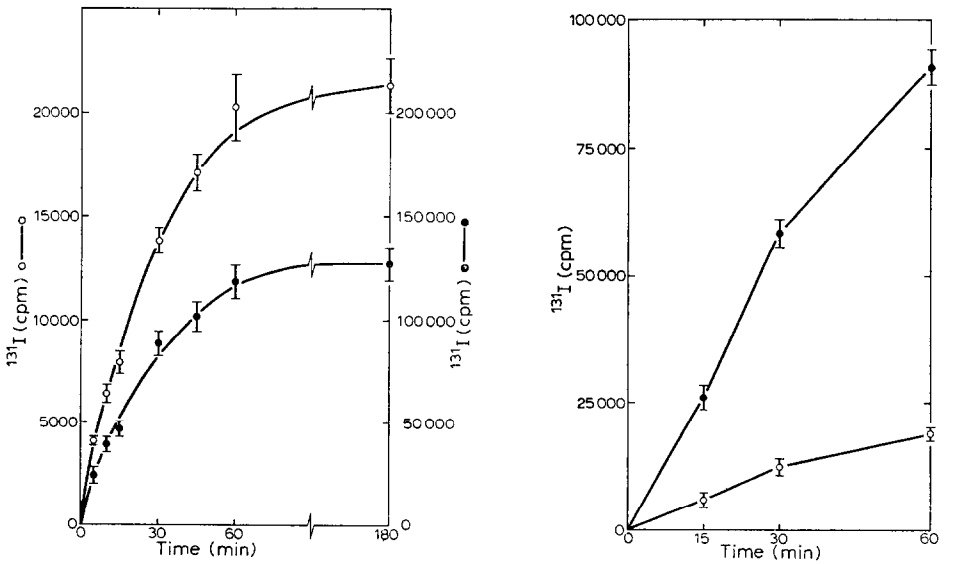



Fig. 1. The accumulation of $^{131}I^-$ by thyroid cells plotted as a function of time. The cells were incubated with $^{131}I^-$ at 37 °C, then rinsed and assayed for protein content and radioactivity. Each of the points on the graphs represents the mean ± S.E. of 5–6 determinations expressed as cpm per mg cell protein. Control thyroid cells, ○—○; thyroid-stimulating hormone-grown thyroid cells, ●—●.

Fig. 2. $I^-$ accumulation by thyroid cells incubated in a low-$Na^+$ medium. Accumulation of $^{131}I^-$ by thyroid-stimulating hormone-grown cells was measured using either a Krebs–Ringer phosphate medium (●—●) containing 150 mM $Na^+$ or a low-$Na^+$ medium (○—○) containing 30 mM $Na^+$. The latter was derived from Krebs–Ringer phosphate buffer by replacement of 120 mM NaCl with 120 mM choline chloride. Influx rates are expressed as cpm per mg cell protein. Each result shown is the mean ± S.E. of 5–6 determinations.

TABLE III

EFFECT OF VARIOUS INHIBITORS ON THE RATE OF $I^-$ INFLUX

Each estimation of $I^-$ influx was made using a 5 min uptake value as described in the text with thyroid cells grown in the presence of thyroid-stimulating hormone. When NaCN was added to the incubation medium appropriate changes in the concentration of NaCl were made to maintain the same osmolality. All concentrations denote the final concentration of the reagent in the incubation medium. Each result is the mean of 5–6 determinations ± S.E. The influx values were calculated from the known specific activity of $^{131}I^-$ in the medium.

| *Addition to incubation medium* | *$I^-$ influx × $10^{11}$ (moles/mg cell protein per min)* |
|---|---|
| None (control) | 15.9 ± 1.8 |
| 0.1 mM $KClO_4$ | 0.132 ± 0.007 |
| 0.1 mM KCNS | 0.120 ± 0.005 |
| 10 mM NaCN | 17.0 ± 1.9 |
| 0.1 mM 2,4-dinitrophenol | 16.0 ± 1.8 |
| 0.05 mM ouabain | 16.3 ± 1.5 |

periments the influx data were corrected in this manner for non-active influx so that the values reported would reflect the process of active transport alone.

*$I^-$ accumulation in low-$Na^+$ media*

Fig. 2 shows that thyroid cells accumulated much less $I^-$ from a medium containing 30 mM $Na^+$ than from a medium containing 150 mM $Na^+$ at all time intervals studied.

*Effects of extracellular $Na^+$ on $I^-$ influx*

When thyroid cells were incubated with $^{131}I^-$ in an all-$K^+$ medium the rate of $I^-$ influx into the cells was low (Table IV) comparable in magnitude to the rate found in the presence of $ClO_4^-$ or $CNS^-$. Substitution of approximately one-half the $K^+$ of the medium by isoosmolar amounts of choline$^+$, $Li^+$, Tris$^+$ or mannitol had no effect on the influx rate. However, a similar substitution of $K^+$ by $Na^+$ markedly stimulated influx, suggesting a specific stimulatory effect of $Na^+$.

TABLE IV

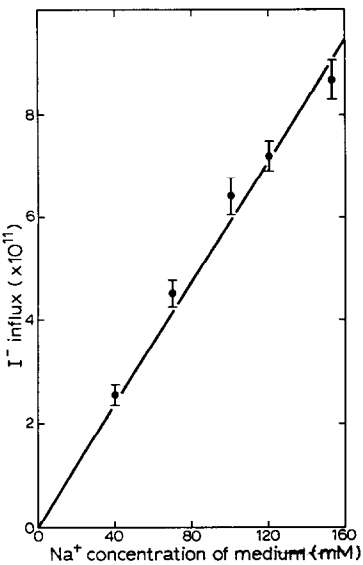
THE EFFECT OF ADDING VARIOUS SUBSTITUENTS TO THE INCUBATION MEDIUM ON THE RATE OF $I^-$ INFLUX

Measurements of iodide influx were made using thyroid cells that had been grown in the presence of thyroid-stimulating hormone. The cells were incubated in all-$K^+$ media (as described in the text), or in similar media in which a part of the $K^+$ had been replaced by various substituents. The $I^-$ concentration of all media was 0.01 mM. Each result is the mean of 5–6 determinations ± S.E. The influx values were calculated from the known specific activity of $^{131}I^-$ in the medium.

| *Composition of incubation medium* | | *$I^-$ influx × $10^{11}$ (moles/mg cell protein per min)* |
|---|---|---|
| *Concentration of $K^+$ (mM)* | *$K^+$ replacement* | |
| 155 | 0 | 0.41 ± 0.04 |
| 85 | 70 mM choline$^+$ | 0.51 ± 0.04 |
| 85 | 70 mM $Li^+$ | 0.40 ± 0.03 |
| 85 | 70 mM Tris$^+$ | 0.38 ± 0.04 |
| 85 | 140 mM mannitol | 0.53 ± 0.04 |
| 85 | 70 mM $Na^+$ | 3.82 ± 0.32 |

The same conclusion was reached when influx measurements were made using a Krebs–Ringer phosphate medium in which approximately one-half the $Na^+$ of the buffer was replaced by isoosmolar amounts of other substituents (Table V). Substitution of $Na^+$ by equivalent amounts of choline$^+$, $Li^+$, Tris$^+$ or mannitol produced almost equal depressions in the rate of $I^-$ influx. Use of $K^+$ as a substituent led to an even greater decrease in influx rate suggesting a specific inhibitory effect of $K^+$.

Fig. 3 illustrates the rate of influx of $I^-$ into thyroid cells incubated in media containing different concentrations of $Na^+$ but a constant concentration of $I^-$. $I^-$ influx was abolished completely in a $Na^+$-free medium, and was directly proportional to the extracellular $Na^+$ concentration over the range 0–150 mM.



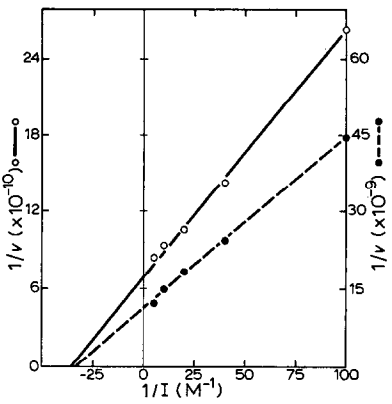




Fig. 3. Variation of the rate of $I^-$ influx with the concentration of $Na^+$ in the medium. $I^-$ influx rates (expressed as moles of $I^-$/mg cell protein per min) were determined for thyroid-stimulating hormone-grown thyroid cells incubated in either Tris–choline media (containing no $Na^+$) or media containing different concentrations of $Na^+$. The latter were derived from Krebs–Ringer phosphate buffer by replacing an appropriate amount of NaCl with an equivalent amount of choline chloride. Each result shown is the mean ± S.E. of 5–6 determinations.

Fig. 4. Variation of the rate of $I^-$ influx with concentration of $I^-$ in the medium. The rate of $I^-$ influx was determined by measuring the accumulation of $I^-$ by thyroid cells during a 5-min incubation at 37 °C in Krebs–Ringer phosphate buffer containing $^{131}I^-$ of known specific activity. Each result shown is the mean ± S.E. of 5–6 determinations. The velocity of influx ($v$) is expressed in units of moles of $I^-$/mg cell protein per min. I is the molar concentration of $I^-$ in the incubation medium. Control thyroid cells, ○—○; thyroid-stimulating hormone-grown thyroid cells, ●—●.

TABLE V

THE EFFECT ON THE RATE OF $I^-$ INFLUX OF REPLACING $Na^+$ FROM THE INCUBATION MEDIUM WITH VARIOUS SUBSTITUENTS

Measurements of $I^-$ influx were made using thyroid cells that had been grown in the presence of thyroid-stimulating hormone. The incubation media employed were Krebs–Ringer phosphate buffer modified as indicated below. The $I^-$ concentration of all media was 0.01 mM. Each result is the mean of 5–6 determinations ± S.E. The influx values obtained were calculated from the known specific activity of $^{131}I^-$ in the medium.

| *Composition of incubation medium* | | *$I^-$ influx × $10^{11}$ (moles/mg cell protein per min)* |
|---|---|---|
| *Concentration of $Na^+$ (mM)* | *$Na^+$ replacement* | |
| 150 | 0 | 22.3 ± 2.4 |
| 80 | 70 mM $K^+$ | 5.9 ± 0.5 |
| 80 | 70 mM choline$^+$ | 10.9 ± 0.8 |
| 80 | 70 mM $Li^+$ | 10.2 ± 0.9 |
| 80 | 70 mM Tris$^+$ | 10.8 ± 1.2 |
| 80 | 140 mM mannitol | 11.2 ± 0.7 |

$I^-$ influx was found to follow Michaelis–Menten kinetics (Fig. 4). Values of the apparent Michaelis constant ($K_m$) were found to be similar for both control and thyroid-stimulating hormone-grown thyroid cells. The maximal velocity of influx ($V$) was however much higher in the latter (Table VI). Reduction of the $Na^+$ concentration of the medium resulted in an increase in $K_m$ with no change in $V$ (Table VII).

*Tracer efflux from thyroid cells*

Plots of $-\ln [I^*/I_0{}^*]$ *vs* time (Fig. 5) were linear suggesting that tracer efflux obeyed first order kinetics. The rectilinear nature of the plots of Fig. 5 also suggests

TABLE VI

KINETIC PARAMETERS OF $I^-$ INFLUX

Each horizontal line represents the results of one experiment.

| *Type of cells* | $V \times 10^{11}$ *(moles/mg cell protein per min)* | $K_m \times 10^5$ *(moles)* |
|---|---|---|
| Control thyroid cells | 14.1 | 2.80 |
| | 11.1 | 3.20 |
| | 10.6 | 3.20 |
| Thyroid-stimulating hormone-grown thyroid cells | 87.3 | 2.93 |
| | 66.7 | 3.33 |
| | 93.6 | 3.64 |

TABLE VII

VARIATION OF THE KINETIC PARAMETERS OF $I^-$ INFLUX WITH CHANGES IN THE EXTRACELLULAR CONCENTRATION OF $Na^+$

Each horizontal line represents the results of a single experiment.

| *Concentration of $Na^+$ (mM)* | $V \times 10^{11}$ *(moles/mg cell protein per min)* | $K_m \times 10^5$ *(moles)* |
|---|---|---|
| 40 | 93.4 | 14.0 |
| | 70.0 | 12.3 |
| | 62.2 | 11.2 |
| 70 | 61.8 | 8.24 |
| | 82.2 | 7.62 |
| | 88.0 | 6.64 |
| 100 | 76.3 | 4.84 |
| | 58.0 | 5.40 |
| | 91.6 | 5.36 |
| 150* | 87.3 | 2.93 |
| | 66.7 | 3.33 |
| | 93.6 | 3.64 |

* Results reproduced from Table VI.

that there was no appreciable recycling of the tracer from the medium into the cells during the experiment. If significant recycling had occurred the graphs would have shown a downward curvature with time.

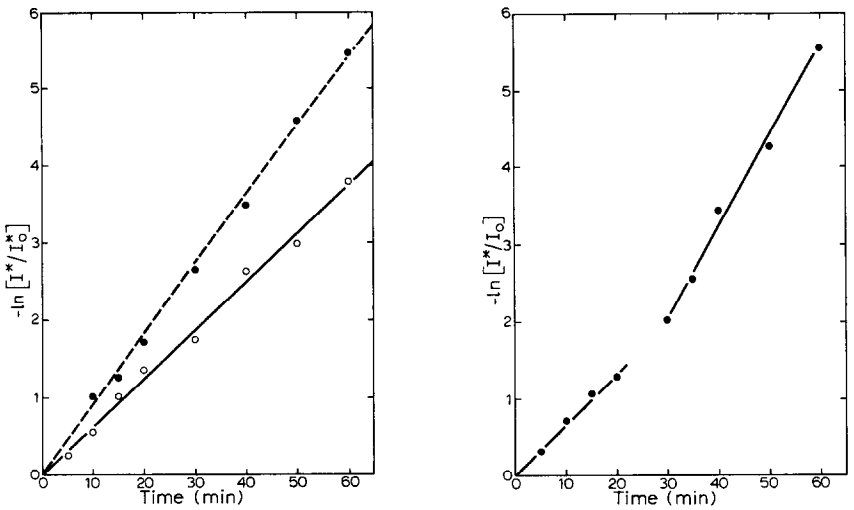



Fig. 5. The rate of $^{131}I^-$ efflux from thyroid cells. Thyroid cells, adherent to a coverslip, were preloaded with $^{131}I^-$ by incubating them with 0.05 mM NaI and 10 $\mu$Ci/ml $^{131}I^-$ for 30 min at 37 °C. The cells were then washed and transferred to a beaker containing 10 ml of the appropriate medium at 37 °C. 1-ml aliquots of the medium were removed at the times indicated on the abscissa. The radioactivity of each aliquot, as well as that remaining on the coverslip after 60 min, was measured. [$I^*/I_0{}^*$]ratios (see text) were calculated from these data. Tris–$Na^+$ medium, ○—○; Tris–choline medium, ●—●.

Fig. 6. The effect of $Na^+$ depletion of the external medium on $^{131}I^-$ efflux. The measurement of efflux was carried out as described in Fig. 1. A Tris–$Na^+$ medium was used for the first 20 min of incubation. The cells were then transferred to a second beaker containing 10 ml of Tris–choline medium and the experiment continued for an additional 40 min.

The rate of efflux was higher when the medium was $Na^+$-free (Fig. 5). The rate of efflux increased from 0.06/min to 0.11/min when the external medium was changed from Tris–$Na^+$ to Tris–choline (Fig. 6). Conversely, the rate of efflux decreased from 0.11/min to 0.06/min when the cells were transferred from a Tris–choline medium to a Tris–$Na^+$ medium (Fig. 7). The results from several experiments of this type are summarized in the first two lines of Table VIII; the rate of efflux was consistently higher for cells incubated in a $Na^+$-free medium.

*Effect of inhibitors on tracer efflux*

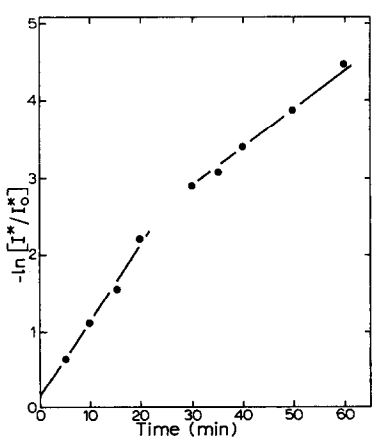
The rate of $^{131}I^-$ efflux from thyroid cells incubated in a Tris–$Na^+$ medium was promptly increased by the addition after 20 min of 0.1 mM NaI (final concentration) to the medium. A plot of the results was similar to Fig. 6. The addition of 0.1 mM $KClO_4$ or KCNS to the medium, instead of NaI, caused a similar increase in the efflux rate. The results from several experiments of this type are summarized in Table VIII, Lines 3, 5 and 7. In contrast, the addition of $I^-$, $ClO_4^-$ or $CNS^-$ to a $Na^+$-

TABLE VIII

RATE CONSTANTS FOR EFFLUX OF $^{131}I^-$ FROM THYROID CELLS

The rates of $^{131}I^-$ efflux were measured as outlined in the text. In each experiment the change of medium, or addition of reagent, was done after the cells had been incubated for 20 min under the initial conditions. When NaI, $KClO_4$ or KCNS was added to the medium its final concentration was 0.1 mM. Each result is the mean ± S.E. of 5–6 determinations.

| *Condition* | *Initial efflux rate* ($min^{-1}$) | *Final efflux rate* ($min^{-1}$) | *Change from initial to final efflux rate* (%) |
|---|---|---|---|
| Change from Tris–$Na^+$ to Tris–choline medium | 0.066 ± 0.003 | 0.110 ± 0.004 | +75 |
| Change from Tris–choline to Tris–$Na^+$ medium | 0.103 ± 0.004 | 0.057 ± 0.003 | −45 |
| Addition of NaI (Tris–$Na^+$ medium) | 0.064 ± 0.002 | 0.099 ± 0.004 | +55 |
| Addition of NaI (Tris–choline medium) | 0.104 ± 0.004 | 0.108 ± 0.005 | + 4 |
| Addition of $KClO_4$ (Tris–$Na^+$ medium) | 0.061 ± 0.004 | 0.103 ± 0.005 | +69 |
| Addition of $KClO_4$ (Tris–choline medium) | 0.106 ± 0.005 | 0.104 ± 0.004 | − 2 |
| Addition of KCNS (Tris–$Na^+$ medium) | 0.061 ± 0.002 | 0.102 ± 0.002 | +67 |
| Addition of KCNS (Tris–choline medium) | 0.110 ± 0.004 | 0.106 ± 0.005 | − 4 |



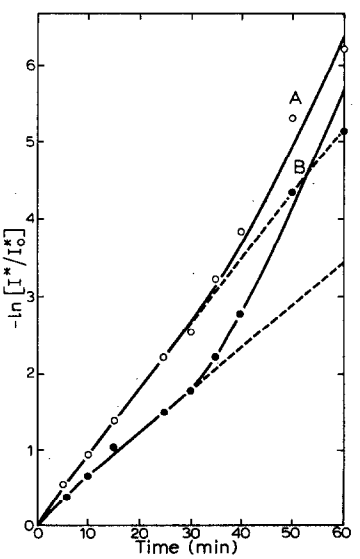




Fig. 7. The effect of $Na^+$ on $^{131}I^-$ efflux. The experiment was carried out as described in Fig. 3 except that a Tris–choline medium was employed for the first 20 min of incubation after which the cells were transferred to a Tris–$Na^+$ medium.

Fig. 8. The effect of ouabain on $^{131}I^-$ efflux. The measurement of $^{131}I^-$ efflux was carried out as described in Fig. 1 except that after 15 min 1 ml ouabain was added to the medium (final concentration 0.05 mM). The media employed were Tris–$Na^+$ with (Curve A) or without (Curve B) 0.01 mM NaI.

deficient medium (Tris–choline) had no effect on the rate of $^{131}I^-$ efflux (Table VIII, Lines 4, 6 and 8).

Fig. 8 (Curve A) illustrates that the addition of 0.05 mM ouabain (final concentration) to a Tris–$Na^+$ medium led to an increase in the rate of $^{131}I^-$ efflux from thyroid cells, but only after a lag period of 20 min. In six replicate experiments the lag period varied between 20 and 25 min. Similar rate plots were obtained when the external medium (Tris–$Na^+$) was supplemented with 0.1 mM NaI from the beginning of incubation (Fig. 8, Curve B). However, in other experiments not reported here, when a Tris–choline medium was used in such experiments, there was no increase in the rate of $^{131}I^-$ efflux following the addition of ouabain to the medium.

Similar efflux plots resulted from parallel experiments in which 10 mM NaCN or 0.1 mM 2,4-dinitrophenol (both final concentrations) were added instead of ouabain to the incubation media.

## DISCUSSION

Monolayer cultures of thyroid cells differ greatly in their ability to transport $I^-$ and to form iodoproteins[4,9,10]. It was thus necessary to study the activity of the present culture system with regard to these functions. Although no iodoprotein formation could be detected in the cultured cells, they were able to concentrate $I^-$ from the medium. The accumulation of $I^-$ was sensitive to inhibitors such as $ClO_4^-$ and $CNS^-$, was dependent on energy metabolism and on the function of ($Na^+$–$K^+$)-ATPase, and was stimulated when the cells were grown in the presence of thyroid-stimulating hormone. These properties are those exhibited by the active transport system of the thyroid gland *in vivo*. The culture system was thus considered to be a valid model with which to carry out further studies of $I^-$ transport.

The present investigation confirms previous observations[2], that net $I^-$ transport by the thyroid is stimulated by external $Na^+$. This effect of $Na^+$ was found to be due, at least in part, to an absolute and specific $Na^+$ requirement of the $I^-$ influx process. Further, external $Na^+$ was confirmed to augment net $I^-$ transport by simultaneously inhibiting the rate of $I^-$ efflux. Agents like $ClO_4^-$ and $CNS^-$ diminished net $I^-$ transport by inhibiting $I^-$ influx into the cell and also, in $Na^+$-containing media, by stimulating $I^-$ efflux out of the cell. In contrast, ouabain and inhibitors of energy metabolism reduced net $I^-$ transport in $Na^+$-containing media solely by increasing the rate of $I^-$ efflux. Such effects of $ClO_4^-$, $CNS^-$, ouabain and metabolic inhibitors on the process of $I^-$ efflux have been reported previously[3,11,12].

### *A model for $I^-$ transport*

Schultz and Curran[1] have discussed the role of $Na^+$ in solute transport on the basis of a general model, which is shown in Fig. 9a as modified for $I^-$ transport. The present observations on $I^-$ transport are, however, compatible only with one variant of this general model shown in Fig. 9b. As in the general model, existence of a mobile carrier molecule is assumed and the translocation reactions of the carrier or the carrier complexes are taken to be rate limiting.

Our observations, that $I^-$ influx has a specific and absolute requirement for $Na^+$, are compatible with this model. Further, since only one $Na^+$ is postulated to combine with each carrier molecule, the rate of $I^-$ influx would vary directly with the first

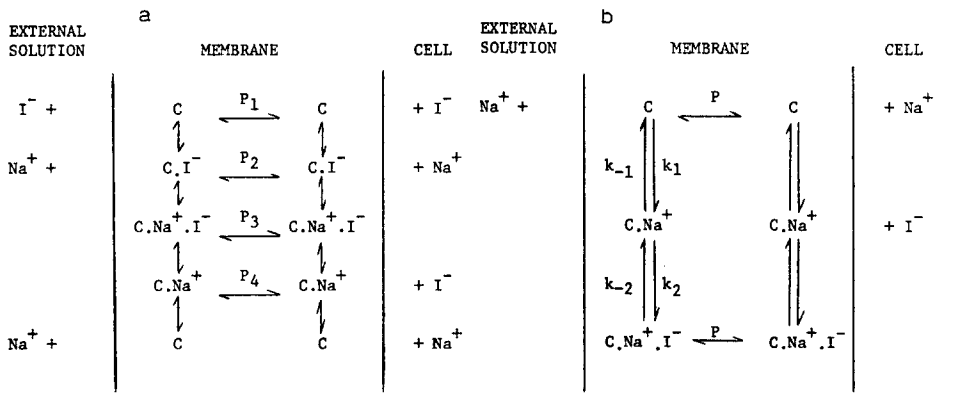



Fig. 9. Kinetic models for thyroidal $I^-$ transport. (a) General model. The carrier molecule $C$ is able to combine with either $Na^+$ or $I^-$ to form binary complexes, which in turn can combine with $I^-$ or $Na^+$, respectively, to form the ternary complex $C \cdot Na^+ \cdot I^-$. $P_1, P_2$, *etc.*, are the rates of translocation of the various complexes across the cell membrane. (b) Proposed model (a variant of the general model). $k_1, k_2$, *etc.*, are the rate constants for the various reactions. $P$ is the rate of translocation of the carrier and the ternary complex across the cell membrane.

power of the concentration of $Na^+$ in the medium. Again this is in accord with the experimental results. $ClO_4^-$ and $CNS^-$ would compete with $I^-$ for combination with the $C \cdot Na^+$ complex and would thus inhibit influx of $I^-$. The proposed model would also satisfactorily explain the trans-inhibitory action of external $Na^+$ on $I^-$ efflux on the basis of the formation of a $C \cdot Na^+$ complex. Formation of such a complex, which is not capable of translocation, would be equivalent functionally to a reduction in the amount of the carrier available for transport. $I^-$, $ClO_4^-$ and $CNS^-$ would relieve such inhibition by promoting formation of a ternary complex from the binary form.

Although the role postulated for $(Na^+-K^+)$-ATPase in relation to solute transport is, on the basis of such a model, an indirect one, it is of fundamental importance and has been discussed at length by Schultz and Curran[1]. Briefly, activity of this enzyme would produce an intracellular $Na^+$ concentration that was much lower than the extracellular. Influx rate, being proportional to the much higher extracellular concentration would greatly exceed the efflux rate resulting in a net accumulation of $I^-$ by the cell. Inhibitors of energy metabolism or ouabain do not alter the influx rate. However, by interfering with the action of $(Na^+-K^+)$-ATPase, they would gradually raise the intracellular concentration of $Na^+$. After a lag period the efflux rate of $I^-$ would rise and eventually equal the influx rate; at this point net $I^-$ uptake by the cell would be abolished. These predictions from the model pertaining to the action of such agents are in complete accord with the results of the present studies.

On the basis of the model, the influx of $I^-$ into thyroid cells would be expected to increase the corresponding influx of $Na^+$. Although an attempt was made to demonstrate this effect by measuring the uptake of $^{22}Na^+$ by thyroid cells in the presence or absence of $I^-$, no increased accumulation of $Na^+$ was found. However, the amount of $Na^+$ which would be co-transported with $I^-$ in this manner would be very small in comparison to the total uptake of $Na^+$ by the cell. This may explain our failure to demonstrate the stimulation.

A kinetic analysis of co-transport models, such as the one proposed (Fig. 9b),

has been discussed by Stein[13]. The following relationship can be deduced for our proposed model:

$$\text{Rate of } I^- \text{ influx} = \frac{p \cdot [\text{total } C]\,[I^-]}{\left(1 + \frac{K_1}{[Na^+]}\right) K_2 + [I^-]}$$

where $p$ is the rate of translocation of the $C \cdot Na^+ \cdot I^-$ complex, total $C$ is the total amount of carrier in the membrane, and $K_1$, $K_2$ are the dissociation constants of the $C \cdot Na^+$ and $C \cdot Na^+ \cdot I^-$ complexes, respectively.

This expression predicts Michaelis–Menten type kinetics for $I^-$ influx as found in the present studies. The maximal velocity of influx equals $p \cdot$ [total $C$] and would thus not be affected by the concentration of $Na^+$ in the medium. $K_m$, which is equivalent to $(1 + K_1/[Na^+])\, K_2$ would decrease as the $Na^+$ concentration of the medium was increased. Both these predictions have been shown experimentally in the present studies. Fig. 10 is a plot of $K_m$ *vs* $1/[Na^+]$ using data from Table VII. From the slope and intercept of this plot it was calculated that $K_1$ was 1.6 M and $K_2$, 3.0 $\mu$M. The high value of $K_1$ would explain why the rate of $I^-$ influx increased linearly with the concentration of $Na^+$ in the medium over the range 0–150 mM without showing evidence of saturation. It is also apparent from the values of $K_1$ and $K_2$ that binding of $Na^+$ by the carrier would result in an intermediate which could avidly bind $I^-$ and that the latter reaction would be virtually complete even at a very low concentration of $I^-$. Such a system would be eminently suitable from a functional standpoint for the cellular concentration of an ion such as $I^-$ which is distributed in nature only in trace amounts.

The present studies show that the kinetic parameters of $I^-$ influx for cells cultured in the presence of thyroid-stimulating hormone were characterized by a higher value for $V$, but a similar value for $K_m$, when compared to cells cultured without thyroid-stimulating hormone. According to the preceding analysis an increase in $V$

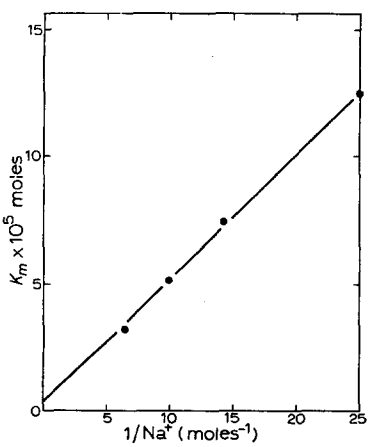



Fig. 10. The variation of $K_m$ for $I^-$ influx with the concentration of $Na^+$ in the incubation medium. The mean values of $K_m$, obtained from Table VII are plotted *vs* the reciprocal of the corresponding $Na^+$ concentrations of the medium.

could result either from an increase in the concentration of carrier, or from an increase in the rate of translocation of the ternary complex. Knopp *et al.*[14] have shown that the acute stimulatory action of thyroid-stimulating hormone on $I^-$ influx for freshly isolated thyroid cells was abolished by actinomycin D or cycloheximide. This would suggest that thyroid-stimulating hormone probably acts by increasing the total amount of carrier in the cell membrane.

The proposed model for $I^-$ transport is bidirectionally symmetrical. $I^-$ accumulation by the cell can result only when a gradient for $Na^+$ is maintained across the cell membrane. The energy inherent in the $Na^+$ gradient is utilized for active transport of $I^-$. It is quite possible, however, that other asymmetries across the cell membrane, *e.g.* the $K^+$ gradient and the electrical potential gradient, may contribute to the energy requirements of the process.

The nature of the interactions among $Na^+$, $I^-$, and the carrier molecule precludes the involvement of covalent linkages. However, if the interaction were electrostatic in nature, then $Na^+$, by binding to an anionic group with neutralization of the negative charge, might make binding of $I^-$ to an adjacent site possible. Such coulombic interactions have been postulated for the active transport of certain anionic amino acids which show a high degree of $Na^+$-dependence[15].

The chemical nature of the thyroidal carrier molecule remains speculative. In bacterial systems a number of proteins have been proposed as the active carriers involved in transport systems for a variety of amino acids, sugars, and ions[16]. Relatively little is known about the nature of such carriers in mammalian cells. Certain phospholipids, which have been isolated from thyroid tissue, were reported to possess specific $I^-$-binding properties[17,18]. The transport system may well involve a multiple-component complex consisting of both protein and phospholipid. A search for such a membrane complex from thyroid tissue based on its apparent $Na^+$-binding and $I^-$-binding properties, may be rewarding.

ACKNOWLEDGEMENTS

The authors wish to thank Miss Eva Dolinski for her skillful technical assistance. The work was supported by the Medical Research Council of Canada (Grant MA-1855).

REFERENCES

1 Schultz, S. G. and Curran, P. F. (1970) *Physiol. Rev.* 50, 637–718
2 Alexander, W. D. and Wolff, J. (1964) *Arch. Biochem. Biophys.* 106, 525–528
3 Iff, H. W. and Wilbrandt, W. (1963) *Biochim. Biophys. Acta* 78, 711–725
4 Tong, W., Kerkof, P. and Chaikoff, I. L. (1962) *Biochim. Biophys. Acta* 60, 1–19
5 Tsan, M. F. and Berlin, R. D. (1971) *Biochim. Biophys. Acta* 241, 155–169
6 Greenberg, D. M. (1957) *Methods in Enzymology* (Colowick, S. P. and Kaplan, N. O., eds), Vol. IV, pp. 708–710, Academic Press, New York
7 Tong, W. (1964) *Endocrinology* 75, 527–536
8 Paul, J. (1970) *Cell and Tissue Culture*, p. 356, E. and S. Livingston, Edinburgh and London
9 Rabin, B. S., Kite, J. H. and Rose, N. R. (1966) *Proc. Soc. Exp. Biol. Med.* 121, 88–89
10 Lissitzky, S., Fayet, G., Giraud, A., Verrier, B. and Torresani, J. (1971) *Eur. J. Biochem.* 24, 88–99
11 Wollman, S. H. and Reed, F. E. (1959) *Am. J. Physiol.* 196, 113–120

12 Scranton, J. R. and Halmi, N. S. (1965) *Endocrinology* 76, 441–453
13 Stein, W. D. (1967) *The Movement of Molecules Across Cell Membranes*, pp. 192–198, Academic Press, New York and London
14 Knopp, J., Stolc, V. and Tong, W. (1970) *J. Biol. Chem.* 245, 4403–4408
15 Schultz, S. G., Yu-Tu, L., Alvarez, O. O. and Curran, P. F. (1970) *J. Gen. Physiol.* 56, 621–639
16 Oxender, D. L. (1972) *Annu. Rev. Biochem.* 41, 777–814
17 Vilkki, P. (1962) *Arch. Biochem. Biophys.* 97, 425–427
18 Schneider, P. B. and Wolff, J. (1965) *Biochim. Biophys. Acta* 94, 114–123