Biochimica et Biophysica Acta, 419 (1976) 349–357

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MOLECULAR TRANSPORT IN THYROID SLICES

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(Received April 1st, 1975)
(Revised manuscript received August 22nd, 1975)

SUMMARY

The aim of this work is to describe quantitatively, from a physical point of view, molecular transport in thyroid slices The study of the release of [^{3}H]sucrose, [^{3}H]inulin and ^{131}I-labeled albumin leads to the following conclusions the molecular transport in the slices is not due mainly to free diffusion Indeed, as these molecules are retained in the interfollicular spaces, the transport of matter is a mechanical process due to the agitation of the medium in which the slices are incubated It depends on the elastic properties of the thyroid tissue, the frequency of the agitator and the thickness of the slice This transport process can be described by a diffusion equation with an empirical diffusion coefficient, we call it diffusivity These findings must be taken into account in any in vitro kinetic study of thyroid metabolism and of its regulation by effectors such as thyrotropin The possibility of interference of such mechanical processes in the interpretation of kinetic tracer studies with tissue slices or fragments should be considered

INTRODUCTION

Most experimental investigations on intact thyroid cells in vitro are carried out using the slice system [1] In kinetic studies of thyroid metabolism and its regulation by various effectors, such as thyrotropin, the penetration of those effectors is generally assumed to be quasi instantaneous [2] Considering the high level of complexity of extracellular space in thyroid and the variability of slice thickness from one laboratory to another this assumption seem clearly an oversimplification [3] The purpose of this work is to analyze the kinetics of penetration and the spatial distribution of extracellular molecules in thyroid slices The results of this analysis could be applied to the interpretation of kinetic data on metabolism and extended to other highly organized tissues

* Publication no BIO 1144

MATERIALS AND METHODS

Slices of dog thyroid (± 220 mg tissue wet weight) were prepared and incubated at 0 C under an atmosphere of O_2/CO_2 (95 5, v/v) in 10 ml Krebs-Ringer bicarbonate buffer containing 8 mM glucose, 0 5 g/l serum albumin and 5 μCi/ml of [^{3}H]-sucrose or [^{3}H]inulin The slices were then transferred, after gently blotting to another flask with 20 ml Krebs-Ringer bicarbonate buffer and incubated at 37 °C Aliquots of 200 μl taken off at definite time intervals were counted in scintillation medium (1 ml Soluene 350 + 12 ml toluene + 48 mg Omnifluor) The thickness of the slices was estimated by dividing the wet weight by the surface assuming a specific gravity of 1 When several slices were incubated together, mean thicknesses were considered The range of thicknesses from one slice to another was within 10 % of the mean The radioactivity was released by the slices, thus giving an ascending curve of medium radioactivity

The data were corrected for aliquot withdrawals The transport of markers out of the slices was also expressed as the decrease of radioactivity in the slices Although the slices were blotted before being dropped into the discharge medium, the exact value of the amount of tracer present in the slice at zero time is unknown Moreover, although it is generally admitted that the markers used do not enter the cells and that the charge preincubation is done at 0 °C, after 2 or 3 h discharge there was still some radioactivity in the slices (from 3 to 10 %), presumably intracellular [4] The discharge for very short times probably comes from open follicles The continuous extrapolation of the first experimental points to zero time is always far from the origin of coordinates (dashed lines in Fig 2) This difference is larger for thin slices where the relative number of open follicles is greater This effect is particularly clear in the

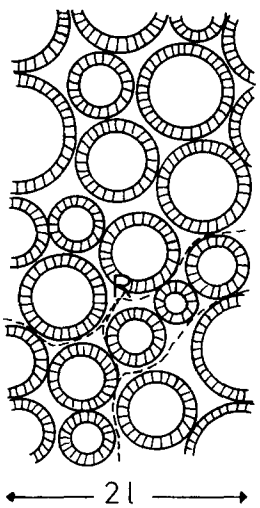

Fig 1 Schematic representation of a cross-section of a thyroid slice (thickness equal to 2*l*) The mean follicular radius is about 100 μm We have also represented (dashed lines) four possible paths of passage of thyrotropin from the incubation medium to a receptor (R) situated on the cellular membrane

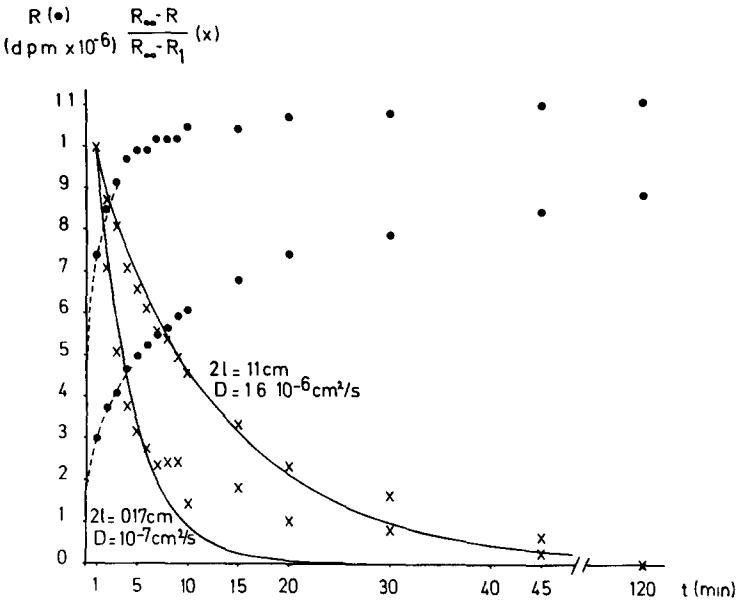

Fig 2 Evolution in time (t, min) of radioactivity R of [^{3}H]sucrose (ordinate) in the incubation medium (●) for two group of slices of thicknesses equal to 0 11 and 0 017 cm We also have represented (×) the value $(R_\infty - R)/(R_\infty - R_1)$ where R_∞ is the radioactivity in the medium after 2 h of incubation and R_1 the radioactivity after 1 min The continuous lines are the simulated curves obtained from Eqn 5 with $2l = 0\ 11$ cm, $D = 1\ 6\ \ 10^{-6}$ cm^2/s and $2l = 0\ 017$ cm, $D = 1\ \ 10^{-7}$ cm^2/s, respectively The dashed lines are the first part of the continuous curves expressed in units R They show that the extrapolation to time zero is very far from the origin of the coordinates

experiment represented in Fig 2 where measurements were performed every minute during the first 10 min To calculate the discharge of the extracellular space of the slices we therefore take the radioactivity (R_1) in the tissue at the shortest discharge time (1, 2 or 5 min) of the experiment as 1, and zero at the longest time (R_∞) (2 or 4 h). Thus, by dividing the radioactivity remaining in the slices at any time ($R_\infty - R$) by $R_\infty - R_1$, we express the disappearance of tracers out of the slices as fractions varying between 1 and 0 (Fig 2)

The [^{3}H]sucrose (spec act 1 2 Ci/M), the [^{3}H]inulin (spec act 300 Ci/M) and the ^{131}I-labelled albumin (spec act 20–80 μCi/mg) were obtained from Radiochemical Center (Amersham, U K) Soluene 350 and Omnifluor were obtained from Packard Instrument Co (Downers, Grove, U S A) and NEN Chemicals GmbH (Frankfurt am Main, Germany)

MATHEMATICAL SIMULATION

The geometrical boundaries of the domain where extracellular effector concentrations are not negligible is of extreme complexity Some possible paths of such effectors are represented on Fig 1 In the experimental conditions discussed below, the problem can be reduced, in a first approximation, to the resolution of the classical one-dimension diffusion equation

$$\frac{\partial C(x, t)}{\partial t} = D \frac{\partial^2 C(x, t)}{\partial x^2} \tag{1}$$

where D is the diffusion coefficient and $C(x, t)$ the concentration, function of time t and of the spatial coordinate x (distance to the center of the slice)

The following assumptions are sufficient conditions for the validity of the consideration of Eqn 1 in our analysis the thickness of the slice ($2l$) should be much smaller than the square root of its surface and the slice medium should be homogeneous and isotropic Indeed, the surface of the thyroid slices used in the experiments is of the order of 1 cm^2 and the thickness of 1 mm On the other hand, as the follicular radius is about 0 1 mm one can assume that, relative to the slice dimensions, the medium can be considered as homogeneous and isotropic Indeed, if the molecular transport is due to free diffusion in the interfollicular medium, the follicles constitute only an obstacle to diffusion The diffusion coefficient in Eqn 1 should then be lower than the real diffusion constant in the interfollicular medium We shall see later that this reasoning is incompatible with the experimental results It is expected, a priori, that the calculated diffusion coefficients of extracellular molecules in the slices are compatible with the classical Einstein-Stokes formula

$$D = \frac{RT}{6\pi\eta N r} \tag{2}$$

R is the absolute gas constant, T the absolute temperature, η the viscosity of water, N Avogadro's number and r the molecular radius It is assumed that the molecules are spherical and diffusing in a continuous medium

It is well known, from the classical theory of diffusion, that the order of magnitude of the distance covered by diffusion in a time t is given by $\sqrt{2Dt}$ As both sides of the slices are in contact with the medium, it is reasonable to consider l as the characteristic length of the system then $l^2 = 2Dt$ We call $l^2/2D$ the characteristic time of diffusion The assumption of uniform concentration in the slices is only valid for times greater than the characteristic time For thyroid slices of 1 mm thickness [5] the characteristic time is greater than 1 h (10^{-2} $cm^2/2$ 10^{-6} cm^2 per s $\simeq$ 5 10^3 s) In this case the classical interpretation in terms of kinetic equations, where the assumption of instantaneous homogenization is implicitly admitted, is not longer adequate It is then necessary to consider one kinetic equation for each portion of the slice, at equal distance from the center, and to integrate with respect to the spatial coordinate x in such a way as to reconstruct the whole slice The global result can then be interpreted

We shall solve Eqn 1 with appropriate initial and boundary conditions corresponding to the experiments under study The origin of coordinates is chosen at the center of the slice, the coordinates of the borders being $x = \pm l$

The time of incubation (2 h) is sufficient for a quasi-uniform concentration of the markers in the slice We call this concentration C_0 and the initial condition is given by $C(x, 0) = C_0$, $-l < x < l$ The incubation medium can be considered as infinite because of the large dilution space This assumption also implies that the tracer concentration in the discharge medium will always be close to zero Furthermore, as the medium is continuously agitated, the concentration near the slices borders is close to zero, so that the boundary conditions are $C(+l, t) = 0$ The preceding

initial and boundary conditions completely define the problem from a mathematical point of view and the solution of Eqn 1 can be found in the literature [6]

$$C(x, t) = \frac{4C_0}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \left[\exp -(2n+1)^2\pi^2 \frac{Dt}{4l^2}\right] \cos \frac{(2n+1)\pi}{2l} x \tag{3}$$

The function $C(x, t)$ is represented in Fig 3 for different values of t and for $D = 10^{-6}$ cm^2/s and $2l = 0\,1$ cm For $t = 3600$ s (1 h) the concentration in the center of the slice ($x = 0$) is close to $0\,1\ C_0$ However, we wish to know the evolution, in time, of the quantity of tracer in the slice We shall call it $R_s(t)$ and it is given numerically by the area defined by the curve $C(x, t)$ and the abscissa axis multiplied by the slice surface S

$$R_s(t) = S \int_{-l/2}^{+l/2} C(x, t)\mathrm{d}x \tag{4}$$

The integral in Eqn 4 is well known [6] and $R_s(t)$ can be written explicitly

$$R_s(t) = SC_0 \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp -(2n+1)^2\pi^2 \frac{Dt}{2l^2} \tag{5}$$

The above expression states that the evolution of the quantity of diffusible matter in the slice is given by the sum of an infinite number of exponentials The slices medium is assumed to be homogeneous, isotropic and infinite This series converges very quickly We have represented in Fig 2 the curves $R_s(t)/R_s(60\text{ s})$ for $t > 60$ s, where

$$\frac{R_s(t)}{R_s(60\text{ s})} = \frac{R_\infty - R(t)}{R_\infty - R_1} \tag{6}$$

We have used a visual criterion to fit these curves to experimental points This criterion is, in our opinion, better adapted to the present problem Indeed, the classical least square criterion will overestimate the role of the last points of the curve which depend in a crucial way on what is considered as the equilibrium value It would be necessary to weigh the different points, but an objective criterion is missing It appears very

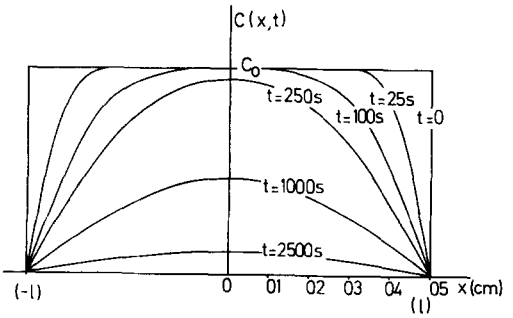

Fig 3 Solutions of diffusion Eqn 1 with $D = 1\ \ 10^{-6}$ cm^2/s and for $-0\,05$ cm $\leqq x \leqq 0\,05$ cm and $t = 25$, 100, 250, 1000 and 2500 s The initial condition is $C(x, 0) = C_0 - 0\,05$ cm $\leqq x \leqq 0\,05$ cm and $C(x, 0) = C_0 - 0\,05$ cm $> x > 0\,05$ cm and the boundary conditions are $C(l, t) = 0$ and $C(-l, t) = 0$, $t \geqq 0$

clearly from Fig 4 that a visual criterion introduces a small error, probably much smaller than all the other inaccuracies associated with this procedure

RESULTS

(a) *Transport of different molecules (sucrose, inulin and albumin)*

In order to verify the possibility of using the Einstein-Stokes equation for different molecules in thyroid slices, the efflux of labeled sucrose (M_r 342), inulin (M_r 5000) and albumin (M_r 70 000) from prelabeled slices has been studied The experimental results and the theoretical simulations are represented in Fig 4

It is clearly apparent from Fig 4 that there is no significant difference between the experimental points of sucrose, inulin and albumin release The three independent experiments made with sucrose show only a small dispersion of the results If the transport were described with a diffusion coefficient given by the Einstein-Stokes equation, the diffusion coefficient corresponding to the curves of sucrose and albumin would differ by a factor 5 9 If the release of sucrose followed the lowest curve ($D = 2\ 10^{-6}$ cm^2/s), the release of albumin would to be close to the second curve from the top ($D = 0\,4\ 10^{-6}$ cm^2/s), the inulin curve being an intermediate It is clear that none of the preceding assumptions allows for an explanation of the coincidence of the curves corresponding to molecules of different molecular weights

The simplest explanation of Fig 4 consists of the assumption that the tracer molecules are sequestered in the interfollicular spaces Most of these spaces communi-

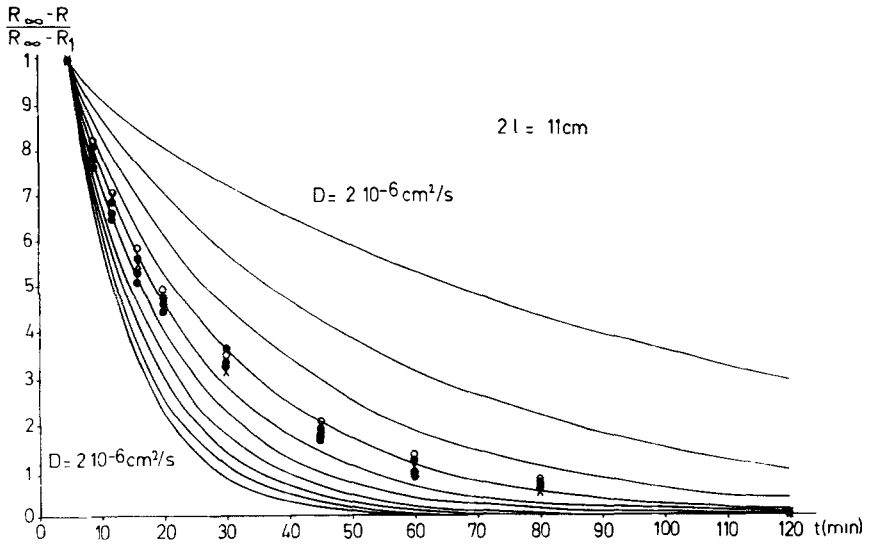

Fig 4 Evolution of the radioactivity in the slices incubated with sucrose (●), inulin (×) and albumin (○) The abscissa axis is the time (t, min) after the moment where the slices are dropped in the discharge medium The ordinate axis is the radioactivity remaining in the slice normalized as in Fig 2 The shortest measured time is 5 min in all these experiments and then R_1 corresponds to $t = 300$ s The continuous curves are equal to $R_s(t)/R_s$ (300 s) and calculated from Eqn 5 D is equal to 0 2 10^{-6} cm^2/s for the upper curve and 2 10^{-6} cm^2/s for the lower one The variation of D between successive curves is equal to 0 2 10^{-6} cm^2/s All the slices had thicknesses close to 0 11 cm

cate between themselves only because of their volume changes due to the mechanical action of the agitator Indeed, the continuous agitation induces mechanical constraints which are randomly distributed in the slice These constraints establish communications between neighbouring interfollicular spaces As there is no preferential direction in the slice (isotropy) and the interfollicular spaces are homogeneously distributed in the slice, such a process can still be described by a diffusion equation However, the diffusion coefficient is no longer related to the particular characteristics of the molecules and consequently the Einstein-Stokes equation cannot be used This explanation is compatible with the shape of the experimental curves which are close to the solutions of the diffusion equation There is, however, a systematic difference for long times which could be interpreted as due to the slow release of phagocytized molecules It should be noted that if we normalize all the curves so that concentration is assumed to be zero at 80 min instead of 120 min, the theoretical curves fit much better the experimental points This is compatible with the preceding explanation

The slices can be considered as a network of compartments (interfollicular spaces) each one sending periodically a pulse to neighbouring compartments It can be shown from the random walk theory that such a process can be described by a diffusion equation [7] This picture implies that inside each interfollicular space the molecular mixing is perfect and instantaneous Such a description constitutes probably the simplest explanation compatible with the experimental results If simple molecular diffusion was the only mixing mechanism in the interfollicular spaces one would expect some significant difference for the discharge curves corresponding to molecules of different molecular weights

It is difficult to describe the other possible mixing mechanisms even from a qualitative point of view However, one can exclude large convective movements between interfollicular spaces Indeed, if a large part of the interfollicular volumes flowed in and out during each agitation, a significative decrease in concentration should be observed after some oscillations of the agitator or some seconds in time scale It is clear from Fig 4 that a significant decrease of the radioactivity in the slices is observed only after some minutes The convective velocity of the molecules is then small It is, however, possible that some turbulence exists in the interfollicular spaces, particularly because of the non-rigidity of the geometrical boundaries of these spaces Such a turbulence would greatly enhance molecular mixing

The new diffusion coefficient D is now an empirical parameter which we call diffusivity and can be calculated by fitting the simulated curves to the experimental points If our hypothesis of the mechanical role of agitation on transport in the slices is correct, a significant dependence between the diffusivity and the frequency of the agitation should be observed

(b) *Dependence between diffusivity and frequency of the agitation*

With similar slices two different frequencies of the agitator have been tried 70 and 100 min^{-1} In two experiments, we found, respectively, $D = 0\,8\ \ 10^{-6}$ and $1\ \ 10^{-6}$ cm^2/s for the lower frequency and $D = 2\,8\ \ 10^{-6}$ and $3\ \ 10^{-6}$ cm^2/s for the higher frequency This means that, to cover the same distance in the slice, the molecules need about 300 % more time when the frequency of the agitator changes from 70 to 100 min^{-1} (43 %) The dependence between the transport in the slices and the frequency of the agitator is then clearly established

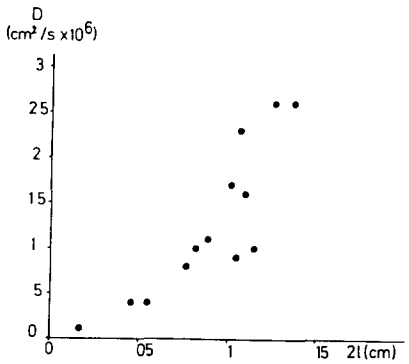

Fig 5 The diffusivity (D) calculated from 13 independent experiments is plotted in function of the corresponding slice thickness ($2l$)

(c) *Dependence between diffusivity and thickness of the slices*

We have grouped in Fig 5 all the diffusivities calculated from 13 experiments The amplitude and the frequency of the agitator was the same A highly significant linear correlation ($r = 0\,853$, $P < 0\,001$) between diffusivity and slices thickness was found However, as there is no physical justification for a linear relationship we have not drawn the linear regression line This is the expected result if our interpretation holds Indeed, the mean distance between the center of interfollicular spaces is about 100 μm The description in terms of a diffusion equation is only valid if the thickness is much greater than the interfollicular distance Only in this case does the assumption of homogeneity of the medium hold For very thin slices, the relative distances to be covered in relation to thickness are greater It is also possible that the mechanical factors are relatively less important, in this case

DISCUSSION

It is generally assumed that the penetration or release from the extracellular space of tissue slices are instantaneous or obey the laws of simple diffusion [2, 3, 8–11] This study shows that classical diffusion theory cannot explain the similar efflux from thyroid slices of molecules of such widely different molecular weights as albumin, inulin and sucrose However, solutions of the diffusion equation can be fitted to experimental points if one attributes a different meaning to the diffusion coefficient We call it diffusivity It cannot be related to the molecular weight by the Einstein-Stokes equation and depends on the frequency of the agitator and on the thickness of the slices

The conclusions outlined above have a direct bearing on the kinetic analysis of the action of various effectors such as thyrotropin on thyroid slices metabolism [8, 12] The different results published in the literature are only compatible if they are obtained with slices of the same thickness and with identical agitators Furthermore, even in such conditions difference of structure between thyroids of different animals could introduce an important dispersion of the calculated diffusivity This consideration should imply some caution in the comparison of kinetic results obtained in different laboratories or in the same laboratory using slices of different thicknesses

for different metabolic measurements [12, 13] An evaluation of diffusivity in thyroid slices would permit one to allow for this factor in the study of effectors of thyroid metabolism Similarly in any metabolic study, for which kinetic data or uptake or release are measured during the first 30 min, diffusivity should be taken into account

A similar analysis could be applied to kinetic studies of slices of other highly organized tissues (e g brain, kidney, lung, etc) The situation in vivo is very different and certainly more complex Agitation is absent but there is the action of pulsatile pressure, diffusion must occur through the capillary walls but, the distance to be covered by any molecule between capillary and follicle is smaller, etc

ACKNOWLEDGEMENTS

The authors would like to thank Mrs Y Van Passen for revising the English text, Mr L Szabo for his technical collaboration, and Mrs D Legrand for the drawing of the figures and the typing of the manuscript This work was supported by the contract of the Ministère de la Politique Scientifique within the framework of the Association Euratom-University of Brussels-University of Pisa

S S is a Fellow of the Institut pour la Recherche Scientifique dans l'Industrie et l'Agriculture (I R S I A)

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