A dialysis rate method for the measurement of free iodothyronine and steroid hormones in blood

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Summary. From the dialysis rate of a small amount of tracer hormone from a serum sample through a semipermeable membrane towards an identical serum sample, the free hormone fraction can be calculated. This method is free from a number of artefacts that may occur in equilibrium dialysis.

Dialysis of diluted or undiluted serum containing a tracer amount of radioactive labelled iodothyronine or steroid hormone against an identical sample without added tracer will ultimately result in equal distribution of radioactivity over the 2 dialysis compartments. The rate at which this equilibrium state is approached depends not only on the permeability of the dialysis membrane, its area and sample volume, but also on the fraction of added tracer that is not bound to serum proteins. As labelled and unlabelled hormones are equally partitioned over free and protein-bound forms, the dialysis rate of tracer hormone depends on the free hormone fraction. This may be understood from the fact that half of the amount of tracer present must migrate to the other compartment, but it can diffuse through the membrane only when it is in the free form. Consequently, when only a small proportion of the hormone is free, it will take longer before final equilibrium is reached than with higher free hormone fractions. The dialysis rate is conveniently expressed by the (radioactive) isotope distribution as a function of time. A mathematical expression, describing the relationship between tracer distribution, dialysis time, membrane permeability, free hormone fraction and other constants of the system can be readily derived, as will be shown below. Once the permeability constant of a cell is established, this relation permits calculation of the free hormone fraction f from tracer distribution and dialysis time.

Theory. A dialysis cell consisting of 2 identical compartments with volume V, separated by a semipermeable membrane with area A, is filled on both sides with the same sample (diluted or undiluted serum). The concentrations of tracer are c_1 and c_2, respectively. Initially, tracer is added to the 1st compartment so at $t = 0$, $c_1 = c_0$ and $c_2 = 0$. Isotope distribution I is defined as $(c_1 - c_2)/c_0$. A fraction f of the total hormone present is unbound. The same applies for the tracer hormone alone. It is assumed that f is constant and uniform throughout the whole process. This implies that the following conditions must be fullfilled: a) Addition of tracer does not result in an increase of the free fraction due to the saturation effect. b) Equilibration between the hormone and its binding proteins is instantaneous compared with the diffusion rate of tracer through the membrane, i.e. equilibrium is maintained in spite of the fact that the free tracer concentration continually falls in the 1st compartment and rises in the 2nd. c) The contents of the cell are well stirred.

Besides it is assumed that diffusion through the membrane follows Fick's first law, which implies a 1st order process. According to this law, the net flow of material through unit cross-section per unit time, or flux, is proportional to the concentration gradient of diffusible material.

In the present case, the tracer flux is proportional to the concentration difference of free tracer between the 2 compartments, i.e. $f \cdot (c_1 - c_2)$. The net flow of tracer S through the membrane per unit time is then given by:

$$S = A \cdot D \cdot f \cdot (c_1 - c_2) \qquad (1)$$

Free hormone fractions measured in serum pools (for explanation see materials)

Sample	Percentage free hormone			
	Aldosterone	Cortisol	T3	T4
A	31.74	5.77	0.233	0.0223
B	31.44	5.64	0.192	0.0180
C	31.14	6.24	0.212	0.0228

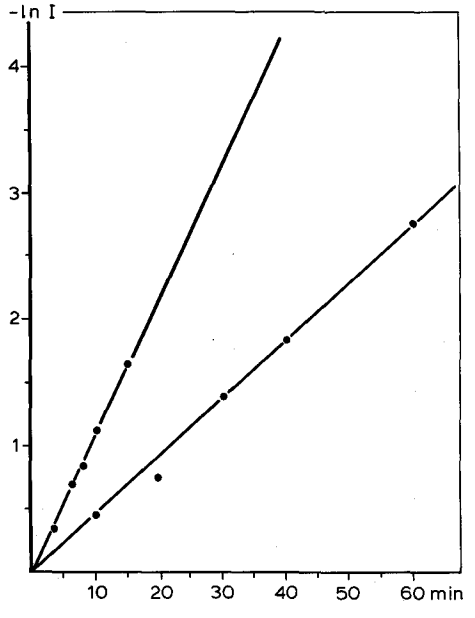

Fig. 1. Log tracer distribution vs. dialysis time for T4 tracer in buffer in micro cells (upper curve) and macro cells (lower curve).

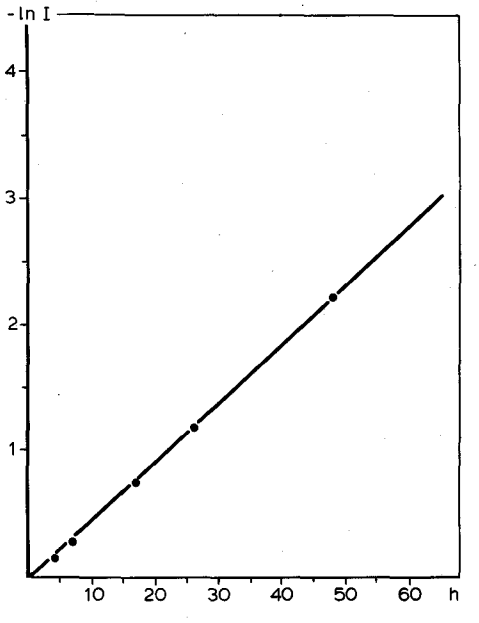

Fig. 2. Log tracer distribution vs. dialysis time for T4 tracer in 1:50 diluted poolserum (macro cells).

The proportionality factor D comprises the diffusion coefficient of the hormone in the solvent used, membrane thickness and a factor relating the effective diffusion area of the membrane to its total area. It will be referred to as the 'membrane diffusion coefficient'.

The increase in total tracer concentration in the 2nd compartment, dc_2, in a time dt is given by the material balance:

$$dc_2 = (S/V)dt \quad (2)$$

Putting $c_1 = c_0 - c_2$, combining (1) and (2) and rearranging gives:

$$dc_2/(c_0 - 2c_2) = (ADf/V)dt \quad (3)$$

which on integration yields:

$$-\ln(c_0 - 2c_2) = 2ADft/V + C \quad (4)$$

The integration constant C is found from the initial condition $c_2 = 0$ as $t = 0$. Consequently:

$$-\ln((c_1 - c_2)/c_0) = 2ADft/V \quad (5)$$

Eq. (5) makes it possible to calculate the free hormone fraction f from tracer distribution $I = (c_1 - c_2)/c_0$ and dialysis time t, if the product 2AD/V which will be called 'cell permeability constant', is known. This constant in its turn is calculated from I and t when f is known, which is the case in the absence of binding proteins so that $f = 1$. The 'membrane diffusion coefficient' D should be independent of cell dimensions and can be calculated when A and V are known. The proportionality between ln I and time t should be tested experimentally in order to verify the assumption of a 1st order process. According to Oppenheimer and Surks[2], f may be divided by the dilution factor of the serum in the cell to obtain the free hormone fraction f_0 for undiluted serum. This is mainly of interest for the thyroid hormones, which have extremely low free fractions, so that these are often analysed in dilute serum. This well-known hypothesis combined with (5) predicts a proportionality between ln I and serum dilution. The constancy of f during the whole procedure is not easily demonstrated, but the assumption is supported by the observation that dialysis rates are very much lower than dissociation rates of the hormone-protein complexes which were estimated in separate experiments.

Materials. A 'Dianorm' (Diachema AG, Switzerland) apparatus consisting of Teflon precision dialysis cells was used for all experiments. Either cells with a working volume of 2×1 ml and a membrane fitting of 4.5 cm^2 or microcells with a working volume of 2×0.2 ml and membrane fitting of 2 cm^2 were used, together with membranes cut from 'Spectrapor 2' dialysis tubing, which excludes substances with molecular weights above 12,000–14,000. All experiments were carried out at 37 °C. Tests were conducted with aldosterone, cortisol (tritium label), thyroxine (T4) and 3,5,3′ triiodothyronine (T3) (^{125}I label) tracers obtained from different sources. Percentages of free hormone were measured in a poolserum from healthy males (A), healthy pregnant subjects (B) and a large pool of hospitalized and outpatient clinic patients (C).

Results and discussion. Figure 1 shows the linearity of log tracer distribution with dialysis time for thyroxine (T4) tracer in phosphate buffer without any protein. As $f = 1$, the slopes of these curves give, according to (5), the 'cell permeability constant' 2AD/V for the micro cells (upper curve) and the larger cells (lower curve). The cell constants were 6.25 and 2.87 h^{-1} and values for D calculated therefrom were 0.325 and 0.319 cm h^{-1}, respectively. Similar results were obtained when cortisol or aldosterone tracers were used, which indicates that no specific interactions between these hormones and the membrane occur. The linearity of log tracer distribution with dialysis time is also shown in figure 2 where T4 tracer was added to 1:50 diluted poolserum and tracer distribution was measured after a number of different time intervals. Not shown here is the observed linearity of ln I and serum dilution. Attention is drawn to the time-scales of figures 1 and 2, which differ by a factor 60. It can be shown that highest precision in the measurement of the isotope distribution is attained for values near 0.5, i.e. when the system is about halfway to its final equilibrium. Therefore, dialysis time and/or serum dilution should be properly adjusted to obtain values of I in that range. Accordingly, the values in the table were obtained with undiluted serum for cortisol and aldosterone, with dialysis times of 2 h and 0.5 h respectively and in serum diluted 1:20 for T3 and T4. Here the dialysis times were 2 h and 18 h. The values shown in the table, which were obtained with this method, lie in the range of those reported by others using different techniques, for aldosterone[3], cortisol[4], T3[5,6] and T4[2,5–8].

Not all aspects of the present method are entirely new. Serum to serum dialysis was described by Korsgaard Christensen[9]. However, this author made use of the proportionality between the diffusion rate across the membrane and free hormone fraction, which exists at the start of the process when c_2 is still negligible. Moreover, no attempt was made to measure the cell permeability constant, so the method, which was applied for T4, gave values expressed in arbitrary units instead of true free T4 fractions.

Methodologically, in our opinion, this dialysis rate method compares favourably with conventional equilibrium dialysis in the following respects: Except for the tracer, the solutions on both sides of the membrane are identical, which means that a) the composition of these solutions is constant during dialysis and b) no osmotic pressure differences and Donnan effects can occur, which in equilibrium dialysis may lead to dilution of dialysand[10] and unequal distribution of free hormone over dialysand and dialysate[11]. Furthermore, c) the presence of binding proteins on both sides of the membrane completely prevents adsorption of ligand to membrane or cell walls, and d) the method is intrinsically less susceptible to interference by radioactive contaminants of the tracer because a much larger proportion of (intact) tracer migrates to the other compartment than with the equilibrium method; so tracer impurities affect the observed tracer distribution to a much lesser degree. This is of particular importance for free T3 and even more for free T4[2,6,7,9]. On the other hand, the equilibrium dialysis method is independent of membrane diffusion constants and does not involve assumptions about the reaction rates of hormone and binding proteins (vide supra). In all instances where these assumptions are valid, i.e. when hormones interact with normally occurring serum proteins, the dialysis rate method forms a useful alternative to conventional equilibrium dialysis.

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