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Studies of rates of transfer of D_2O, H^{3*}, Cl^{36}, Na^{22}, and Mg^{28} across the isolated pericardium of dogs

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A method is described for the study of the rates of transfer of tracer isotopes (D_2O, H^3, Cl^{36}, Na^{22}, and Mg^{28}) across the isolated pericardium of dogs. This method has applications in tracer studies in other biologic fields. The mean coefficients of transfer across the isolated pericardium in vitro were as follows: D_2O, 3.06×10^{-2}; H^3, 2.31×10^{-2}; Cl^{36}, 1.95×10^{-2}; Na^{22}, 1.75×10^{-2}; and Mg^{28}, 1.00×10^{-2} (milliliters per centimeter² per minute). The coefficients of transfer of D_2O and H^3 were essentially the same and faster than those of Cl^{36} and Na^{22}, which in turn were faster than those of Mg^{28}. These coefficients of transfer were compared with those determined in vivo, which were consistently lower. The coefficient of transfer of Cl^{36} across a pericardial segment frozen overnight and thawed was higher than that across the freshly isolated segment of pericardium.

The transfer of water and electrolytes across biologic membranes has been studied extensively in recent years, but the pericardium has been neglected except for recent studies in this laboratory.[1] In a continued investigation of the transfer of fluid and electrolytes across the pericardium of dogs, isolated segments of pericardium have been utilized for the determination of rates of transfer of D_2O, H^3, Cl^{36}, Na^{22}, and Mg^{28} as tracers. This report summarizes the results of the observations to date. In addition, by way of comparison, the results of some studies of rates of transfer across the intact pericardium in vivo are included, although these will be reported separately in more detail.

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Materials and methods

The assembly used is shown in Fig. 1. A suitable piece of freshly isolated parietal pericardial segment from dogs (approximately 5 by 5 cm.) was mounted as a membrane between the ground edges of the 2 cylindrical chambers *A* and *A'*, and 150 ml. of the test solution was placed in each chamber. The serous surface of the pericardium faced chamber *A'*. Two small magnetic bars *M* and *M'* were placed in the chambers to provide stirring. This apparatus was placed in water bath *W* which was maintained at 37° C. throughout the experiment. Then 150 ml. of modified Ringer's solution (pH 7.4) containing the test isotope was placed in the chamber *A'*. Immediately after the test solutions were placed in the respective chambers, the first samples (0.1 ml.) from the chambers *A* and *A'* were removed. This procedure was repeated at 30 minute intervals during the experiments, which lasted 240 minutes.

The methods used in the studies with the intact pericardium in vivo have been described previously.[1] Briefly, the pericardium was exposed through a substernal incision and a soft polyethylene catheter (1.8 mm. outside diameter) inserted and secured in a leak-proof, purse-string suture. Fifty milliliters of Ringer's solution containing the isotope was instilled in the pericardial cavity and the thorax was closed. Sampling was carried out at intervals of 2 minutes for the first 10 minutes, then at intervals of 5 minutes for the remaining period of 20 minutes. The volume of fluid remaining at the end of the 30 minute period was determined by aspiration.

D_2O concentration in the test fluid was determined by measuring the freezing point with the Fiske osmometer as previously described.[2] The accuracy of the osmometric method for measuring the D_2O concentration in the electrolyte solution was verified by repeat measurements of D_2O concentration in water distilled from the samples. The presence of electrolytes and other substances in the test fluid did not alter the accuracy of the method. The initial apparent osmolality of the test fluid was 164 ± 10 mOsm. per liter (5.0 ± 0.2 per cent D_2O in Ringer's solution).

H^3 was measured with Tri-Carb liquid scintillation spectrometer, and Cl^{36}, Na^{22}, and Mg^{28} were measured with a Baird-Atomic gas flow automatic counter.

The mathematical analysis used in this study is outlined on the following pages. These symbols are used in the equations:

t = time
M_1 = amount of tracer in compartment 1,
M_2 = amount of tracer in compartment 2,
V_1 = volume of compartment 1,
V_2 = volume of compartment 2,
C_1 = M_1/V_1 concentration of tracer in compartment 1,
C_2 = M_2/V_2 concentration of tracer in compartment 2,

Table 1. Mean coefficients of transfer in milliliter per centimeter² per minute of D_2O, H^3, Cl^{36}, Na^{22}, and Mg^{28} across the freshly isolated pericardium and the intact pericardium of dogs

Isotope	Coefficient of transfer (k)	
	Isolated pericardium*	Intact pericardium†
D_2O	3.06×10^{-2}	1.55×10^{-2}
H^3	2.81×10^{-2}	—
Cl^{36}	1.95×10^{-2}	0.27×10^{-2}
Na^{22}	1.75×10^{-2}	0.27×10^{-2}
Mg^{28}	1.00×10^{-2}	0.20×10^{-2}

*Mean of 10 experiments for D_2O, Cl^{36}, Na^{22}, and Mg^{28}, and 5 for H^3.
†Mean of 5 experiments for D_2O, 10 for Cl^{36}, 8 for Na^{22}, and 9 for Mg^{28}.

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A = area of membrane boundary between compartments 1 and 2,
k = coefficient of transfer across membrane expressed in amount of tracer per unit time, area, and concentration. It has the dimensions of volume per unit area and per unit time (milliliters per centimeter² per minute).
e = base of natural log.

(The additional subscript o is used to indicate an initial mass (t = o), concentration, or volume. Thus C_{o1} is the initial concentration of tracer in compartment 1.) For transfer, "diffusion-like," processes (which obey the laws of first-order kinetics) between two compartments in both of which instantaneous and complete mixing is assumed to occur, the following equations can be written:

$$\left.\begin{aligned} \frac{dM_1}{dt} &= -kA\ (C_1 - C_2) \\ \text{and} \quad \frac{dM_2}{dt} &= +kA\ (C_1 - C_2). \end{aligned}\right\} \qquad (1)$$

Since $$M_1 = C_1V_1 \qquad M_2 = C_2V_2 \qquad (2)$$

$$\left.\begin{aligned} \text{therefore,} \quad C_1 \frac{dV_1}{dt} + V_1 \frac{dC_1}{dt} &= -kA\ (C_1 - C_2) \\ \text{and} \quad C_2 \frac{dV_2}{dt} + V_2 \frac{dC_2}{dt} &= kA\ (C_1 - C_2). \end{aligned}\right\} \qquad (3)$$

The solution of Equation 3 is straightforward and depends on boundary conditions and the time variation of those terms which may be considered to be parameters. Solutions for the cases in this paper are as follows:

Case 1. If $V_1 = V_2 = V$ (constant), $C_{o1} = 0$, then

at time $t = \infty$, $\quad C_1]_{t=\infty} = C_2]_{t=\infty} = \frac{1}{2} C_{o2}$.

$$\text{Therefore, } C_1 = \frac{1}{2} C_{o2} \left(1 - e^{-\frac{2kA}{V}t}\right) \qquad (4)$$

$$C_2 = \frac{1}{2} C_{o2} \left(1 - e^{-\frac{2kA}{V}t}\right) \qquad (5)$$

$$\text{and} \quad k = -\frac{V}{2At} l_n \left(\frac{C_{o2} - 2C_1}{C_{o2}}\right)$$

$$= -\frac{V}{2At} l_n \left(\frac{2\ C_2 - C_{o2}}{C_{o2}}\right). \qquad (6)$$

Case 2. If $V_2 \gg V_1$ and $C_{o2} = 0$, but V_1 is varying linearly with time according to $V_1 = V_{o1}\ (1 - at)$, then C_2 is always essentially 0.

$$\text{Therefore, } C_1 = C_{o1}\ (1 - at)^{\frac{kA}{aV_o} - 1} \quad \text{for } at \leq 1 \qquad (7)$$

$$\text{and} \quad k = \frac{aV_{o1}}{A} \left[\frac{l_n \frac{C_1}{C_{o1}}}{l_n\ (1 - at)} + 1 \right] \qquad (8)$$

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$$\text{or} \qquad k = \frac{aV_{01}}{A}\left[\frac{\left.\frac{dC_1}{dt}\right|_{t=0}}{-a\,C_{01}} + 1\right].$$

Case 1 applies to the in vitro experiments with the isolated pericardial segment whereas Case 2 applies to the in vivo experiments with the intact pericardium. The coefficient of transfer k was calculated by Equation 6 for the in vitro experiments, and by Equation 8 for the in vivo experiments. The assumption of linear volume change with time in Case 2 is arbitrary but the possible error introduced is felt to be within the general range of error of the experiments. The area of the pericardial segment in the in vitro experiments was 15.2 cm.^{2} In the in vivo experiments the pericardial area was estimated by the method previously described[1] and ranged around 300 cm.^{2}

Results and discussion

The mean coefficients of transfer, k, for the various isotopes across the isolated pericardium are listed in Table I. Also included in the table for comparison are corresponding coefficients of transfer obtained for the in vivo experiments. Fig. 2 shows the mean concentration time course curves and standard deviations for each isotope for the in vitro studies. In all experiments the concentrations of tracers in both compartments approached equality ($Ct/C_0=0.5$) and no concentration gradients were observed for the ions studied.

The mean values of k for D_2O (3.06 times 10^{-2} ml. per centimeter^2 per min-

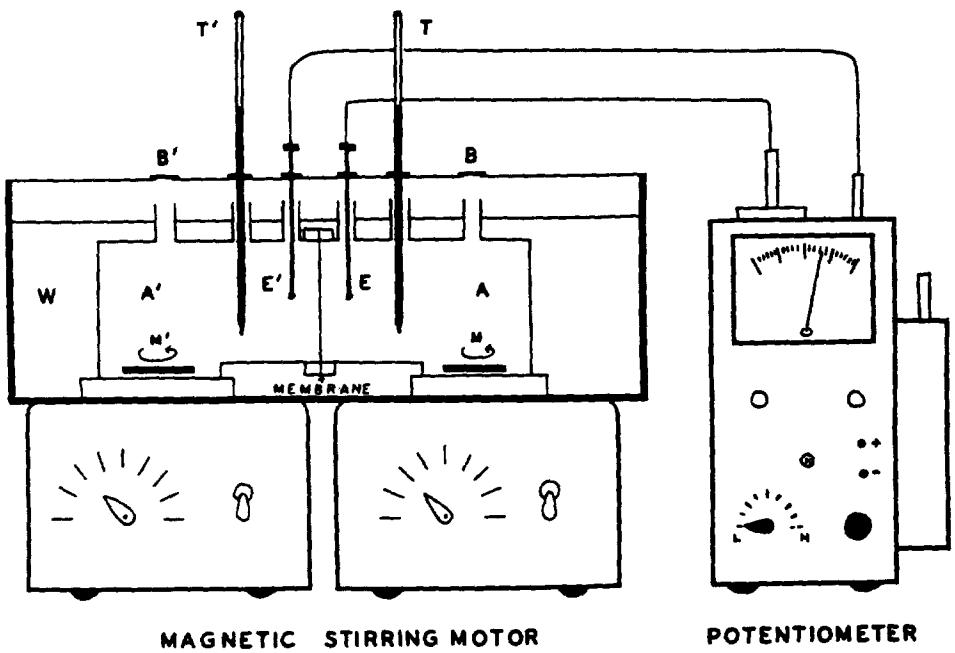

Fig. 1. A method for measuring the transfer of D_2O, H^3, Cl^{36}, Na^{22}, and Mg^{28} across freshly isolated pericardium of dogs. Small magnets provide mixing in each chamber *A* and *A'*. Consult text for details.

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ute and H^3 (2.81×10^{-2} milliliter per centimeter^2 per minute) were sufficiently close to justify the assumption that they behaved similarly as tracers of H_2O for the concentrations used. As derived in these studies k has the significance of a permeability coefficient.[3, 4] For the sake of comparison a few permeability coefficients for other membranes derived from tracer studies without pressure gradients across the membrane have been collected from the literature. Permeability coefficients for water determined in experiments with osmotic or hydrostatic pressure across the membranes are higher than those determined with tracer studies and equal activity of water on both sides of membrane, so that one must exercise caution when making comparisons.[5-7] Reduced to a common

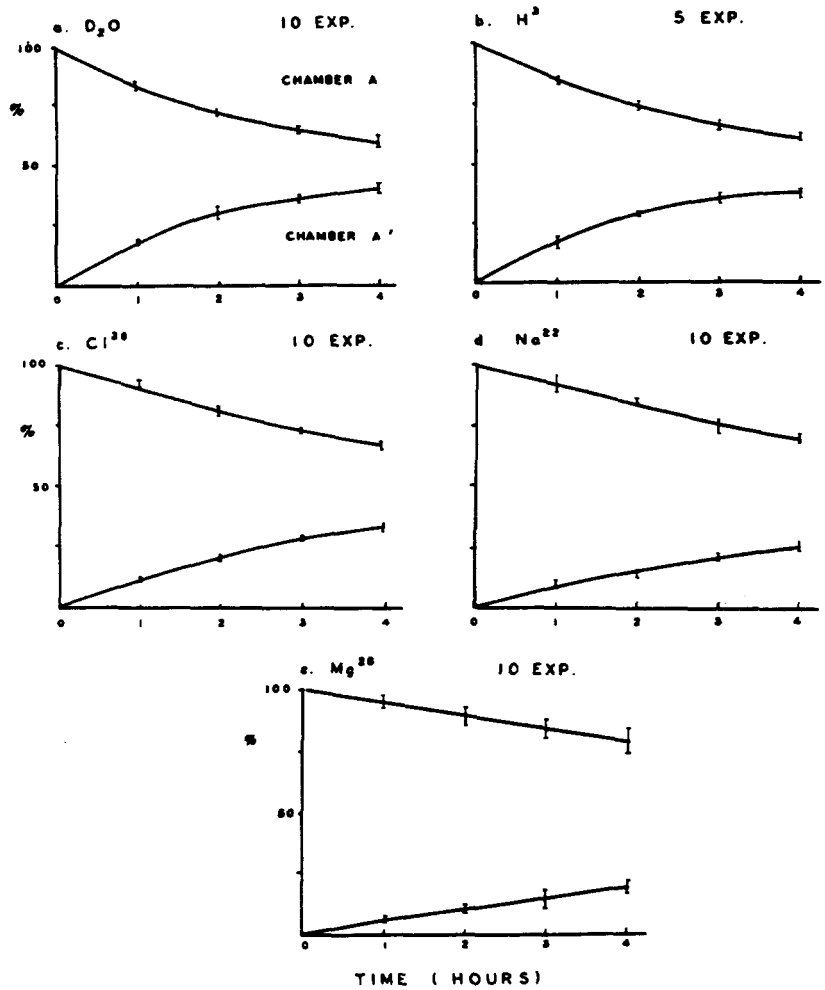

Fig. 2. Concentration time course curves of D_2O, H^3, Cl^{36}, Na^{22}, and Mg^{28} in Ringer's solution with freshly isolated pericardium of dogs. The short bars on the curves are the standard deviations for each point. Ten experiments were performed with D_2O, Cl^{36}, Na^{22}, and Mg^{28}, and 5 experiments were performed with H^3.

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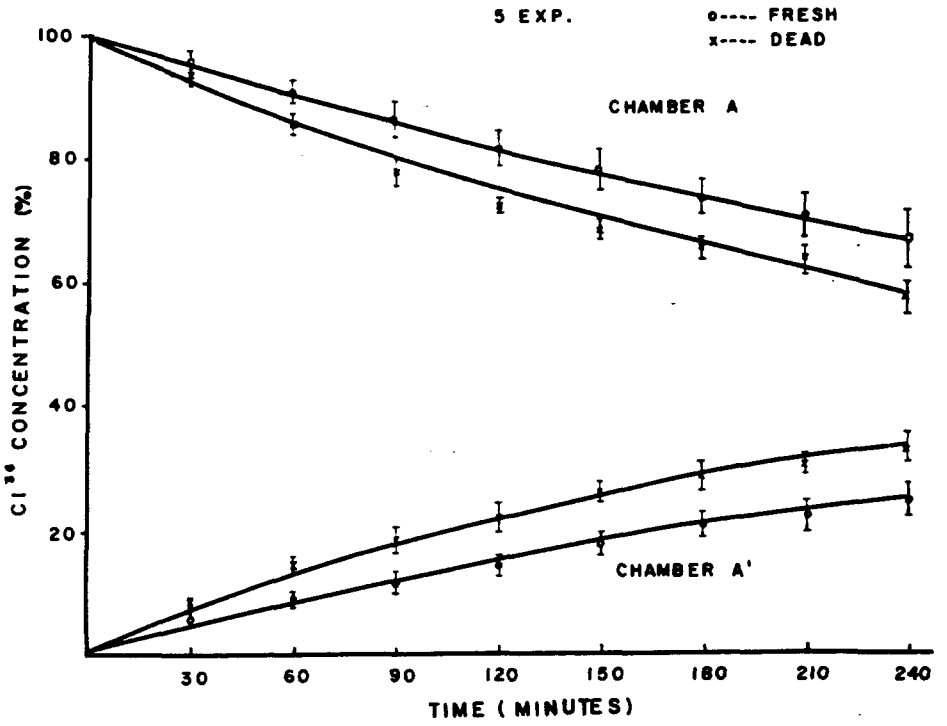

Fig. 3. Comparative studies of rate of transfer of Cl^{36} across the isolated "fresh" and "dead" pericardium of dogs. The short bars on each experimental period represent the standard deviations.

dimension of μ per second, the permeabilities to water reported for other membranes are as follows: the red cell of dog, 63[8]; fibroblast in tissue culture, 62[9]; the pericardial membrane of dog, 5; frog skin, 0.73[10] and 1[6]; frog gastric mucosa, 0.48[11], and dog ileum, 0.3.[12] The above values are all from experiments performed at 20 to 25° C. except for the present studies. The permeability of the pericardium to water seems to be substantially less than that of the cell membrane of the fibroblast or red cell, but greater than the complex membranes of frog skin and frog gastric mucosa and dog ileum.

The coefficients of transfer of Na^{22} and Cl^{36} across the isolated pericardium wer similar (1.75×10^{-2} ml. per centimeter^2 per minute and 1.95×10^{-2} ml. per centimeter^2 per minute, respectively) and less than those for D_2O and H^3, but greater than that for Mg^{28} (1.00×10^{-2} ml. per centimeter^2 per minute). The k values determined in vivo were lower for each isotope tested than the corresponding k values determined in vitro (Table I). Because of the small number of experiments, the data are not suitable for statistical analysis, but the consistency of the results is apparent. The reasons for a lower k in vivo are speculative, but they may include slow mixing in vivo, artificial increase in permeability because of alterations of physiologic functions in vitro, or inhomogeneity of the membrane with regard to permeability. For example, the visceral and parietal

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pericardium may differ in their permeability characteristics (only the parietal pericardium was used for the in vitro studies).

An attempt was made to assess the importance of the "freshness" of the pericardial segment, by comparing the coefficient of transfer of Cl^{36} across freshly isolated pericardium with the coefficient of transfer of Cl^{36} across segments previously frozen overnight and thawed. The average coefficient of transfer of Cl^{36} (3.76×10^{-2} ml. per centimeter^2 per minute) was higher after overnight freezing (Fig. 3). This lends support to the contention that there may be alterations of the pericardium in the isolation procedure which increases the permeability.

The electrical potential difference across the isolated pericardial membrane separating Ringer's solution on both sides was measured on numerous occasions and never found to exceed a few millivolts.* The significance of this low potential difference across the membrane is unknown.

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