Rate of Exchange of Sodium and Potassium Between Amniotic Fluid and Maternal System.* (21122)

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In a previous communication(1) we demonstrated that the mechanism of deuterium exchange between amniotic fluid and maternal circulation is that demanded by the theory of multicompartment systems, a general treatment of which has been presented by Sheppard and Householder(2). The exchange rate between the 2 compartments in 2 women at term was determined to be about 30 moles of water per hour, indicating a continuous formation, flow and reabsorption of the amniotic fluid.

There exists, in all probability, an electrolyte equilibrium between amniotic fluid and maternal plasma, which is analogous to that of lymph, cerebrospinal-, and extracellular fluid. The pathologic depletion or accumulation of water in these hypothetical compartments is associated with or conditioned by a disturbance in the electrolyte transfer, changes in hydrostatic pressure and the osmotic equilibrium. A fundamental step for further investigations on the etiology of these pathologic states would be a study of the rate of exchange of electrolytes, particularly that of sodium and potassium.

A comparison of these exchange rates could be used to evaluate the hypothesis that the amniotic fluid is not a transudate or formed by ultrafiltration of plasma, although the concentration of electrolytes in plasma and amniotic fluid is of the same order of magnitude. The present investigation was intended to give information on the quantity of sodium and potassium exchanged per hour and to demonstrate that sodium, potassium, and water exchange at their own characteristic rates.

The assumptions necessary for the application of tracers to the study of multicompartment systems are essentially the same as those previously cited(2). The expression for a 2-compartment system in a steady state

$$\log_e\left[\frac{a_1 - a_2}{a_{1(0)}}\right] = \rho t\left[\frac{1}{S_1} + \frac{1}{S_2}\right] = \frac{0.693\ t}{T_{1/2}}$$

is applicable to a multiplicity of tracers provided the designation of a and S is consistent(1). S_1 and S_2 refer to the amount of exchangeable species in compartments 1 and 2, and a_1 and a_2 to the specific activity of the tracer in these compartments at time t; $a_{1(0)}$ is the specific activity in compartment 1 at time zero. ρ is the exchange rate, the dimensions of which are determined by the units of t and S, and $T_{1/2}$ is the time necessary to replace ½ of the exchangeable species. If the logarithmic expression of the above equation is plotted against time, a straight line is obtained whose slope, the disappearance constant, is proportional to the exchange rate. If the amniotic fluid were formed by processes of filtration or diffusion, one would expect the slope of these lines for all constituents to be nearly equal though the exchange rates, expressed as moles per unit of time, may differ. In order to minimize the introduction of errors due to biologic variations, multiple simultaneous tracer experiments were carried out, using isotopes of hydrogen, sodium, and potassium. The tracers used in these experiments were chosen on the basis of the availability of selective analytic methods. Transabdominal puncture was performed on 2 pregnant volunteers at term who had been considered for caesarean section. The isotopes were administered into the amniotic fluid compartment by a method previously described(1,3). Samples of amniotic fluid and maternal blood were collected at predetermined intervals and the concentration of the tracers determined. Deuterium oxide was estimated by the falling drop method. Sodium-22, sodium-24, and potassium-42 were determined by their radio-

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activity. Because of the low dosage administered (3 μc) a Q-gas counter was used to determine the specific activity of the samples containing potassium-42. For the experiment where sodium-22 and sodium-24 were the only radio-tracers a well-type scintillation counter was found to be most advantageous.

The samples were counted immediately after collection and recounted after an interval of about 10 half life times to allow for the decay of sodium-24 or potassium-42. The remaining counts were due to sodium-22, which, when subtracted from the corrected total, permitted the estimation of potassium-42 or sodium-24 activity. The volume of the amniotic fluid was determined by the Congo Red method of Neslen *et al.*(3), and the total body water, total exchangeable sodium and total exchangeable potassium were estimated by extrapolation. The concentration of sodium and potassium in the amniotic fluid was determined by the flame-photometric technic.

Experimental. The first experiment was intended to test the correctness of the assumption that there exists a steady state which implies a constant amount of exchangeable species in both compartments and equal and opposite exchange rates. The experimental verification of this hypothesis is relatively simple for the exchange of sodium, because 2 easily detectable isotopes are available. Sodium-24 was injected into the maternal system and sodium-22 and deuterium oxide introduced into the amniotic fluid. The amount of radio-tracers injected into each compartment was proportional to the size of its pool (milliequivalents or moles of exchangeable sodium). In the determination of

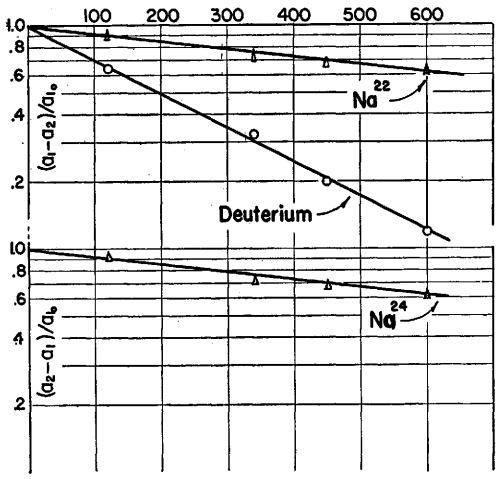

FIG. 1. Semi-log plot of function indicated on left against time in min. Upper cycle refers to transfer from amniotic fluid to mother; lower cycle designates reverse transfer.

TABLE I. Summary of Pertinent Data and Results Relating to Experiments Described in Text. Abbreviations "A.F." and "M.S." Refer to Amniotic Fluid and Maternal System, Respectively.

	Exp. I 30 cc D_2O and 3 μc Na^{22} inj. into A.F. 90 μc Na^{24} inj. into maternal circulation.	Exp. II 30 cc D_2O, 3 μc Na^{22} and 3 μc K^{42} inj. into A.F.
Disappearance constant in min.$^{-1}$ for		
Deuterium (A.F. → M.S.)	.00354	.014
Sodium (A.F. → M.S.)	.000907	.00273
Sodium (M.S. → A.F.)	.000822	—
Potassium (A.F. → M.S.)	—	.00284
Total body water (moles)	1680.0	1720.0
Vol of A.F. (")	126.0	39.0
Total exchangeable sodium (mEq)	2380.0	2460.0
Sodium concentration in A.F. (mEq/lt)	150.0	130.0
Total sodium in A.F. (mEq)	350.0	91.0
" exchangeable potassium (mEq)	—	2920.0
Potassium concentration in A.F. (mEq/lt)	—	3.4
Total potassium in A.F. (mEq/lt)	—	2.4
Exchange rate in moles/hr		
Water (A.F. → M.S.)	25.0	31.9
Sodium (A.F. → M.S.)	.016	.014
" (M.S. → A.F.)	.015	—
Potassium (A.F. → M.S.)	—	.00041

the transfer rate from amniotic fluid to maternal pool, the concentration of the tracer in the latter can be neglected while the determination of the opposite transfer rate necessitates a knowledge of the specific activity in both compartments as a function of time. Fig. 1 represents a semi-log plot of the function $(a_1-a_2)/a_{1(0)}$ against time for sodium-22, sodium-24, and deuterium oxide. The slopes of the lines for the 2 sodium tracers appear to be identical within the limits of error of the method, thus indicating that the transfer rates *in* and *out* of the amniotic fluid compartments are equal. By means of the other essential data listed in Table I the transfer rates were calculated as .016 and .015 mole per hour and 25.0 moles of water per hour.

In a second experiment deuterium oxide, sodium-22, and potassium-42 were injected into the amniotic sac. Since there was no detectable rise of radioactivity in the maternal serum, the value of a_2 was negligible for the 2 radiotracers. In the calculation of the disappearance constant for deuterium oxide, a rise in its concentration in maternal serum was taken into consideration. Fig. 2 represents a semi-log plot of the data obtained by the simultaneous application of deuterium, sodium-22, and potassium-42. The disappearance constants for the 3 tracers from amniotic fluid to maternal system were found to be .014 min.$^{-1}$ for water, .00273 min.$^{-1}$ for sodium and .00284 min.$^{-1}$ for potassium. Substitution of the data of Table I in the above equation permitted the calculation of the exchange rates as 31.9 moles of water per hour, 0.014 mole of sodium per hour and 0.00041 mole of potassium per hour.

Discussion. The transfer of sodium to the amniotic fluid has been investigated by Vosburgh, Flexner, Cowie, Hellman, Proctor, and Wilde(4). They reported that an average of about 6.9% of the sodium of the amniotic fluid is replaced per hour during gestational periods of 12 to 40 weeks. The transfer of water in another series of patients was found by these authors to be about 5 times faster.

There is an appreciable variation in the sodium and potassium concentration of the amniotic fluid which does not always parallel the electrolyte concentration of maternal

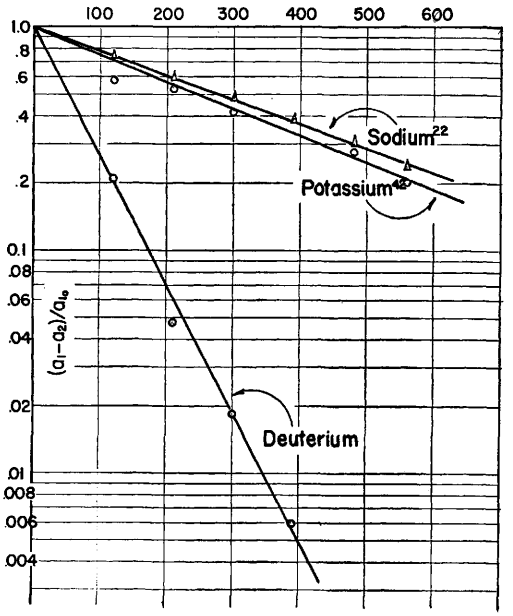

FIG. 2. Semi-log plot of function indicated on left against time in min. All tracers injected into amniotic sac.

serum. The application of average values for the sodium concentration of amniotic fluid and maternal serum may lead to errors, which, when added to the inherent errors in the determination of the compartmental size, would render the analysis worthless. For purposes of comparison the transfer of a species from one compartment to another should not be expressed as per cent of the total amount of the species within the compartment, but expressed as the rate of exchange, the dimensions of which are moles per unit of time. This incorporates variations in the amount of exchangeable species and its disappearance constant. The necessary prerequisite for the application of the theoretic considerations is a demonstration that the rates of exchange in both directions are identical.

The first of our experiments demonstrates that the exchange rates for sodium ion to and from the amniotic fluid are equal, within the experimental error of the methods. In the second experiment the transfer rates of deuterium, sodium, and potassium were determined. A comparison of the data for sodium of this and the preceding experiment shows that the transfer rates (14, 15 and 16 mEq./

hr) are of comparable magnitude while the disappearance constants differ by a factor of 3 (.00273 and .000907 min.$^{-1}$). The disappearance constant for potassium is roughly of the same magnitude as that of sodium, but a comparison of the exchange rates shows that the number of milliequivalents of sodium exchanged per minute is 35 times greater than the number of milliequivalents of potassium. The ratio of these exchange rates is about the same as the ratio of their respective concentrations in the amniotic fluid.

Summary. The exchange rate of sodium to and from the amniotic fluid was determined by the simultaneous application of sodium-22 and sodium-24. This exchange rate, in opposite directions, was found to be 15 and 16 mEq. of sodium per hour, respectively. The transfer rate of hydrogen, sodium, and potassium from the amniotic fluid to the maternal system was determined by the simultaneous application of tracers for each of these elements. The disappearance constants for sodium and potassium were found to be nearly equal, indicating that the same percentage of these elements is exchanged per unit of time, while that for deuterium was found to be 5 times greater. The exchange rates were calculated as 31.9 moles of water, .014 mole of sodium, and .00041 mole of potassium per hour. It is concluded that the water and electrolytes of the amniotic fluid are in dynamic equilibrium with maternal plasma, each exchanging at its own characteristic rate.

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Propagation of Salivary Gland Virus of the Mouse in Tissue Cultures.* (21123)

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The presence of greatly enlarged cells containing large intranuclear inclusions in the salivary glands of several species of rodents has been shown to be associated with a transmissible filterable virus(1-3). In each case the virus has proved to be entirely species-specific. In most species of animals basophilic cytoplasmic inclusions also occur in the cytoplasm of many of the enlarged cells, but in the experimental infections they appear later than the intranuclear inclusions(4). They are seen only occasionally in the salivary glands of infected mice. Intracellular inclusions resembling those occurring in the salivary glands of rodents have been observed in the salivary glands in 10 to 30% of autopsies of infants and young children(5-8) regardless of the cause of death and are present in other organs, as well as the salivary glands, in cases of inclusion disease of infancy. Transmission of the etiologic agent of the human disease to experimental animals has not been reported. The only reported attempt to propagate any of the salivary gland viruses of rodents in tissue cultures is that of Andrews(9). He tried to cultivate the salivary gland virus of the guinea pig in surviving tissue in a modified Maitland medium. Intranuclear inclusions appeared in mononuclear cells in the interstitial tissue in cultures of guinea pig testis and less constantly in cultures of guinea pig ovary, brain, or salivary glands. The results of all attempts to subculture the virus were negative. No inclusions were found in the

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