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Water Exchanges and Barriers as Studied by the Use of Hydrogen Isotopes[1]

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SINCE THE DISCOVERY OF deuterium by Urey in 1931 this isotope has been widely used in the biological sciences for studies in water exchanges and intermediary metabolism. The first biological experiment in which deuterium was employed as a tracer for water appeared in the literature in 1933 (27). Shortly thereafter there were numerous other reports of its use in water exchange studies (5, 10, 12, 13, 19, 28–32, 41, 44, 50) and in investigations of intermediary metabolism (4, 56, 62–64). A third isotope of hydrogen, of mass three and called tritium, was discovered in 1939 (1) and within a few years began to take its place with deuterium as a tracer for hydrogen in biological experimentation (6, 48, 52, 53, 71). It is to be expected that tritium will be more widely used as it becomes available to more investigators.

The research which has been done with deuterium and tritium as tracers of hydrogen in water exchange studies is the area of primary concern in this review. Those aspects of exchange of deuterium and tritium with labile H atoms in organic and inorganic molecules and with normally nonlabile H atoms in organic molecules which become labile during chemical reactions involved in intermediary metabolic processes will be discussed only as these pertain to studies of water balance per se. References to original articles have in many cases been omitted when these articles are covered by reference to subsequently published reviews. Excellent bibliographies covering the literature on application of deuterium and tritium in all fields of science prior to 1950 are available (34, 69, 70).

PROPERTIES OF DEUTERIUM AND TRITIUM

Deuterium, a stable isotope of hydrogen of atomic mass two, is found in nature mixed with hydrogen of mass one (protium) in the ratio of 15 parts deuterium to 100,000 parts protium. In all natural substances in which hydrogen occurs this ratio is essentially the same (8). When deuterium combines with oxygen to form deuterium oxide (D_2O) a molecule of molecular weight 20 is obtained. Deuterium oxide has a specific gravity approximately 11 per cent greater than that of pure H_2O and it is this physical difference in density which has been most widely employed in measuring the deuterium content of water in biological research. When mixed with H_2O the deuterium in D_2O readily exchanges with the hydrogen in H_2O and hence in solu-

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tions dilute with respect to D_2O one obtains deuterium principally in a molecule represented by the symbol HDO (59, 65) of molecular weight 19. Detailed comparisons of the physical properties of D_2O and H_2O and of mixtures of these two waters are available (11, 36, 65).

Tritium is the radioactive isotope of hydrogen of mass three. It has a physical half-life of about 11 years and decays to helium with emission of a soft beta particle of about 18 kev. maximum energy. No gamma radiation is emitted and consequently tritium presents no radiological working hazard as long as it is prevented from entering the body. The radioactivity of tritium is its most marked physical characteristic from the standpoint of its toxicity within biological systems and also from the standpoint of its assay in the study of such systems. Calculated on the basis of its physical half-life, one finds that 1 milligram of pure T_2O, if available, would contain about 3 curies (1 curie = 3.7×10^{10} disintegrations per second). If one assumes for tritium an average energy release per disintegration of one-third the maximum energy, which has been observed to hold true for other beta emitters, a radiation dose of about 10^4 roentgens equivalent physical (rep) per second would be obtained in pure T_2O. Thus in body fluids tritium must be extremely dilute or objectionable radiation doses will be obtained. A concentration of tritium in body fluids equivalent to 0.2 μc. per ml., if maintained, will result in a radiation dose to the body of about 0.05 rep per day (52, 53). In this situation tritium is present in the ratio of about 1 atom of tritium to 10^{18} atoms of ordinary hydrogen. In the interest of health safety this concentration should probably not be exceeded in experiments on man. In animal experiments higher concentrations in body fluids may be employed always keeping in mind that excessive radiation doses will produce physiological alterations which may affect the result one obtains.

Methods of Assay

The ratio of deuterium to ordinary hydrogen in a mixture of these two isotopes, utilizing the mass difference between them, measured on a mass spectrometer provides one of the most sensitive means for assay of deuterium content (60, 67). This method of assay involves conversion of these isotopes to the gas. Use is also made of the physical differences between HDO and H_2O for assay of deuterium content. The specific gravity or density difference is most widely employed (29, 33, 56, 58, 60, 72). The falling drop method for densiometric determination requires only small amounts of fluid for analysis (33, 59) and gives adequate sensitivity for most biological work. It is cheaper than the mass spectrograph in terms of initial cost of equipment and of personnel and time required to obtain analyses. In densiometric measurements by the falling drop method water of unusual purity is essential for accurate results and is usually obtained by double distillation of the biological fluid. All distillations must be carried to completion to avoid errors due to isotopic fractionation. With the techniques developed it is possible to determine the deuterium oxide content of a D_2O:H_2O mixture with a sensitivity of about one part in 10^5 (33, 59, 60). Since D_2O may be used in concentrations up to about 1 per cent in biological systems without expectation of toxic effects this gives a working range of about 10^3 between usable concentrations in the body fluids and the measuring sensitivity. The details of all methods used for deuterium assay have been assembled and compared elsewhere (36, 65).

The most sensitive assay for tritium relates to its radioactivity. Because of the low energy of the emitted beta particle tritium must be introduced into a counter

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ion chamber for assay. It has been measured in Geiger counters, proportional counters, and ion chambers (57) as HTO vapor (48), as tritiomethane (57, 70), as tritiobutane (57), as tritioacetylene (71) and as HT (52, 53). When assaying tritium as HTO vapor one is limited to a low vapor pressure in counters and may have difficulty with "memory effects" (48). When incorporated in the methane, butane, or acetylene molecule memory effects are reduced and higher pressures may be utilized in the chamber, but in the conversion process a dilution of the specific activity or concentration of the tritium results in some loss in sensitivity. The gas HT is usually obtained by reduction of water over hot zinc or magnesium without dilution of specific activity and its ionization may be measured at high pressures with negligible memory effects in an ion chamber. Where maximal sensitivity is desired a counter utilizing any of the gases mentioned above may be expected to produce better results than an ion chamber. The ion chamber employed with a dynamic condenser (vibrating reed) electrometer (49) has proven quite satisfactory for tritium assay in the range of specific activities necessary for biological experimentation and is relatively free of mechanical and electronic difficulties. It is roughly a factor of 10 less sensitive than one might expect of the best counter system. With the ion chamber and dynamic condenser electrometer one can determine the tritium content of an H:T mixture with a sensitivity of about 1 part tritium in 10^{13} parts protium (24, 53). This gives one a working range of about 10^3 between the usable concentrations in body fluids which may be employed safely in experiments on man and the measuring sensitivity. For animals this working range may be increased (53) up to the point where the radiation dose during the time period covered by the experiment could be safely expected not to influence the physiological process being studied. For experiments of brief duration the working range may be increased to 10^7 or more.

Theory of Use of Isotopic Tracers as Related to Deuterium and Tritium

Isotopes are usually considered to be ideal tracers. It is generally assumed that one isotope of an element reacts just the same in exchange and chemical reactions as any other isotope of that same element. Such a result is expected because of the close similarity of the chemical properties of isotopes. Such assumptions for isotopes of hydrogen may to some degree be false. Considering the large percentage mass differences between ordinary hydrogen and deuterium or tritium, one might well be skeptical of the degree to which any one of these isotopes exactly parallels another. There is some evidence to indicate that H_2O and D_2O or HDO do not diffuse across membranes at the same rate (5, 25, 31, 41, 50) and do not enter into chemical reactions in precisely the same way (2, 3, 16, 22, 39, 41, 72). Pure D_2O is reported to produce hemolysis in mammalian erythrocytes 44 per cent more slowly than H_2O (50) and solutions of D_2O in H_2O (HDO) hemolyze erythrocytes more slowly than ordinary water (5). Since hemolysis is produced by the net transfer of water into the erythrocyte as a result of the differential in the rate of diffusion of water into and out of the cell, the time differential required for hemolysis is not a measure of the differential in rate of diffusion of D_2O versus H_2O. The result obtained indicates that D_2O diffuses into the erythrocyte more slowly than H_2O but the magnitude of the difference is unknown. To determine this one would need to know the total amount of water exchanged across the cellular membrane in relation to the amount of net transfer of water into the cell required to produce hemolysis. Considering the diffusion coefficient of water in water (45, 46), it seems likely that the amount of water ex-

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changed must be very large compared to the net transfer. Hence the differential between the diffusion rate of D_2O, or HDO versus H_2O across the boundary of the erythrocyte may be quite small. A difference in the permeability of the skin of the frog, in either direction, to D_2O and H_2O has also been recorded (31) in which the same considerations advanced above also apply.

There is evidence that the metabolic processes of living organisms are considerably altered by appreciable concentrations of deuterium in H_2O (2, [illegible], 15, 22, 39, 60, 72). Concentrations of D_2O in H_2O of 1 to 25 per cent depress metabolism in mice (2), retard growth of lower organisms (3, 16, 22) and reduce the rate of germination of tobacco seeds (16, 39). Higher concentrations, if maintained, are generally lethal to warm-blooded animals (16, 20, 39, 60). The basic explanation for such results has not been elucidated but the results per se are ample indication that protium and deuterium do not react exactly the same in animal organisms and appropriate consideration of this fact in biological research is necessary. The fact that biological systems do not react the same to HDO as to H_2O has been pointed out by several workers in this field (26, 38, 61). Some have suggested that the differences noted were due to the gross physical differences between HDO and H_2O, such as viscosity (5), degree of association (5), density, and coefficient of diffusion (45, 46) and have proposed that in solutions where HDO is present with large excess of H_2O, and only very small gross physical differences are measurable, that biological differences measurable at the higher concentrations may not prevail (32). Such assumptions seem unwarranted in as much as an HDO molecule may react the same with the organism whether it be in a dilute or concentrated solution. The fact that the difference noted in concentrated solutions is not measurable in dilute solutions gives no assurance that it does not prevail. Others have proposed that the difference noted between deuterium and protium in biological systems can be explained entirely on the basis of a rate difference, i.e. of diffusion or reaction (26). Deuterium and protium, within the limits of measuring sensitivity, are present in all organic and inorganic biological systems in the same ratio as these two isotopes exist elsewhere in nature (8, 10, 37). This suggests that if their rates differ equilibrium of the two is always attained in time in biological exchanges and reactions.

Tritium, having become available to biological investigators only recently, has not yet been used extensively but it seems reasonable to expect differences between protium and tritium as large as or larger than those observed between protium and deuterium. A comparison of the toxic effects of tritium versus deuterium at concentrations approaching those where deuterium is toxic may never be made because the radiation dose delivered by tritium at such concentrations would probably mask any other toxic effects. With tritium available as an isotopic tracer it now becomes possible to compare simultaneously the respective rates with which deuterium and tritium diffuse across barriers, mix with various body fluids, exchange with protium, or engage in chemical reactions. So far such comparisons have not been made but when accomplished they may be expected to clarify to some degree the status of deuterium and tritium as ideal tracers for protium in various biological processes. It has already been shown that deuterium and tritium become distributed in the same diluent volume in the body of the dog after equilibrium is attained (57) but the relative rate at which equilibrium is attained for these two isotopes has not been carefully compared.

In summary it appears, from evidence presently available, that HDO does not differ markedly from H_2O as regards the rate with which the two diffuse across

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membranes in biological systems. That deuterium and ordinary hydrogen differ appreciably in chemical reactions concerned in body metabolic processes is suggested by the toxic effects produced in various organisms when deuterium is substituted in high concentrations for ordinary hydrogen. To what extent HTO may differ from H_2O in body metabolic processes has yet to be investigated.

Water Exchange Between the Body and External Environment

In a consideration of water balance and barriers one may, for convenience, arbitrarily divide the discussion into those aspects of exchange which exist between the organism and the exterior environment and those aspects of exchange which exist within the organism itself. In the first of these two, absorption and excretion of water by the organism are involved. In the latter distribution and metabolism of water within the organism are involved. Animals exchange water with the external environment through the lungs, skin, gastrointestinal tract and kidneys.

Water Exchange Through the Lungs. Dry air inhaled into the alveoli of the lungs is found to be saturated with water vapor on expiration, suggesting a rapid transfer of water from body fluids across the alveolar membranes and diffusion of water vapor into alveolar air. If the body fluids contain HTO the concentration of tritium in expired water vapor is found to be the same, within the measuring sensitivity, as the concentration of tritium in the body fluids (52, 53). If there exists a differential in the rate at which HTO and H_2O transfer across alveolar membranes and diffuse into alveolar air this differential is small compared to the times involved in the transfer and hence isotopic equilibrium is attained during the period that inspired air remains in the alveoli. If one measures the HTO transfer in the reverse direction across the alveolar membrane by having the animal inhale an atmosphere containing HTO vapor it is found that the rate of appearance of HTO in the body fluids indicates an absorption of 85 to 100 per cent of the HTO in the total air inspired (6, 52, 53). Thus a rapid exchange of water vapor between body fluids and alveolar air in either direction sufficient to permit establishment of a fairly complete equilibrium for both HTO and H_2O, in the time available during the respiratory cycle, is indicated. Inhalation of HTO vapor at high specific activity is one way of introducing this isotope into the general circulation very rapidly.

Water Exchange Through the Skin. The passage of water outward through the skin, independent of sweat gland activity, is called water loss by insensible perspiration. Water vapor presumably diffuses through the skin barrier and the rate of loss in man has been shown to be related to the vapor pressure differential between body fluids and the exterior environment (51). If a diffusion process only is involved then diffusion of water from outside to inside should take place resulting in an exchange of water vapor across the skin barrier. This phenomenon has been observed in man by exposing a skin area to an outside environment saturated with dilute HTO vapor and measuring the rate and amount of HTO appearing in the fluids of the body. In such experiments the amount of HTO absorbed is proportional to the concentration of HTO in the saturated environment, to the length of time of exposure, and to the area exposed. The rate of HTO passage inward through the skin on exposure of the skin to an atmosphere saturated with HTO vapor at 32°C. is found to approximate (54) the rate at which H_2O passes out through the skin normally as insensible perspiration. It makes little, if any, difference in the rate of HTO transfer inward through the skin whether the skin is immersed in a solution at a given temperature containing HTO or is exposed to an atmosphere saturated, at the same temperature,

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with water vapor from the same [illegible] other case it takes about 10 minutes from the beginning of the exposure [illegible] HTO to begin to appear in the urine. After the skin exposure is ended the concentration of HTO excreted in the urine continues to increase for 30 minutes to several [illegible] the exposed skin is warmed after exposure the HTO concentration in the urine increases rapidly and reaches a constant value in about an hour. If the exposed skin is cooled after exposure the HTO concentration in urine increases slowly for a period of more than 5 hours. This is presumed to represent a retardation of the rate of pick up in the general circulation of the HTO which was transferred from the environment to the extravascular fluids in the skin during the exposure.

These results suggest a barrier in the skin surface, between the exterior and the fluids beneath the skin, across which water diffuses in the vapor state; hence no large difference in transfer rate is noted whether the outside environment be liquid water or water vapor at the same temperature and pressure. These results also suggest another barrier, perhaps liquid, between the skin surface and the general circulation. Water exchange between this second barrier and the general circulation is considerably altered by cooling the skin. A reduction in blood flow through the superficial skin capillaries caused by cooling the skin is probably primarily responsible for the reduction in the rate of transfer of the HTO from the extravascular fluids in the superficial skin area to the general circulatory system and thence to other body fluids.

Water Exchange Through the Gastrointestinal Tract. Ingestion was the mode used to introduce HDO into the body in the first reported biological use of this tracer in man (28, 29). Equilibration of the ingested HDO with body fluids was observed to take place in 2½ hours after ingestion of 2 liters of fluid (29). When lesser volumes are ingested, equilibrium between the gastrointestinal contents and blood is obtained in less than an hour (40, 44, 53, 60). The time required to transfer the water from the gastrointestinal tract to the blood probably increases with the volume of water ingested. The transfer may be linear with time, exponential with time, or follow some less well-defined function depending on the processes involved. The increase in HDO or HTO content of venous blood following ingestion of the heavy isotopes in 200 and 450 ml. of water is linear with time and equilibrium is attained in 40 to 45 minutes (40, 53). The simplest and most likely deduction is that the net transfer of water from the gastrointestinal tract to the blood is linear with time until the transfer is completed. Whether the rate differs for H_2O, HDO or HTO has not been determined. The disappearance of HDO from stomach pouches due to exchange is found to be exponential with time and to have a half-time of approximately 20 minutes (7). Using HDO as a tracer, the rate of water movement from gut to blood and from blood to gut as well as the net movement has been measured and according to the observer (74) the results support the hypothesis of a forced flow of fluid across the intestinal epithelium in both directions simultaneously. This postulates an exchange of water across the intestinal epithelium which cannot be explained on the basis of a simple diffusion or osmotic process but which is presumably dependent on some special characteristic of the intestinal epithelium which forces water across it in both directions. From data available it appears that the greater amount of water absorption takes place through the intestine and that here the net transfer to the blood is linear with time.

Other Water Exchanges with the Environment. It has been observed that the HTO concentration of water of urine, sputum, sweat, feces and insensible perspiration of man is the same, within the measuring sensitivity, as that existing in the water

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of the blood at the time of collection of these excreta (52, 53). The same has been found for HDO in the urine and gastric juice of man (28, 42, 43, 60) and in the milk of cows (10). If a rate difference exists for transfer of H_2O, HDO, or HTO across membranes involved in the formation and excretion of these fluids the time periods involved in the net transfer are sufficiently long to permit isotopic equilibrium to be established. Thus either HDO or HTO at equilibrium in body fluids serve as excellent tracers for H_2O in these transfers so far as can be ascertained from data available.

Since equilibrium of HDO and HTO with all body fluids is reached rapidly (37, 40, 52, 53) and all fluid lost from the body shows a concentration of HDO and HTO the same as that prevailing in body fluids (10, 29, 43, 53, 60), these isotopes are eliminated from the body fluids at a rate which is exponential with water intake or water excretion (53), i.e. if the logarithm of the concentration of HDO or HTO in body fluids is plotted against H_2O intake one obtains a straight line. The decrease in concentration of HDO or HTO in body fluids is also exponential with time at any constant rate of H_2O intake (53). The half-time, or time required to reduce the concentration of HDO or HTO in body fluids by one-half, at normal rates of H_2O intake is 8 to 14 days for man (29, 53, 60) with an average of about 10 days; 3 to 5 days for the rat (4, 53); and 1 to 2 days for the mouse (53, 71).

Water Exchanges Within the Body

When HDO or HTO is introduced into the body by ingestion, injection, inhalation, or in any other way the deuterium and tritium are diluted by exchange with H atoms in body water and with the exchangeable H atoms in organic molecules. These latter include the hydrogen atoms in amino, carboxyl, hydroxyl, imino, sulfhydryl, and in other positions generally where the hydrogen atom is not directly bound to a carbon atom. The rapidly exchangeable hydrogen atoms in the organic constituents of the body are estimated to correspond to a water equivalent of 0.5 to 2.0 per cent of the body weight in man (32, 60). This value is calculated assuming that proteins comprise 15 per cent of the body weight (61), that 6 per cent of protein is hydrogen, and that 6 to 15 per cent of the H atoms in proteins are readily exchangeable (37, 73). This gives roughly 0.1 per cent (15% × 6% × 11%) of the body weight as exchangeable H atoms in proteins, corresponding to a water equivalent of about 1 per cent of body weight. For carbohydrates, it is assumed that 350 gm. are present in the body giving 0.5 per cent of the body weight as carbohydrate, that 6 per cent of the carbohydrate is hydrogen, and that 35 per cent of the hydrogen is exchangeable (4). This gives about 0.01 per cent (0.5% × 6% × 35%) of the body weight as exchangeable H atoms in carbohydrate, corresponding to a water equivalent of about 0.1 per cent of body weight. The rapidly exchangeable H atoms in fat are assumed negligible in amount. By adding the above values, a figure between 1 and 2 per cent of the body weight is obtained as a water equivalent for the rapidly exchangeable H atoms in the organic constituents of the body. In almost all reports giving the percentage of body weight appearing as body water and obtained by dilution of deuterium or tritium as tracers, the above equivalent has not been subtracted and hence the values reported are high to this extent.

Where hydrogen atoms are bound to carbon atoms directly, exchange takes place not at all or only very slowly as such atoms become labile during processes involving chemical reactions in the body metabolism. These exchanges are of utmost importance in studying the intermediary metabolism of the body (4, 21, 61–64)

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but are too slow to be of any great consequence in water balance experiments of short duration and hence can usually be ignored in such measurements.

If water molecules containing deuterium or tritium exchange across body membranes just as does an H_2O molecule these isotopes may be used to measure the rates of exchange of water between various water compartments in the body. The rates of exchange thus measured may be influenced by many factors such as the rate and characteristic of blood supply to the area concerned, the thickness and area of the membranous barrier involved, the thickness of the fluid layer on each side of the barrier, the type of barrier, and the diffusion coefficient.

HDO or HTO in the body usually comes into equilibrium in about an hour with all rapidly exchangeable H atoms (40, 53, 60). Since water is the principal diluent, the amount of water required to give the concentration noted at equilibrium is calculated and expressed as a percentage of the body weight. Such determinations give body water values ranging from 45 to 60 per cent (average 52%) of body weight for women and from 56 to 70 per cent (average 62%) of body weight for men in one series of measurements (60). Others have reported 63 ± 4 per cent for man (29); 57 per cent for man (53); 72 per cent for man (40); 72.5 per cent for man (43); 65 per cent for the guinea pig (12); and 57 to 72 per cent for rats (53). These results indicate considerable variation in the percentage of body weight which is measured as water. Since body fat has a low water content it has been suggested that body fat is largely responsible for the wide variations in percentage of body water measured. The percentage of body water in the fat free body or 'lean body mass' of rabbits and man is found to be 73.2 (48). There is some indication that the lean body mass does not vary widely with regard to its water content (47, 48, 55). If correction is made for the rapidly exchangeable H atoms in organic constituents of the body the values listed above for percentage of body weight appearing as water would be reduced by 0.5 to 2.0 per cent.

Water Exchange Between Individual Body Fluids and Blood. The rate of appearance or disappearance of HDO or HTO in a given body fluid is used to measure the rate of exchange of that fluid with other body fluids. The tracer isotope is either injected into the blood stream and its rate of appearance in the specific fluid under study noted or injected into the fluid being studied and the decrease in concentration with time noted as water exchanges with the blood stream. If the tracer isotope is placed, for example, in the aqueous of the eye and the water exchange between the eye and the circulating blood proceeds at a constant rate with no large net transfer of water, one expects an exponential decrease in concentration of the tracer isotope in the aqueous of the eye with time. The rate constant for this function will depend on the volume of water in the aqueous of the eye and the volume of water exchanged between the eye and the blood per unit time. The rate constant has been measured for water in the eye compartments, in the cerebrospinal fluid, and in amniotic fluid. The half-time, or the length of time necessary for the HDO concentration of the water in these water compartments to be reduced by one-half by exchange with the circulating fluid, is 2.7 minutes for the aqueous of the rabbit eye (35), 10 to 15 minutes for the vitreous of the rabbit eye (35), less than 7 minutes for the aqueous of the monkey eye (35); 1.5 minutes for the cerebrospinal fluid in the region of the cisterna magna in man (68); 8 to 11 minutes for the cerebrospinal fluid in the ventricular region in man (68); and 18 to 26 minutes for the cerebrospinal fluid in the lumbar region in man (68). Water exchange between the amniotic fluid and the maternal circulation indicates a half-time for the amniotic fluid of 40 minutes in

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the guinea pig (13) and 2 hours in the human (75). Because these areas represent fluid volumes in contact with the circulating blood only on the periphery, these half-times are probably representative of some of the longer half-times existent in the body.

The water exchanged across the placenta between the human fetus and the maternal circulation is found to increase from 101 ml./hour at a gestation age of 14 weeks to 2500 ml./hour at a gestation age of 31 weeks and to decline to 1580 ml./hour at term (23). At a gestation age of 31 weeks the fetus requires only 0.66 ml. of water per hour for the increase in fetal weight noted due to growth. Therefore, the volume of water exchanged across the placenta at this time is 3800 times the volume of water retained by the fetus for growth (23).

The erythrocyte is observed to equilibrate with HDO in the blood in less than 1 minute (18) and, considering its size together with the diffusion coefficient for HDO in water (45, 46), probably equilibrates in a very few seconds. The fluids discussed above represent only a small fraction of the total fluid in the intracellular and extracellular space of the body but direct measurement of the rate of equilibration of the intracellular and extracellular fluids with blood in other individual organs of the body has not yet been reported. Such measurements can probably be made with the aid of deuterium or tritium as tracers. This represents a field for future work.

Water Exchange Between Vascular and Extravascular Fluids. The sum total of water exchange between the blood and all extravascular water has been determined by isotope dilution of HDO in the blood after intravenous injection. Two observers report independently that two rates, both exponential with time, are sufficient to describe the dilution noted in the blood of man. The data obtained are described by the equation

$$C_t - C_{eq} = ae^{-\lambda_1 t} + be^{-\lambda_2 t} \qquad (14, 60)$$

where C_t is the concentration of deuterium in vascular fluids at time t after intravenous injection, C_{eq} is the concentration of deuterium in all body fluids after equilibrium is established, a and b are constants, and λ_1 and λ_2 are the two rate constants which describe the speed with which the vascular fluid is exchanging with extravascular fluids. The half-time for disappearance of HDO from the blood calculated from each of these two rate constants is approximately 30 seconds and 3 minutes according to one observation (14) and approximately 75 seconds and 9½ minutes according to the other (60). Taking the longer of the two half-times in each of the above observations we can calculate that 99 per cent equilibrium will be established between the vascular and extravascular fluids in about 20 minutes in the first case and in about 63 minutes in the second case. These values correspond to the equilibrium time generally observed for man by others using HDO (40) and HTO (48, 53). Utilizing the observed rate constants it is possible to calculate that a volume of water larger than the volume of plasma water is exchanged per minute between the vascular and extravascular fluids of man (14). Such large exchanges most likely take place by diffusion back and forth across capillary walls. That only two rate constants are sufficient to describe the water exchange process between vascular and all extravascular fluids is rather surprising considering the fluids involved in the diverse areas of the body. The two rates determined may well be only average values around which rates in the diverse areas of the body tend to scatter. In any case the two rates found suggest two general extravascular fluid areas in the body each of which has a characteristic rate of exchange with the vascular fluid. The difference in

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exchange rate noted may result from differences in amount of blood flow to the two general areas or from differences in the diffusion rate into the two areas due to variation in barrier permeability or barrier thickness. Some observers (132) have interpreted the two rates to be measures of mixing of vascular fluid with extracellular and intracellular fluid, respectively.

Actual measurement of the rate of exchange between the vascular fluid and fluid in various body organs of animals appears feasible with HDO or HTO as tracers and would undoubtedly give some indication of the relative importance of blood flow versus diffusion as a limiting factor in the exchange. What would be the effect on the water exchange rate between vascular and extravascular fluids in the skin if the skin is cooled? It was noted above, under water exchange through the skin, that cooling of the skin reduced markedly the rate at which HTO in the skin appeared in other body fluids. It is presumed that HDO or HTO injected into the blood stream might come into equilibrium with extravascular fluids in the skin very much slower when the skin is cooled than when the skin is warmed.

Radioautographs have been used to localize tritium-labeled compounds in paramecium and yeast cells (9). It may be possible to immobilize HTO in various body organs during the process of establishment of equilibrium by quick freezing with carbon dioxide or nitrogen and then make radioautographs of the frozen sections to determine the relative concentration of the tritium in various parts of the tissue. If such a procedure proves feasible it might be useful in further clarifying some of the factors relating to water exchange in the body.

Summary

The physical properties of deuterium and tritium in relation to concentrations of these isotopes which may be toxic to biological organisms and which determine the sensitivity of methods of assay are indicated.

The concept of the use of isotopes as related to the use of deuterium and tritium is examined in the light of biological studies already made with these heavy isotopes of hydrogen. Where it has been possible to compare the rates of diffusion of HDO or HTO with H_2O across various barriers no great difference has been found in the rates with which these molecules move across such barriers.

Studies of water exchange between the animal organism and the external environment using HDO and HTO as tracers show that *a*) the exchange of water between the inspired air and body fluid in the lungs is nearly 100 per cent, *b*) the amount of water transferred from the gastrointestinal tract to the blood stream following ingestion is linear with time, *c*) a permeable barrier exists in the outer skin through which water vapor may diffuse in either direction, and *d*), within the limits of measuring sensitivity, all fluids lost from the body contain the same isotopic concentration of HDO or HTO as that prevailing in the water of the blood at the time of loss.

The rate of water exchange between the vascular fluid and the water of the eye compartments, cerebrospinal fluid, and amniotic fluid is so fast that within a few minutes or at most a few hours the volume of water exchanged is as great as the volume of water maintained in these water compartments. HDO or HTO introduced into the blood stream normally attains 98 per cent equilibrium with extravascular body fluids in about an hour indicating rapid water exchange between vascular and extravascular fluids. The rate of this exchange indicates that the volume of water passing in and out of the blood stream per minute is larger than the volume of water normally maintained in the blood stream as plasma water.

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HDO or HTO are useful as tracers in measuring water exchange across barriers which cannot be measured with H_2O due to one's inability to distinguish one H_2O molecule from another. With HDO or HTO one can differentiate between the water exchanged and net water transferred. By using the radioactive character of tritium it may be possible in the future to localize the position of water molecules as they proceed through various barriers in exchange and equilibrium-establishing processes.

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