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A Survey of Deuterium Chemistry

1. Historical Introduction.
2. The Separation of Deuterium from Hydrogen.
3. The Analysis of Deuterium and Deuterium Oxide.
4. Applications of Deuterium as a Tracer in the Study of Intermediary Metabolism.
5. The Physiological Effects of Deuterium Oxide.

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Index

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General Introduction

A great deal of work has been done using isotopes to study fundamental scientific problems. For example, isotopes have been used to study the mechanism of reactions, chemical exchange, structure of compounds, thermodynamic equilibrium, stereochemical factors, etc. The use of isotopes in these fields has by no means been exhausted, but the bulk of the work is such that it is difficult to see all that has been done. In scientific research, there is little to be gained from the needless repetition of experiments. For this reason, it was decided that a comphrehensive survey of the literature would be of much benefit. Not only would such a survey tie together related data, but it would also act as a convenient stepping stone for future work.

The present survey deals with the use of deuterium and includes the following topic:

1. Historical Introduction.
2. The Separation of Deuterium from Hydrogen.
3. The Analysis of Deuterium and Deuterium Oxide.
4. Applications of Deuterium as a Tracer in Intermediary Metabolism.
5. The Physiological Effects of Deuterium Oxide.

Several additional topics will be added later.

Chapter I

Historical Introduction

In 1929 Aston determined the molecular weight of hydrogen with his mass spectrograph. Basing his calculations on the assumption that oxygen had an atomic weight of 16.0000, he obtained a molecular weight very close to the chemically determined molecular weight. This was taken as proof that hydrogen had no isotopes.[1, 2]

	Aston	Chemically Accepted
$\frac{\text{Mass H}}{\text{Mass O}}$	$\frac{1.00778}{16.00000}$	$\frac{1.00777}{16.00000}$

But in 1929 Giaugue and Johnston discovered the heretounknown fact that oxygen had three isotopes of masses 16, 17 and 18; and that these were all present in the naturally occuring oxygen.[1, 3, 4] The abundance ratio determined by Meeke and Childs[1] was found to be:

$$O^{16} : O^{17} : O^{18} = 3150 : 1 : 5$$

This meant that the average atomic weight of naturally occuring oxygen was:[1, 2]

$$\bar{O} = \frac{3150 \times 16 + 1 \times 17 + 5 \times 18}{3156} = 16.0035$$

According to this, the atomic weight of hydrogen calculated by Aston on the basis of $O^{16} = 16.0000$, would be in error by $\frac{16.0000}{16.0035} = \frac{1}{1.00022}$. If the chemical atomic weight is

recalculated on the basis of O^{16} = 16.0000, the ratio obtained is:

$$\frac{\text{Mass H}}{\text{Mass } O^{16}} = \frac{1.00799}{16.00000}$$

which differs from Aston's by 0.00021. After checking the limits of error in Aston's work, Birge and Menzel[1] concluded that the difference between Aston's determination and the chemical determination could be accounted for by the presence of an isotope of mass 2 and abundance 1:4500.

Later workers determined the oxygen isotopes by the molecular spectrum methods, and other work in the field led to the discovery of new isotopes, for example C^{13}, N^{15}, Be^{8}, until isotopes were known for all the elements up to and including sulfur. All this data completed the picture of the proton-neutron plot of the lighter elements, and the regularities thus indicated strengthened the hypothesis that hydrogen had an isotope of mass 2; and possibly one of mass 3. However, because of the low abundance ratio, H^2:H^1, in naturally occuring hydrogen, the presence of the isotopes, as yet only hypothesized, had still not been detected.

Then in 1932 Urey, Brickwedde and Murphy[1], using the Debye theory, calculated the relative vapour pressures of H_2^1, H^1H^2, and H^1H^3 in the solid state; and found that appreciable differences could be expected in the three. As a result, fractional distillation of liquid hydrogen near the triple point should be an effective method of concentrating the heavier isotopes. Using Bohr's

theory, it was next possible to calculate the expected position of the lines of the atomic spectra for these hypothesized isotopes. Furthermore, because lines in the atomic spectra are very intense, a photograph of this spectra should give a very sensitive, unambiguous method of detecting the isotopes. These workers photographed the lines of tank hydrogen, and of the residual from a triple point evaporation of 4000 cc. of liquid hydrogen. In both cases isotopes of masses 2 and 3 were detected, the lines being much more intense in the latter case. Because of the importance that was considered attached to these isotopes, they were given special names, deuterium (D_2) for H^2; and Tritium (T_2) for H^3.

Bleakney[1] examined ordinary electrolytic hydrogen and the enriched samples of Urey, Brickwedde and Murphy with a mass spectrograph, and was able to confirm their conclusions. Later investigators soon detected deuterium by other methods, for example, in the infra-red spectra of HCl and DCl, and by using a high dispersion prism spectrograph.[1] The enrichment by fractional distillation of liquid hydrogen at the triple point was also confirmed by others.[1]

In the spring of 1932 Washburn[1] discovered that pure deuterium could be prepared by the electrolysis of aqueous solutions. By this method Lewis and MacDonald[1] prepared pure deuterium oxide, and their demonstration of the simplicity of the preparation led to the concentration of deuterium oxide in large quantities.

The atomic weight was determined by Bainbridge,[1, 5] and later redetermined by Aston.[6]

Table 1

Atomic Wt. Reference	Ref. No.	At. wt. H	At. wt. D	At. wt. T	Mean At. wt. W
O^{16} = 16.0000	5	1.007775	2.01363 ±0.00008	-	-
O^{16} = 16.0000	6	1.00812 ±0.00004	2.01471 ±0.00007	-	-
$\overline{O}$ = 16.0000	5	1.007504	2.01309	3.0151	1.00770

By the most recent determination the abundance of deuterium in naturally occuring hydrogen is 1:5000[1] (1:6900)[7]. Emeléus[1] has made a careful survey of differences in abundance for waters from different sources. He found that differences in water densities of more than 1 ppm are very common, but that there are not many regularities observable. Mineral waters show some enrichment probably because the evaporation of salt lakes leaves the residual water somewhat enriched. Waters of vegetable origin also show some enrichment. In this case, the enrichment could be caused by the different vapour pressures of the two waters influencing the rates of evaporation from the leaves, the rates of diffusion through the stomata, or the equilibrium constants for exchange reactions between the water and the organic substances of the plant. There is also an increased density observable in waters from commercial sources due to such processes as fractionation, distillation, electrolysis, absorption, or chemical fractionation. (For example, the water of crystallization of alum contains more deuterium than the mother liquor.)

It is interesting to note that hydrogen gas in equilibrium with water should contain from 1/3 to 1/4 as much deuterium as the water.(8)

Chapter II

The Separation of Deuterium from Hydrogen

1. The Electrolytic Method

a) Experimental

Washburn and Urey[2] are given the credit for discovering the electrolytic preparation of deuterium. They determined that the residual water from electrolysis had a higher specific gravity than the original; and by spectroscopic analysis were able to show that this phenomenon was related to deuterium enrichment. The enrichment is measured in terms of the fractionation factor α:

$$\alpha = \frac{H}{D} \text{ final} / \frac{H}{D} \text{ original}$$

This method was used by Lewis and MacDonald[1, 9] to prepare practically pure deuterium oxide, and their research led to a widespread utilization of the method. It is this method that has made possible the extensive use of deuterium as a tracer in chemical and biological research.[1]

Although several adaptations of the electrolytic method are available, the most widely used is a batch process involving electrolysis of a dilute alkaline solution. When the alkali concentration becomes too great for further operation, part of the alkali is neutralized and the process continued. On the basis of the Rayleigh distillation formula, the following relationship between initial and final volumes and mole fractions of deuterium holds:

$$\frac{(1-N)^{\frac{1}{\alpha'-1}}}{(1-N_0)}\,\frac{(N)^{\frac{\alpha'}{\alpha'-1}}}{(N_0)} = \frac{W_0}{W}$$

α' - constant fractionation factor
N_0, N - initial and final mole fractions of deuterium
W_0, W - initial and final volumes of the solution

Note: α' is the effective fractionation factor which differs from the true fractionation factor, α, because of the evaporation of water.

Urey and Teal have calculated, on the basis of this equation, that in a cell of $\alpha' = 6$, it would be necessary to electrolyse 25 litres of 0.07% D_2O to a residue of 1 cc. to obtain 99.9% D_2O. This illustrates the great loss of D_2O occuring, as the initial water contained 17.5 cc. of D_2O.(1)

When the process starts, the escaping gas contains less deuterium than natural water, but as the process proceeds and the deuterium content of the water increases, even the escaping gas becomes considerably enriched in deuterium. To prevent the loss of too much deuterium, the escaping gases are burned and reworked.(1, 2) This can be conveniently done by burning the escaping hydrogen and oxygen at small nozzles after the gases have passed through a short sand trap. The sand trap stops explosion waves resulting in the event of plant shut down. The jets are mounted in banks so that any jet extinguished by the water of combustion may be relighted by its neighbours. This prevents any unlooked for loss of deuterium. Starting with water enriched in deuterium oxide, this method leads step-wise to 99.9% D_2O.

Other electrolytic methods tried involve continuous flow and cascade techniques. These methods are less convenient, and more wasteful because of the difficulty encountered in burning and reworking the escaping deuterium-rich gases.[1]

b) Theoretical Calculations

The electrolytic separation depends primarily on the following exchange reaction between water and hydrogen gas:

$$H_2O + HD \rightleftharpoons HDO + H_2 \qquad (1)$$

This exchange reaction favours an increased concentration of deuterium in the liquid phase, as shown by the equilibrium constant.[1, 8]

$$K = \frac{(HDO)(H_2)}{(H_2O)(HD)} = 3$$

The equilibrium constant may be determined not only experimentally but also from basic statistical mechanical considerations.[2, 10] By means of these calculations, experimental data are correlated with the thermodynamical properties of the system as determined from molecular spectra.[11] The following section deals with the development of this method using equation (1) as an example. In the electrolytic process this reaction reaches equilibrium because of the catalytic effect of the electrodes.

Consider any general reaction:

$$a\,A + b\,B \rightleftharpoons c\,C + d\,D \qquad (2)$$

The equilibrium constant for the general reaction is given (12, 13) by:

$$R \ln K_p = -\frac{\Delta F^\circ}{T}$$

$$= -\frac{\Delta(F^\circ - E_0^\circ)}{T} - \frac{\Delta E_0^\circ}{T} \qquad (3)$$

However:

$$F^\circ = E_0^\circ + RT \ln N - RT \ln Q$$

and substituting in (3):

$$K_p = \frac{\dfrac{(Q_c)^c}{(N_c)^c}\dfrac{(Q_d)^d}{(N_d)^d}}{\dfrac{(Q_A)^a}{(N_A)^a}\dfrac{(Q_B)^b}{(N_B)^b}} \cdot e^{\frac{\Delta E_0^\circ}{RT}} \qquad (4)$$

But:

$$Q = Q_T \cdot Q_I = Q_T \cdot Q_V \cdot Q_R$$

Q - total summation of state
Q_T - translational summation of state
Q_I - internal summation of state
Q_V - vibrational
Q_R - rotational

(4) may also be written:

$$\ln K_p = \ln \frac{\Sigma\left(\frac{Q_T}{N}\right)_p}{\Sigma\left(\frac{Q_T}{N}\right)_r} + \ln \frac{\Sigma\left(\frac{Q_V}{N}\right)_p}{\Sigma\left(\frac{Q_V}{N}\right)_r} + \ln \frac{\Sigma\left(\frac{Q_R}{N}\right)_p}{\Sigma\left(\frac{Q_R}{N}\right)_r} - \frac{E}{RT} \qquad (5)$$

where the subscripts p, r, indicate products and reactants respectively. From Herzberg: (13)

$$Q_T = V\frac{(2\,M\,k\,T)}{h^2} \qquad (6)$$

$$Q_V = \sum_{v_i} e^{- w_i v_i hc/ kT} \qquad (7)$$

$$Q_R = \frac{k T}{\sigma hc B} \qquad (8)$$

V - volume
M - mass
k - Boltzmann constant
T - temperature, °A
h - Planck constant
c - constant
B - rotational quantum number, which depends on I, the moment of inertia
w_i - lowest vibrational energy level
v_i - vibrational quantum number
σ - symmetry number

An examination of these equations leads to the conclusions:

$$\ln (6) = \ln \frac{\sum (M_p)^{3/2}}{\sum (M_r)^{3/2}}$$

$$\ln (7) = \ln e^{- w_i v_i hc/kT}$$

$$= \ln \frac{1}{1 - e^{- w_i v_i hc/kT}}$$

$$\ln (8) = \ln \frac{\sum I_p}{\sum I_r} - \frac{\sum \sigma_p}{\sum \sigma_r}$$

Refering to equation (1), and substituting in (5), the equation for the equilibrium constant becomes:

$$\ln K_p = \ln \frac{(M_{HDO})(M_{H_2})}{(M_{H_2O})(M_{HD})} + \ln \frac{I_{HDO} \cdot I_{H_2}}{I_{H_2O} \cdot I_{HD}} - \ln \frac{\sigma_{HDO} \cdot \sigma_{H_2}}{\sigma_{H_2O} \cdot \sigma_{HD}} \qquad (9)$$

$$+ \ln \frac{1}{1 - e^{-w_i hc/kT}} - \frac{\Delta E_0^\circ}{RT}$$

In the example dealt with here, the third term is zero as the symmetry numbers cancel, and the fourth term is negligible because the first vibrational state is far above the zero point energy.[2] The final equation for the equilibrium constant becomes:

$$\ln K_p = \ln \frac{(M_{HDO})(M_{H_2})}{(M_{H_2O})(M_{HD})} - \ln \frac{(I_{HDO})(I_{H_2})}{(I_{H_2O})(I_{HD})} - \frac{E_0^\circ}{RT} \qquad (10)$$

where the total number of molecules involved is N, Avogadro's number.

Atomic weight determinations supply the data for determining the first term.

Table 2#

Molecular Species	Molecular Weight
H_2	2.01501
HD	3.02059
D_2	4.02618
H_2O	18.01501
HDO	19.02059
D_2O	20.02618

all values based on $\bar{o}$ = 16.00000, the chemical molecular weight standard. (1)

Spectroscopic data is used to calculate the moments of inertia and zero point energies. In the case of the water molecules, the moment of inertia given in the table is the resultant of three dimensional moments of inertia.

$$I = \sqrt{A.B.C.}$$

Table 3

Molecular Species	I g/cm²	Ref. No.	E_0° cal.	Ref. No.
H_2	4.67×10^{-41}	(2)	6183.5	(2)
HD	6.21×10^{-41}	(2)	5366.4	(2)
D_2	9.31×10^{-41}	(2)	4394.5	(2)
H_2O	2.47×10^{-60}	(14)	13097	(2)
HDO	4.09×10^{-60}	(14)	11393	(2)
D_2O	6.48×10^{-60}	(14)	9527	(2)

When the values given in Tables 2 and 3 are substituted in (10):

$$\ln K_p = \frac{3}{2} \ln 0.7045 + \ln 1.2452 + 1.5229$$

$$K_p =$$

Equilibrium constants have been calculated for all possible exchange reactions between these gases and waters. The values are listed below:

Table 4

Reaction	Ref. No.	Equilibrium constant from: Thermodynamics 20° C. (8, 15)	100° C. (2)	(16)	Fugacities 25° C. (17)	100° C. (17)
$H_2 + D_2 \rightleftharpoons$	2 HD	3.27	3.49		3.28	3.44
$H_2 + D_2O \rightleftharpoons$	HD + HDO	1.22			1.09	1.53
$HD + H_2O \rightleftharpoons$	H_2 + HDO	2.67	1.98		2.37	2.87
$D_2 + HDO \rightleftharpoons$	HD + D_2O	2.67		3.00	2.98	2.24
$D_2 + H_2O \rightleftharpoons$	HD + HDO	8.73			7.79	6.45
$D_2 + H_2O \rightleftharpoons$	H_2 + D_2O	7.13			7.08	4.20
$D_2O + H_2O \rightleftharpoons$	2 HDO	3.27	3.37	4.10	2.60	2.87

The most recent calculations were made from a study of the two reactions: (18)

$$FeO + H_2 \rightleftharpoons Fe + H_2O$$

$$FeO + D_2 \rightleftharpoons Fe + D_2O$$

The equilibrium constant for the reaction:

$$H_2O + HD \rightleftharpoons HDO + H_2$$

was determined as a function of the absolute temperature (15) according to the equation:

$$\log K_p = (750/2.3RT) - 0.1335$$

c) Separation and the Cathode Reactions.

Because the separation takes place at the cathode, the anode reactions may be omitted. The cathode reactions may be divided into four chief stages.

I Transport of ions from the electrolyte to the electrode: (1, 2, 14)

$$2\,H_2O \longrightarrow OH^- + H_3O^+$$

(1, 2, 14)

II Discharge of ions at the electrode.

$$H_3O^+ + M^- \longrightarrow H_2O + H\text{-}M$$

Note: H-M indicates atomic hydrogen adsorbed on the electrode metal.

III Formation and liberation of gas molecules at the electrode. (1, 2, 14)

$$2\,H\text{-}M \longrightarrow H_2 + 2\,M$$

IV The exchange reaction: (1, 14)

$$H_2O + HD \longrightarrow HDO + H_2$$

Although this is the most important exchange reaction, (2, 8) the following also occur.

$$H_2 + D_2 \longrightarrow 2\,HD$$

$$H_2O + D_2O \longrightarrow 2\,HDO$$

I Transport of Ions to the Electrode

This process is not the cause of the separation,

as in an alkaline electrolysis only the Na^+ ions migrate to the cathode. In acid solution a separation due to different mobilities of H^+ and D^+ could be accomplished if natural diffusion did not keep the hydrogen and deuterium in normal concentrations on the cathode boundary; a phenomenon never observed.

II and III Ionic Discharge and Liberation of Gas Molecules

The discharge and adsorption of the atomic hydrogen is assumed to occur in one step because this mechanism requires less energy than a two-step reaction involving the formation of free hydrogen atoms in solution.

The actual process is very complicated and has been explained in several ways.[1] Two reactions are generally assumed to take place:

$$M^- + H_2O \longrightarrow OH^- + MH \qquad (1)$$

$$2\,MH \longrightarrow H_2 + 2\,M \qquad (2)$$

It is generally believed that the rate of one of these reactions is slower than that of the other, and that the slow reaction determines the overall rate.

a) A study of the hydrogen overvoltages indicates that the relation between current and overvoltage is independent of the hydrogen ion activity, which is in agreement with equation (1). At low current densities it is possible to explain the current - overvoltage relationship on the basis of (2) being the slow reaction. Hammett gives the following equation:[1]

$$I = k_2 (1 - e^{-2F\Delta E/RT})$$

At high current densities, (1) is assumed to be the slow reaction. Bowden gives, for high overvoltages:

$$I = -k' (e^{-0.5 F\Delta E/RT})$$

$0.01 \rangle E \rangle -0.01$

ΔE - overvoltage
F - number of Faradays passing
0.5 - theoretical derivation not clear.

I - current
k_2, k' - constants

These two equations can be combined to reduce to one or the other depending on the current.

Since the separation of hydrogen and deuterium occurs in the high overvoltage region, it is assumed, on the basis of this reasoning, that the fractionation is associated with the discharge, (1), rather than with (2).

b) Fowler,[1] basing his values on a general study of the reaction, has calculated the fractionation factor obtainable, assuming in turn either (1) or (2) to be the slow reaction. He assumed that the probabilities of deposition of hydrogen and deuterium on the electrodes are proportional to the mole fractions of hydrogen and deuterium in the solution, independent of the molecular predecessor. (H_2O, HDO, or D_2O)

The processes involved in the discharge are:

$$H_2O + M^- \longrightarrow OH^- + MH$$
$$D_2O + M^- \longrightarrow OD^- + MD$$
$$HDO + M^- \longrightarrow OD^- + MH$$
$$HDO + M^- \longrightarrow OH^- + MD$$

A

The formation of molecules then proceeds as follows:

$$\left.\begin{array}{ll} 2\,MH \longrightarrow & H_2 + 2\,M \\ 2\,MD \longrightarrow & D_2 + 2\,M \\ MD + MH \longrightarrow & HD + 2\,M \end{array}\right\} \quad B$$

These reactions must be reversible as a reversible electrode is considered. Using the following symbols:

I_1, I_2 - current carried by the discharge of H^+, D^+ (F/time)

N_{11}, N_{12}, N_{22} - mole fraction of H_2O, HDO, D_2O respectively.

n_1, n_2 - concentration of adsorbed H, D respectively. (atoms/area of electrode)

x_1, x_2 - concentration of OH^-, OD^- respectively.

n_{11}, n_{12}, n_{22} - mole fractions of H_2, HD, D_2 respectively in the gas.

and considering that the following equations hold at equilibrium:

$$H_2O + D_2O \longleftrightarrow 2\,HDO \qquad K_w = 3.28$$

$$H_2 + D_2 \longleftrightarrow 2\,HD \qquad K_g = 3.27$$

and:

$$N_{11} + N_{12} + N_{22} = 1$$

$$n_{11} + n_{12} + n_{22} = 1$$

then, if the rates of reaction follow the mass action laws, for reaction A :

$$\left.\begin{array}{l} I_1 = (2\,k_{11}N_{11} + k_{12}N_{12}) - (\mu_{11}n_1x_1 + \mu_{12}n_1x_1) \\ I_2 = (2\,k_{22}N_{22} + k_{21}N_{12}) - (\mu_{22}n_2x_2 + \mu_{21}n_2x_1) \end{array}\right\} \quad C$$

The twos are introduced to account for phenomena such as the probability of H being discharged from H_2O being twice the

probability of H being discharged from HDO. For the reactions B:

$$I_1 = (2V_{11}n_1^2 + V_{12}n_1n_2) - (2\lambda_{11}n_1 + \lambda_{12}n_{12}) \quad \Big) \; D$$
$$I_2 = (2V_{22}n_2^2 + V_{12}n_1n_2) - (2\lambda_{22}n_{22} + \lambda_{12}n_{12}) \quad \Big)$$

k_{ij}, V_{ij}, λ are velocity constants and functions of temperature.

The terms in the first and second parentheses refer respectively to the forward and reverse reactions. If the terms on the right are all large as compared to I_1, I_2, equilibrium would hold and the separation factor would be approximately 3.[1] However, as the separation depends on relative velocities rather than equilibrium conditions, some reaction must be slow with respect to the other.

Case I The discharge reaction is the slow one.

Here the terms with $\mathcal{M}$ may be set equal to zero and:

$$\frac{I_1}{I_2} = \frac{2\,k_{11}N_{11} + k_{12}N_{12}}{2\,k_{22}N_{22} + k_{21}N_{12}}$$

which reduces to the observed fractionation factor:

$$\frac{I_1}{I_2} = \frac{k_{11}^{\frac{1}{2}}.2\,N_{11} + N_{12}}{k_{22}^{\frac{1}{2}}.2\,N_{22} + N_{12}}$$

if $K_w = 4$, and $k_{12} = k_{21}$, and $k_{12}^2 = k_{11}k_{22}$

In order for this last statement to be true, each of the constants must be in the form $ce^{-E/kT}$, where c is a constant and $E_{k_{12}}$ is the mean of $E_{k_{11}}$ and $E_{k_{22}}$.

This condition is reasonable as the difference in activation energies lies in the change in the zero point energy, and the zero point energy for the mixed molecule HDO is the mean of the values of the two homogeneous molecules, H_2O and D_2O. However, the assumption that $k_{12} = k_{21}$ means that the H and D atoms of HDO can pass the potential hill equally easily if the same energy is supplied. Since, moreover, $K_w = 3.26$ and not 4, these equations are at best approximations: but they do show that the observed facts agree with the assumption taken here.

Case II The velocity of formation of the molecules is the slow reaction.

In this case the λ's of equations D are negligible. n_1 and n_2 are assumed to be proportional to:

$$n_1 = k_1 (2 N_{11} + N_{12})/ 2 = k_1$$
$$n_2 = k_2 (2 N_{22} + N_{12})/ 2 = k_2$$

Then:

$$\frac{I_1}{I_2} = \frac{2 V_{11}\beta^2 + V_{12}\beta(1 - \beta)k_2/k_1}{2 V_{22}(1 - \beta)^2(k_2/k_1)^2 + V_{12}\beta(1 - \beta)k_2/k_1}$$

If $V_{12}^2 = 4 V_{11}V_{22}$, the above reduces to:

$$\frac{I_1}{I_2} = \frac{k_1(V_{11})^{\frac{1}{2}}\beta}{k_2(V_{22})^{\frac{1}{2}}\beta} = \frac{k_1(V_{11})^{\frac{1}{2}}(2N_{11} + N_{12})}{k_2(V_{22})^{\frac{1}{2}}(2N_{22} + N_{12})}$$

which is also in accord with experimental data.

On the basis of the above calculations, Fowler

has indicated that either the discharge reaction or the velocity of formation of molecules may be the slow reaction. It is possible to correlate either case with experimental data.

c) A potential barrier has also been postulated to account for the separation.

1. Erdy-Gruz and Volmer[1] suggest a barrier to the passage of electrons from the solution to the electrode.

2. Gurney[1] holds there is a barrier to the passage of electrons into the solution.

Bell has considered the Gurney theory in connection with the discharge of two water molecules differing in zero point energy. k_{11}, k_{22}, and k_{12} (k_{21}) are calculated and used in the equation:

$$\frac{I_1}{I_2} = \frac{2 k_{11} N_{11} + k_{12} N_{12}}{2 k_{22} N_{22} + k_{21} N_{21}}$$

$$N_{11} + N_{22} + N_{12} = 1, \; K_w = 3.28$$

The result gives the expected fractionation factor. Since the k's are proportional to:

$$\frac{T}{1-\gamma} \cdot e^{\frac{E - E_m + eV}{\gamma kT}} + \phi$$

γ = a constant greater than 1.
E - energy of neutralization of an ion in its lowest energy state.
ϕ = $f(V,T)$, and is independent of mass.
E_m - the work function of the metal.
V - the applied potential.

It may be considered that:

$$E = \text{constant} + E_0^0$$

where E_0^0 depends on the molecule involved. Then:

$$\frac{I_1}{I_2} = \frac{2 N_{11} e^{E_{11}^0/\gamma kT} + N_{12} e^{E_{12}^0/\gamma kT}}{2 N_{22} e^{E_{22}^0/\gamma kT} + N_{12} e^{E_{12}^0/\gamma kT}}$$

When $K_w = 4$

$$E_{12}^0 = (E_{11}^0 + E_{22}^0)/2$$

$$\ln \alpha = + (E_{11}^0 - E_{22}^0)/2\gamma kT$$

This formula is consistent with experimental results dealing with the temperature coefficient of the fractionation factor, and the fractionation factor itself.

This equation is only approximate but might perhaps be made more consistent with experimental results. The theoretical significance of γ is not known and the equation takes no account of any effect of the electrodes on the fractionation factor. This equation does not allow for the possibility that the probabilities of H and D, passing the potential barrier from molecules of H_2O, HDO and D_2O of equal energy, are not equal.

d) A second type of potential barrier has been postulated by Farkas and Farkas.[2] When the electron leaves the metal for the hydrogen ion, it must jump over an energy barrier, which is probably different for H^+ and D^+. Therefore, there is a different probability for

the discharge of each. This mechanism can account for the fact that the efficiency of separation is nearly independent of the electrode metal, and also gives a fractionation factor of the correct magnitude.

IV The Exchange Reaction

$$H_2O + HD \rightleftharpoons HDO + H_2$$

$$K = \frac{(HDO)(H_2)}{(H_2O)(HD)} \cong 3 \text{ at } 20^\circ \text{ C.}$$

This exchange probably takes place to a large extent in the electrolytic process as the metal electrodes will certainly catalyse the reaction. Moreover, the released hydrogen, being "in statu nascendi"[1], has a high reactivity toward exchange. It is therefore possible that complete equilibrium is established. However, it is impossible to assume that the fractionation depends only on this exchange. Were this the case, the value 3 would be a limiting value for the fractionation factor as equilibrium were approached in either direction, and fractionation factors much larger than this have been obtained in actual practice. It is probable that the fractionation factor depends to some extent upon all the theories discussed above.

d) Other Factors Influencing the Fractionation Factor

The fractionation factor varies during the course of the electrolysis and from run to run. The

current density has a slight effect, higher densities favouring increased fractionation. Electrolytic concentration has little effect. Alkaline solutions give better separation than acid solutions. Different authors have found different combinations of electrolyte and electrode to be effective. Topley and Eyring[14] find Pt - KOH best, but Bell[1] found Ni -, Pt -, or Cu - NaOH to be about equally effective. Reported fractionation factors range from 3.4 to 7.6, with 5 or 6 the most common. Lower efficiency is caused by high temperature operation (50 - 60° C.) which results in some carry-over of water as spray or vapour. The effect of temperature is shown in the equation:

$$\ln \alpha = Q/RT + C$$

Q - difference in the activation energy for the discharge of H^+ and D^+.

e) Summary

The electrolytic process, as indicated in the above discussion, is very complex. To a great extent the fractionation depends on exchange between water and gaseous hydrogen with consequent enrichment of the aqueous phase.

$$H_2O + HD \rightleftharpoons HDO + H_2$$

However, the limiting value of the equilibrium constant[#]

[#] the equilibrium constant corresponds to the fractionation factor for the exchange reaction alone.

for the exchange reaction is only 3, and the overall fractionation factor is usually 5 - 6. The greater separation is brought about by some process at the cathode. Several of these reactions have been postulated, and it is conceivable that all, or many of these theories are somewhat involved in the electrolytic separation.

2. Other Methods of Separation

a) Fractional Distillation of Liquid Hydrogen

This was the first method used to concentrate deuterium. Urey, Brickwedde and Murphy[19] tried concentrating deuterium by this method in an effort to detect the presence of the isotope spectroscopically. Using the Debye theory, they calculated the vapour pressure of three species of hydrogen, H^1H^1, H^1H^2, and H^1H^3, of which only H^1H^1 was known. The calculations indicated that at the triple point, the vapour pressures of the three should be appreciably different. Therefore it would seem that a triple point evaporation should considerably enrich the residue in the heavier species. Four litres of liquid hydrogen were evaporated near the triple point to a few millitres. An examination of the spectrum of this residue gave proof of the presence of deuterium by hitherto undiscovered spectral lines in the calculated position. By a very lengthy exposure it was possible to obtain a deuterium spectrum from ordinary tank

hydrogen, and from the length of the exposure, the abundance was estimated to be 1:4500. These authors obtained an enrichment of about 1:800. More recently Keesom, Dijk and Haantjes,[1] using a fractionating column, obtained an enrichment of 1:60 by this method. Taylor, Gould and Bleakney claim there is a slight separation of H_2 and D_2 obtained by fractional desorption from charcoal at liquid air temperature.[2]

b) Distillation of Water

Because of a slight difference in the vapour pressure of H_2O, HDO and D_2O, separation is possible by the fractional distillation of water. The workers in this field are Washburn and Smith, Lewis and Cornish, and Hall and Jones.[1]

The fractionation factor obtained ;

$$\alpha = \frac{2AB + (ABkK)^{\frac{1}{2}} \cdot 2 + (BK)^{\frac{1}{2}}}{2 + (ABkK)^{\frac{1}{2}} \quad 2B + (BK)^{\frac{1}{2}}}$$

A - the vapour pressure of pure H_2O and D_2O
B - the ratio of their mole fractions in the liquid
k - $P_{12}/P_{11}P_{22}$ where P_{12}, P_{11}, and P_{22} are the vapour pressures of HDO, H_2O and D_2O respectively.

At the boiling point A = 1.05 and the fractionation is approximately 1.025 (K = 4, k = 1 Lewis and Cornish[1]). A fractionation column of 100 theoretical plates would give a fractionation factor of about 10. It was formerly thought that columns of this type might be used with sufficient efficiency to compete with the electrolytic

method, but this is no longer the case. However, the method is still important, either by itself, or as an initial stage for the electrolytic process.[20]

c) Diffusion Methods

I Through a porous plug

This method is based on the different molecular velocities of the isotopes. These velocities are inversely proportional to the root of the mass of the isotope.

$$w_{H_2} : w_{HD} : w_{D_2} = \frac{1}{\sqrt{2}} : \frac{1}{\sqrt{3}} : \frac{1}{\sqrt{4}}$$

w_{H_2}, w_{HD}, and w_{D_2} - mean velocities of H_2, HD and D2

When a mixture of gases is pumped from a vessel through a fine nozzle we get a separation expressed by the Rayleigh formula:

$$\frac{(H_0)}{(H)} \cdot \left[\frac{(D)}{(D_0)}\right]^s = \left(\frac{p_0}{p}\right)^{s-1} \qquad s = \sqrt{2} = 1.41$$

p_0, p - initial and final pressure in the vessel.
(H_0), (H), (D_0), (D) - initial and final concentrations of hydrogen and deuterium.

This holds only if the dimensions of the nozzle are small compared to the mean free path of the molecules.

The separation factor, s, is very low, but by a several stage diffusion through porous clay tubes sealed in glass and suitably connected to several diffusion pumps, Herts managed to effect a multiple separation

leading to 100% D_2.[2] (The Balmer lines of hydrogen could not be found in a spectroscopic analysis of the product.) Because the starting material contained a very low concentration of actual D-D molecules, an electric discharge was applied at a suitable point in the system to establish the equilibrium:

$$H_2 + D_2 \rightleftharpoons 2\ HD$$

This method of preparation yields a very pure product but its usefulness is limited in that it is only applicable to small quantities.

II Diffusion Through Palladium.[1, 2]

Diffusion through palladium can also be used to concentrate deuterium. The separation, in this case, is not due to the different molecular velocities alone; and temperature has an important effect. The hydrogen diffuses through the palladium in the atomic form. As this process has an activation energy of roughly - 16,500 cal., the rate of diffusion defined as:

$$s = \frac{(H/D)\ \text{diffused}}{(H/D)\ \text{original}}$$

is expressed by the relationship:

$$s \propto e^{-16{,}500/RT}$$

The activation energy appears to be higher for HD and D_2 because of their lower zero point energies. Assuming an atomic mechanism for the diffusion process, the rate

of diffusion, s, is given by

$$s = e^{(E^{\circ}_{H_2} - E^{\circ}_{D_2})/ 2 RT}$$

The values of the zero point energies are known, and are 6183.4 calories for hydrogen and 4394.5 calories for deuterium. Substituting:

$$s = e^{850/ RT}$$

The form of the equation indicates that the separation factor is roughly proportional to 1/T.

This is a very inefficient method of concentrating deuterium. At low temperatures, the volume of the gas passed is very small; at high temperatures the separation is very poor. However, and 8 -10 fold concentration of deuterium has been obtained by Harris, Jost and Pearse[2] at 300° C. and atmospheric pressure/100.

d) Chemical Methods[1]

Any reaction involving hydrogen and deuterium and having a different rate of reaction for each isotope may be used to separate the two. This refers also to exchange reactions having equilibrium constants different from those calculated from the statistical distribution of the isotopes. An excellant example is:

$$H_2O + HD \rightleftharpoons HDO + H_2$$

statistical K = 4, actual K = 2.67

This might conceivably be utilized by an apparatus similar

to a fractionating column and based on countercurrent principles.[20]

e) More Recent Methods

I An electrolytic method has been studied in which the reaction takes place in a series of cells.[21] The level is kept constant by the flow of electrolyte from one cell to the next, with fresh solution being added only to the first cell in the series. The enrichment obtainable by this method depends on the number of cells. The process tends to be expensive as the amount of current required increases rapidly with the number of cells.

II A slight separation has been reported to occur in the flames rising from the combustion of mixtures of the gases, H_2, D_2 and O_2.[22]

III Thermal diffusion columns have been adapted to the separation of hydrogen and deuterium. Seaborg, Wahl and Kennedy obtained a separation factor of 30 with an apparatus of this nature.[23] The method gives promise of being rapid and efficient for obtaining low enrichments of D_2O.[24].

IV The ultracentrifuge has been suggested for isotopic separation.[20] This method is not very practical for deuterium. The separation obtained depends on the mass difference rather than on the percent mass difference, and as the latter is very great for the hydrogen isotopes,

this method represents a loss in efficiency.

V Fractional adsorption and desorption of steam or hydrogen gas can be utilized for the isotopic separation.[25, 26] The method leads to low concentrations of the heavy isotope.

VI Hydrogen and deuterium exhibit different rates of decomposition and recombination. In a glow discharge tube this phenomenon has been utilized to concentrate deuterium at the cathode.[27]

3. Conclusions

The most widely used method of preparing deuterium is the electrolytic method. Although the theory is very complicated, the method of preparation is simple and convenient, and in regions where electrical power is inexpensive, produces 99.9% deuterium at a low cost. It is this method that is given the credit for making deuterium available for such wide-spread use as it finds to-day. The fact that this method, which is one of the first used, has not been displaced by later methods is in itself proof of its excellence.

During the war period large amounts of deuterium oxide were required for the atomic energy project.[20] The deuterium oxide used in this project was prepared by fractional distillation or by chemical exchange. Although the raw product for fractional distillation is cheap and readily obtainable, the method

is expensive because the slight difference in vapour pressures of the molecular species necessitates a great deal of boiling. In the chemical exchange method, a catalyst is employed to bring about equilibrium between water and hydrogen gas. The reaction takes place in a tower featuring counter-current flow of water from top to bottom, and hydrogen gas from bottom to top. In contrast to the distillation process, this method proved to be very inexpensive.

Deuterium gas and deuterium oxide are both available commercially at the present time, and the comparatively low cost of these substances speaks for the efficiency of the above methods of preparation. (28)

Chapter 3

The Analysis of Deuterium and Deuterium Oxide

Before the hydrogen isotope could be utilized as a scientific tool, it was necessary to devise methods of analysing gas and water samples for their deuterium content. A great number of analytical methods have been developed, a number of which are described below.

1. Early Methods (1)

a) Analysis of the Hydrogen Spectrum

This is a good method of detecting deuterium but a poor method of analysing it. There are many errors and difficulties involved in the determination of relative intensities of light, and it is also very difficult to avoid the partial reversal of the lines of the most abundant isotope.

b) Mass Spectrographic Determination

These methods are similar to those used by Thomson, Dempster, Aston, or Bainbridge. Blaauney used a mass spectrometer and determined the abundance by collecting the ion charges and determining the charge to mass ratios of the ion beams. Masses 1 - 6 are collected, as are tabulated in Table 5.

Table 5

Ion Species	Mass
H^+	1
H_2^+	2
D^+	2
H_3^+	3
HD^+	3
D_2^+	4
H_2D^+	4
HD_2^+	5
D_3^+	6

The intensities of these beams are plotted as functions of the pressure. An analytical and graphical solution of the curves is made and they are compared with the curves determined for natural hydrogen.

c) Pycnometers

Because of the relatively high percentage mass difference of the molecules H_2O and D_2O, the densities of the two waters are considerably different. Very pure D_2O has been prepared, and its density has been carefully measured. (Table 6).

Table 6

Density of H_2O and D_2O

Density H_2O	Density D_2O	Temperature °C.	Date of Determination	Ref. No.
1.0000	1.1079	25	1934	149
0.999980	1.10726	20	1938	169
1.00000	1.10726	20	1942	170
1.00000	1.10765	25	1942	170

The relationship between mole fraction and density has been given by Lewis and Luten.[1]

$$d_{25^\circ C.} = 0.9701 + 0.1087N_2 + 0.0012N_2^2$$

N_2 - mole fraction D

Using this equation and the density as determined with a pycnometer, the D content of water samples may be calculated.

d) Bouyancy Balance[1]

Dufour's bouyancy balance may also be used to determine density differences. A glass float just remains suspended in a reference sample at a convenient temperature. At another temperature it will remain afloat in the unknown. The apparatus is calibrated in terms of the relationship between change of density and change of temperature.

e) Gilfillan and Polanyi - Float Method[1]

A second float method devised by Gilfillan and Polanyi uses the pressure under which the diver just sinks to determine the density. The difference in

compressability of a closed glass float and the water might also be utilized.

f) Refractive Index (1)

The concentration of deuterium may be determined by refractive index measurements, the refractive index of water samples being a linear function of the D_2O concentration.

g) Bombardment Techniques (1)

Rutherford determined the deuterium concentration from the number of 13 cm. range alpha particles resulting from the high voltage bombardment of lithium with ions from an unknown H_2 - D_2 mixture. These alpha particles result only from the collisions involving the deuteron. This is a very rapid method.

h) Hill-Baldes Vapour Tension Apparatus

The different vapour tensions of H_2O and D_2O can be used to analyse for the deuterium content. (29) Because of the difference in vapour pressure, a drop of H_2O on one end of a thermocouple appears cold with respect to a drop of D_2O on the other end. The concentration of D_2O is indicated by the magnitude of deflection of a galvanometer connected to the thermocouple circuit.

2. Some Methods in Use To-day

a) Falling Drop Method (30)

This method makes use of the fact that the rate of fall of a drop through an immiscible medium depends partly on the density of the drop. The density difference of 100,000 ppm between pure H_2O and D_2O makes the method particularly sensitive for D_2O analysis. Barbour and Hamilton developed the method, and Vogt and Hamilton adapted it to D_2O - H_2O analysis in 1935. Other workers have improved the technique until errors are only of the order of 0.1 ppm. This permits a determination of 0.0001% deuterium.

The rate of fall of a drop through a medium at a given temperature is given by Stokes' law:

$$6\pi\eta av = \frac{4}{3}\pi a^3 (d - d_0)g \qquad (1)$$

v - velocity of fall
d - density of drop
d_0 - density of medium
a - radius of drop
g - acceleration of gravity
η - viscosity of medium

The sensitivity of the method is defined as the difference in the velocity of two drops of given density difference.

$$v_2 - v_1 = \frac{k}{\eta} a^2 (d_2 - d_1) \qquad (2)$$

As the parameters must be controlled in order to get reproducible results, the effect of each of these is considered in turn.

I Size of Drop

A small drop is desirable as the original

samples are usually quite small. Since the sensitivity is a function of a^2, a large drop would give theoretically better results. However, as the radius of the drops approaches approximately 1/3 the tube radius, the sensitivity of the method is decreased because of the [illegible] action of the tube walls which effectively increases the viscosity.

The required precision of a few ppm is obtained by use of a mechanical micropipet. If the drops are not uniform, the percentage error introduced into the velocity determinations is 2/3 the size of the percentage volumetric error. There have been several types of pipets described.[31, 32] In general, they consist of a capillary pipet operated by a carefully manufactured screw and piston arrangement which, by changing the level of some mercury in the capillary, can suck up or eject a standard drop of sample.

II Medium

This, of necessity, is a liquid, non-miscible with water, and with a density slightly less than that of the water at just below room temperature. Although mixtures of bromobenzene and xylene have been used successfully, o-flourotoluene is better because the density of the former changes on evaporation. The density of o-flourotoluene is such that 0 - 3% D_2O may be measured at 26.8° C. Other concentrations of D_2O may be determined by changing the temperature.

M-flourotoluene is suitable for determining 0 - 3% D_2O at 19.3° C.

III Gradient Tube

Although the size of the tube is not important, its diameter should be more than 3 times that of the drop to avoid significant wall effects. Because the time measured depends on the length of the path through which the drop falls, the tube should be as long as is convenient for the size of the water bath. To allow the drop to reach temperature equilibrium and to avoid end effects, the drop should fall 20 cm. before timing is started, and timing should be stopped 10 cm. before the end of the tube.

IV Temperature

Since temperature influences the density of both the drop and the medium, and the viscosity of the medium, temperature control is very important. The temperature of the bath is that which will give a suitable rate of fall over the range of densities to be measured. In the attainment of reproducible results, uniformity of temperature is not as important as constancy of temperature. It is advantageous to keep the room temperature about 1° higher than that of the bath. This means that the drop is initially less dense than the medium and therefore will fall very slowly until temperature equilibrium is reached. Under these conditions the drop is more likely to reach temperature equilibrium

in the 20 cm. before timing is started than if it were initially more dense than the medium.

V Time

It has been found experimentally that the relationship between the density and the velocity of fall of the drop is not linear, but is given by:

$$d - d_0 = k'(v + \beta v^2) \qquad (3)$$

The precision of the results is limited by the precision of the stop-watch. This is especially true when the differences in density of drop and medium are large. When the density differences are small, the fluctuations in the other parameters become more important. It has been found that precise measurements can be made with a stop-watch reading 0.01 sec. for drop velocities of 0.1 cm/sec.

VI Concentration of Samples

Samples containing 0 - 3% D_2O can be measured conveniently. More concentrated samples should be diluted to approximately this concentration.

VII Experimental

The sample must be very carefully prepared and purified. (See Chapter 4) Freshly distilled water is used as a standard, and standard and sample are always dropped together. Before use, the pipet is rinsed with some of the sample. The drop is formed under the surface

and released at the surface when the pipet is raised. The time of fall between two designated marks on the tube is measured, and the D_2O concentration is read from a calibration curve. A pycnometer is used to determine the density of a series of primary standards. The calibration curve is determined using these and a series of secondary standards prepared from these by dilution.

VIII Errors

Depending on the density, errors of the order of 1 - 4 ppm can be expected. Usually this error is insignificant as the error inherent in the preparation and purification of the samples is of the order of 20 ppm.

b) Gradient Tube (33)

When a liquid of greater density is layered over another of lesser density, and miscible with the first, a uniform gradient of density develops in the region of the juncture of the two. Density determinations can be made from the equilibrium position of small drops of immiscible liquids in the linear density gradient.

I The Gradient Tube

For the determination of the D_2O concentration of water samples, two bromobenzene-kerosene mixtures are used. The denser is carefully poured on top of the less dense, and the liquid in the tube near the juncture of the two is thoroughly stirred by up and down strokes of a stirrer.

After sitting 24 - 48 hours the density gradient reaches linearity. These gradients may be used for 6 months to one year if they are kept at a uniform temperature and are well stoppered to prevent evaporation and dust contamination.

Error will be introduced if the bromobenzene-kerosene mixtures contain water or water soluble substances. Therefore they must be carefully purified and dried before use. Traces of water will exchange with the D_2O samples introduced, but after a short period of use the gradient will be saturated with equilibrium concentration D_2O at each level and so no further error will be introduced.

II Standard Solutions

These are prepared by diluting 99.9%, or purer, D_2O with carefully distilled normal water. About 10 standards with a density range of 0.998 - 1.01 are prepared.

III Experimental

A very accurate pipet is used to deliver the drops. It is rinsed with each sample before use. Drops of standard samples lying in a range close to the unknown are introduced at the same time as the unknown, and the relative positions of all the drops are determined with a cathetometer. The unknown density is calculated by interpolation.

When the readings have been taken the drops are removed by filter paper rolled on a glass rod. It is

about 6 hours before the gradient can be used again.

c) Thermal Conductivity

The heat that a hot wire can lose to a cold surface depends on the specific heat of the gas surrounding the wire.[2] Since H_2, HD and D_2 exhibit great specific heat differences at liquid air temperature, this principle has been adapted to the analysis of hydrogen and deuterium gas.[1, 2, 34] Farkas and Farkas[2] did the original work on the method. The technique requires very small gas samples (about 0.002 cc. at N.T.P.), and has been widely used.

The apparatus consists essentially of a bare platinum wire stretched in a glass tube. When measurements are being made the tube is immersed in liquid air.[1, 2, 34] The platinum wire is heated by a battery and its resistance is measured by a wheatstone bridge. In the analysis of a sample, the current is determined which will keep the wire at a standard temperature. The D percent is determined from a calibration curve in which %D is plotted against current for several standard samples. Actually the D content can be calculatedoon the basis of the Maxwell-Chapman-Enskog equation:

$$\lambda = A \frac{C_v}{m^{\frac{1}{2}} \sigma^2}$$

λ - temperature gradient
A - apparatus constant
σ - molecular diameter
C_v - specific heat at constmt volume
m - molecular weight

The calculations are fairly long and because of experimental inexactitudes (such as the presence of unknown amounts of heating in parts of the wiring other than the platinum wire; and convection currents in the tube) not too precise. For this reason a calibration curve is employed.

3. Use of a Mass Spectrometer

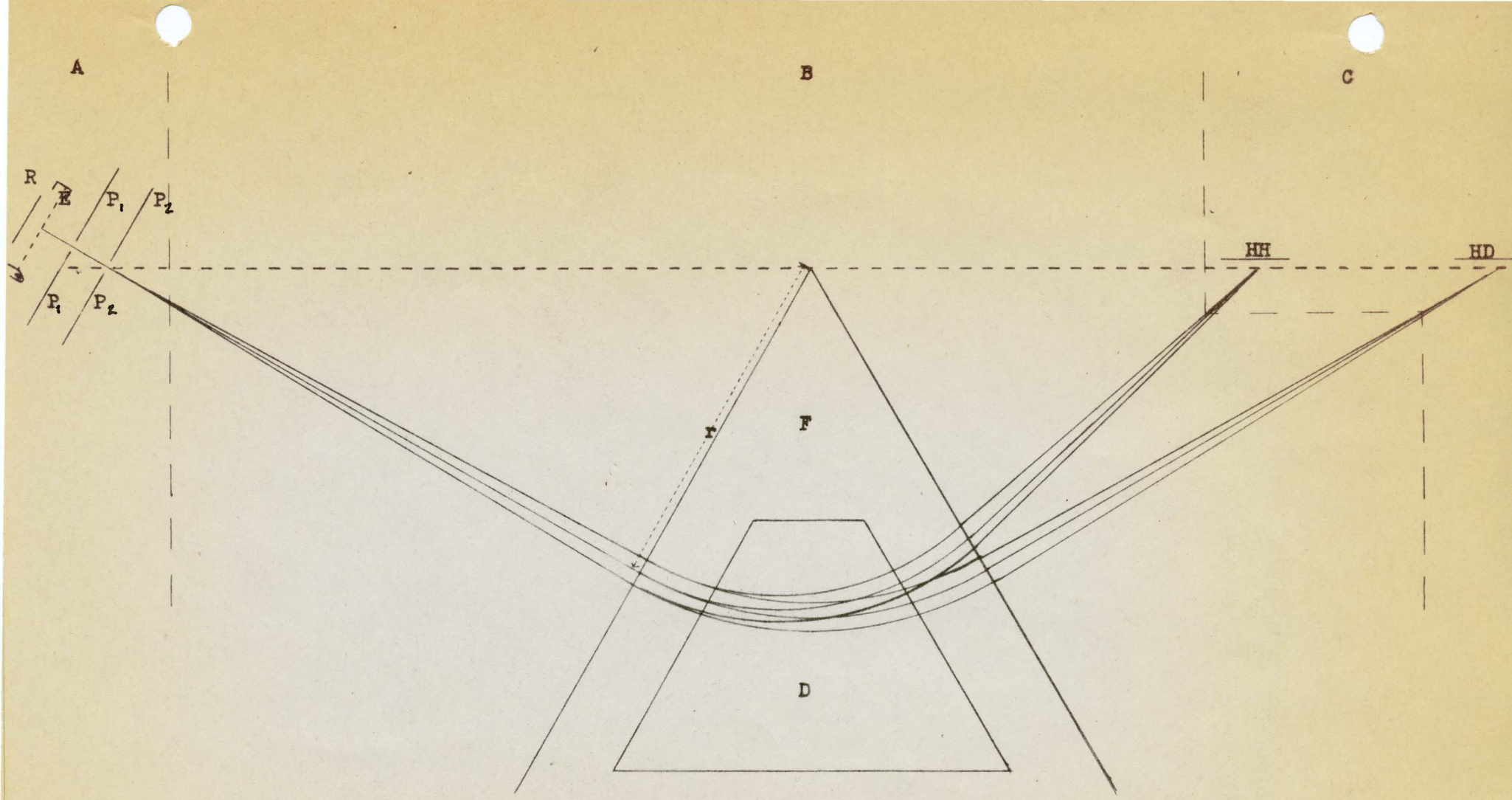
The best method of isotopic analysis available at the present time is the mass spectrometric method.[35] A mass spectrometer is an instrument designed to separate particles of different charge/mass ratios.[105] Since the ratio of charge/mass is different for each isotope of an element, a mass spectrometer can be used for their separation. The separating action depends on the effect of a combination of an electric and a magnetic field on positive ions produced from the isotopic substance by electron bombardment. After the separation, the various isotopes are collected, and the corresponding ion currents are measured. The relative sizes of the ion currents are an indication of the relative isotopic abundances of the sample.

Figure 1 shows a mass spectrometer specially designed by A. O. Nier for the analysis of deuterium.[36] The spectrometer tube has been differentiated into three parts, namely: A - the source end.

B - the analyser tube.

C - the collector end.

D is a small permament Alnico magnet with 60° pole faces.



SCHEMATIC DIAGRAM

NIER TYPE SIMULTANEOUS FOCUSING

DEUTERIUM MASS SPECTROMETER

Fig. 1

At the source end the sample to be analysed, in this case hydrogen gas, is introduced. The gas passes into an ionisation chamber, E, where the gaseous molecules are ionized by electron impact. An electrical field, produced by a potential drop between R and P_2, accelerates the ions toward the analyser tube, the velocity attained by the ions depending on the potential of R.

The relationship of the potential of R to the velocity of the ions is the following:

$$Ve = \frac{1}{2} mv^2 \qquad (1)$$

V - the accelerating potential
e - the charge on the particle
m - the mass of the particle
v - the velocity of the particle

All the positive particles have essentially the same energy because of the controlled conditions of their production.

From the ionization chamber, the ions pass through collimating slits in the plates P_1, P_2 into the analyser tube. Here the ion beam is subjected to the influence of a magnetic field produced by a 60° sector magnet, D. The spectrometer is so designed that the ion beam enters the magnetic field perpendicularly to the field. If the field strength is H, the magnetic force applied to each ion will be Hev.[12] Under the influence of this field, the ions will follow a curved path with radius of curvature, r, such that the centrifugal force, mv^2/r just balances the magnetic force.

$$Hev = mv^2/r \qquad (2)$$

H - magnetic field strength
e - charge on particle
v - velocity of particle
m - mass of particle
r - radius of curvature of particle

It has been shown (37) that there will be a focusing of the ion paths if a slightly divergent beam of ions of the same momentum (under the condition of equal energy as explained on page 48, this implies particles of equal mass) enters a magnetic field at right angles to the field, and is bent through such an angle as to leave the field perpendicularly. This focusing takes place in such a way that the ion source, the apex of the magnetic field fringe, and the point of focusing all lie in one plane.

In most mass spectrometers there is a single collector plate at the point where the beams are focused on leaving the magnetic field. Ions of different masses are focused on this collector either by changing the accelerating potential, or by changing the strength of the magnetic field. In the deuterium instrument, calculations are made to determine the points of focus of the two isotope beams under the condition of field and accelerating voltage (36) both fixed.

The relative values of the radii of curvature of the ions are determined from equations (1) and (2).

From (1): $$e/m = v^2/2V \qquad (3)$$

From (2): $$e/m = v/Hr \qquad (4)$$

From (3) and (4): $v^2/2\ V = v/Hr$

$$v = 2\ V/Hr \qquad (5)$$

substituting in (4): $e/m = 2\ V/H^2r^2$

$$m = eH^2r^2/2\ V \qquad (6)$$

Since e, H, V and m are constant, we can calculate r for any given ion. Let $K = eH^2/2\ V$. Then (6) becomes:

$$m = Kr^2 \qquad (7)$$

Also, by differentiating equation (7):

$$\triangle m = 2\ Kr\ \triangle r \qquad (8)$$

Dividing (8) by (7):

$$\triangle m/m = 2\ \triangle r/r \qquad (9)$$

Using equations (7) and (9) we can calculate the radius of curvature for both the hydrogen and deuterium ion beams.

Using these calculations, two collector plates are placed so that for the fixed values of magnetic field and accelerating potential, the hydrogen ion beam and the deuterium ion beam will be brought to a focus on the two plates simultaneously. These positive ions fall on the collector plates and the resulting ion currents are amplified and measured by the deflection of galvanometers connected to the amplifier outputs. The isotopic abundances can be determined from the relative galvanometer deflections caused by the ion beams.

In the deuterium mass spectrometer the

calculations have been simplified by the incroporation of a null method of analysis.[36, 38] The collector for the smaller ion beam is connected to an amplifier and galvanometer as outlined above. The larger ion beam is connected to a feedback amplifier, and the output of this amplifier is fed into the input of the first amplifier in such a manner as to nullify the signal from the smaller ion beam. (Fig. 2)

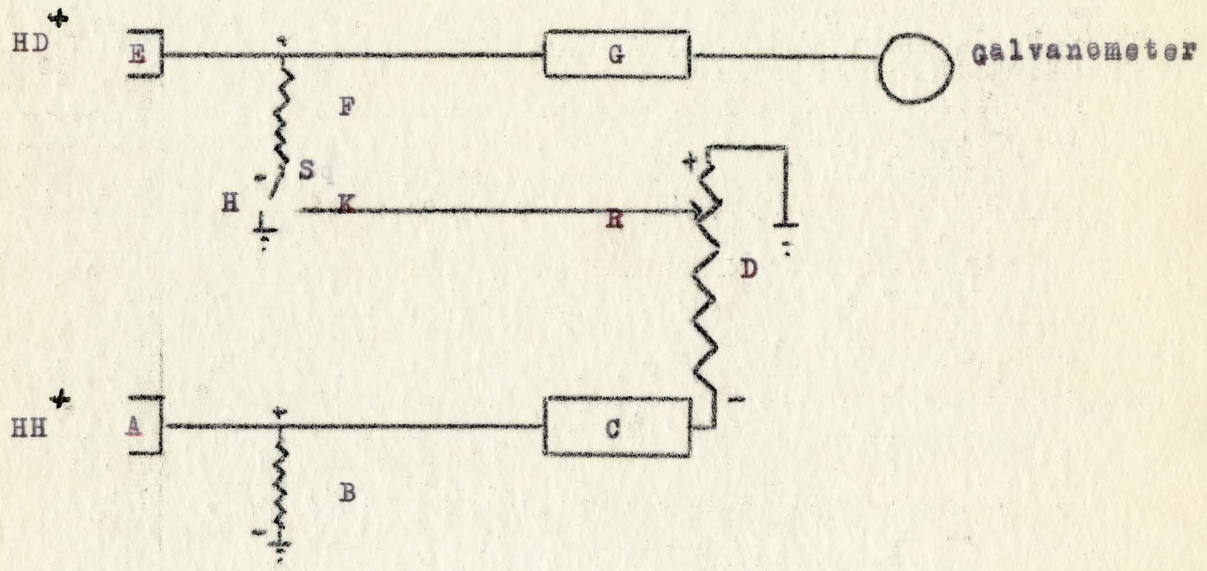


Fig. 2

Simultaneous Collection Principle

When a mixture of hydrogen and deuterium gas is ionized, there are several ionic species formed, the amounts of each depending on the relative concentrations of hydrogen and deuterium present. For gases of low deuterium content, the most abundant ions are HH^+ (Mass 2) and HD^+ (mass 3), and for gases of high deuterium content,

HD^+ and DD^+ (Mass 4) are chiefly formed. In 99% H_2 the concentration of DD^+ is negligible and in 99% D_2 the concentration of HH^+ is negligible. Depending on the samples to be analysed, deuterium instruments are available which collect simultaneously either masses 2 and 3, or masses 3 and 4. [36] [39] An instrument has also been proposed which can be used to collect either pair, depending on the sample to be analysed.# The theory is the same regardless of which instrument is being considered. This paper deals with the 2 - 3 instrument, which is used for the analysis of gas samples containing up to 5% deuterium.

The HH^+ ions are collected on the collector, A. The positive charge resulting causes a potential drop over the grid leak resistor, B, (of the order of 10^{10} ohms) and this potential drop acts as a signal for the feedback amplifier, C. The output of C is fed to ground through the potentiometer, D, causing the appearance of a potential across D. It is to be noted that, although the input to the feedback amplifier was positive with respect to ground, the output is negative with respect to ground.

Similarly, the HD^+ ions are collected on the collector, E, causing a potential drop across the grid leak resistor, F, (4×10^{10} ohms) when the switch, s, is closed to H. This potential drop is the signal for the amplifier, G. The output of this amplifier is fed to a

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#Private communication to this laboratory from A. O. C. Nier.

galvanometer, the magnitude of the galvanometer deflection being a measure of the size of the HD^+ beam. When s is closed to K, however, part of the potential of D is fed into F. This decreases the signal to the amplifier, E, because the potential on F is positive with respect to ground while that of D is negative with respect to ground. By adjusting the contact, M, a potential can be found just equal to that caused by the HD^+ beam, and here the signal for the amplifier, E, and the galvanometer deflection will be zero. By using hydrogen samples of known deuterium content, the potentiometer, D, can be calibrated so that for every setting of M, the ratio HD^+/HH^+ can be read directly for a zero galvanometer deflection.

This null method has several inherent advantages (36, 38) which make it less subject to error. The number of ions of both isotopes at any given time is a function of the ionizing current. Fluctuations in the ionizing current will cause fluctuations in both ion beams of the same relative magnitude; so that the ratio HD^+/HH^+ will not change although the number of ions of each species does change. If each ion beam were measured separately, they could not be measured simulataneously; and if the ionizing current varied between readings, the resulting ratio would be in error by the magnitude of the ionizing current fluctuations. But by the use of the null method, the fluctuations in one ion beam are balanced out against the corresponding fluctuations in the other ion beam, and the HD^+/HH^+ ratio

remains constant in spite of these fluctuations. This method of analysis is also much more rapid as only one reading need be made for each ratio determination, and calculations are simplified by reading the ratio directly.

4. Deuterium Oxide Analysis With the Deuterium Mass Spectrometer

With the instrument described above, it is possible to analyse directly only samples of hydrogen or deuterium gas. Since most uses of deuterium depend on the utilization of deuterium oxide, it is important to have available spectrometric methods of water analysis.

a) Equilibration (40)

When a deuterium gas sample is shaken with a mixture of H_2O and D_2O, hydrogen exchange takes place between gas and water until equilibrium is reached. From a mass spectrometric determination of the HD^+/DD^+ ratio of the gas, the deuterium content of the water can be calculated if the values of the equilibrium constants involved are known.

In the preparation of gas samples by equilibration a catalyst (PtO_2) and a peptizing agent (maleic anhydride) are used. The gas and water are shaken with the catalyst and peptizing agent in a constant temperature bath at 25° C. for at least 4 hours. The deuterium gas for use in equilibrations may be prepared from 99.5% D_2O by the action of very pure calcium metal

in an evacuated system.[41] This gas has a deuterium content of greater than 99 atom % deuterium.

The following reactions are involved in the calculations of the deuterium content of the water from the value of the HD^+/DD^+ ratio given by a 3 - 4 deuterium mass spectrometer. (For simplicity, the HD^+/DD^+ ratio given by the spectrometer is known as R)

$$H_2 + D_2 \rightleftharpoons 2\,HD \qquad K_1 = \frac{(HD)^2}{(H_2)(D_2)} \qquad (1)$$

$$= 3.26 \text{ at } 25^\circ \text{ C.}$$

$$D_{2(g)} + HDO_{(g)} \rightleftharpoons D_2O_{(g)} + HD_{(g)}$$

$$K_2 = \frac{(HD)(D_2O)}{(D_2)(HDO)} \qquad (2)$$

$$= 3.11 \text{ at } 25^\circ \text{ C.}$$

$$HDO_{(l)} \rightleftharpoons HDO_{(g)} \qquad K_3 = \frac{(HDO)_g}{(HDO)_l} = P_{HDO} \qquad (3)$$

$$D_2O_{(l)} \rightleftharpoons D_2O_{(g)} \qquad K_4 = \frac{(D_2O)_g}{(D_2O)_l} = P_{D_2O} \qquad (4)$$

On the basis of these the following calculations can be made:

$$\text{I Atom \% } D_2 \text{ in gas} = \frac{\text{No. of atoms of } D_2}{\text{No. of atoms } D_2 \text{ and } H_2} \times 100\%$$

$$\text{By (1) atom \% D} = \frac{2(D_2) + (HD)}{2(H_2) + 2(D_2) + 2(HD)}$$

(a) Gass99% D or greater

(H_2) is negligible according to the probability of states.

$$\text{atom \% D} = \frac{2(D_2) + (HD)}{2(D_2) + 2(HD)}$$

$$= \frac{2 + \frac{(HD)}{(D_2)}}{2 + 2\frac{(HD)}{(D_2)}}$$

$$= \frac{2 + R}{2 + 2R} \qquad (5)$$

Where R is the ratio determined by the deuterium mass spectrometer.

(b) Gas less than 99% D

In this case (H_2) is not negligible.

From (1) $$(H_2) = \frac{(HD)^2}{(D_2) \times 3.26}$$

$$\text{atom \% D} = \frac{2 + R}{2 + 2R + \frac{2(HD)(HD)}{(D_2) \times 3.26} \times \frac{1}{(D_2)}}$$

$$= \frac{2 + R}{2 + 2R + \frac{2R \cdot R}{3.26}} \qquad (6)$$

II Atom % D in a liquid

$$\text{atom \% D} = \frac{\text{no. of atoms } D_2}{\text{no. of atoms } (D_2 + H_2)} \times 100\%$$

$$= \frac{2(D_2O) + (HDO)}{2(D_2O) + 2(H_2O) + 2(HDO)}$$

(a) Water 99% D or greater

(H_2O) is negligible. Let $\frac{(HDO)}{(D_2O)} = R'$

$$\text{atom \% D} = \frac{2\,(D_2O) + (HDO)}{2\,(D_2O) + 2\,(HDO)}$$

$$= \frac{2 + R'}{2 + 2R'} \qquad (7)$$

From equation (2)

$$K_2 = \frac{(HD)_g x (D_2O)_g}{(D_2)_g x (HDO)_g}$$

From equation (3)

$$(HDO)_g = K_3\,(HDO)_l$$

From equation (4)

$$(D_2O)_g = K_4\,(D_2O)_l$$

Substituting:

$$K_2 = R x \frac{K_4 (D_2O)_l}{K_3 (HDO)_l} \qquad (8)$$

Therefore:

$$R' = \frac{R \cdot K_4}{K_2 K_3} \qquad (9)$$

$$\text{atom \% D} = \frac{2 + RK_4/K_2K_3}{2 + 2RK_4/K_2K_3}$$

$$= \frac{\frac{2K_3K_2}{K_4} + R}{\frac{2K_3K_2}{K_4} + 2R}$$

Since $\frac{K_3}{K_4} = 1.067$ at 25° C.

$$= \frac{2(3.11)(1.067) + R}{2(3.11)(1.067) + 2R}$$

$$= \frac{6.64 + R}{6.64 + 2R}$$

(b) Water less than 99% D

A term is included to account for (H_2O) disregarded above. This is obtained by replacing R with R' in (6); and then substituting for R', in (9).

$$\text{atom \% D} = \frac{2(3.11)(1.067) + R}{2(3.11)(1.067) + 2R + \frac{2R^2}{3.26(3.11)(1.067)}}$$

$$= \frac{6.64 + R}{6.64 + 2R + 0.187\ R^2}$$

On the basis of these calculations, the atom percent deuterium of a gas sample, and of a water sample in equilibrium with the gas, can be determined from the HD/D_2 ratio given by the deuterium mass spectrometer. This means that water samples enriched in D_2O can be readily analysed by equilibration with deuterium gas and subsequent analysis of the gas.

b) Hot Wire Method

The equilibration method works very well for water samples of 5 cc. or larger. For smaller water samples, a technique known as the "hot wire" method can be used. It is based on the decomposition of water by a heated tungsten wire[42], deuterium being liberated, and the oxygen being chemically united with the tungsten. The gaseous deuterium is freed from moisture by passage through a dry-ice-cooled trap before it is admitted to the mass spectrometer. In this case the concentration of the water

is considered to be the same as that of the gas.

c) Use of Zinc Metal

Instead of a hot wire, heated, very pure zinc may be used to decompose the water in a vacuum line.(43) The oxygen and zinc are chemically united, and the hydrogen may be transferred through a drying trap directly to the sample line of the mass spectrometer. This method has an advantage over the hot wire method in that the zinc does not need to be replaced as often as the filament. The method is very rapid, and is capable of dealing with very small water samples. It is possible by this technique to analyse the water of combustion obtained from 3 - 5 mg. of organic tissue.

4. Discussion (43, 35)

The mass spectrometric method is the most valuable.(107) Although only samples of gas can be directly analysed, several techniques are available for determining the concentration of deuterium in water samples through a gas analysis. The method is very rapid and precise, and samples in the neighbourhood of a few milliliters of water are readily analysed. As hydrogen is the only interfering contaminant, there is no need for extensive purification of water samples, and constant temperature baths are not required except for equilibration.

In laboratories where a mass spectrometer is not available, the falling drop or the thermal conductivity

methods are widely used.[107] Both require only small samples and give good precision and accuracy. These methods are more tedious to use as the samples to be analysed must be very carefully purified. The actual analysis also is more time consuming.

Chapter 4

Applications of Deuterium as a Tracer in the Study of Intermediary Metabolism

1. Introduction

For many years there has been great curiosity in the minds of scientists regarding the fate of the food we eat. The mammalian body is such a complex organism, and there are so many compounds involved in its nutrition that it has been necessary to devise special methods of investigation for the solution of this problem.

The earliest technique studied was the classical balance experiment (35, 44). In these experiments, the animal was fed an excess of one type of food, and the excretions were examined to determine the metabolic end product of the nutrient by means of the component found in excess in these waste products. This method had definite limitations. Many body constituents are not produced in excess of the body requirements regardless of the excess intake of precursors (44). Moreover, one substance may induce the formation of an excess of another without being itself involved in the process (35, 44, 45). An excellant example of this is the effect of insulin in inducing the formation of glycogen (44, 46, 47).

An effort was made to overcome the defects of the balance method by labeling food constituents with such things as chloride of phenyl groups (44, 45). Unfortunately, the body organs involved in metabolism can distinguish readily between these compounds and the normal compounds

and therefore treat them in an unnatural manner. Thus it is impossible to depend on results obtained in this manner for a true representation of the metabolic processes.

In order to successfully trace the reactions that take place in metabolism, it is essential to have some method of labeling food components, not detectable by the organism, but readily detectable by the chemist. (44, 45) This desirable result is achieved by the use of isotopically labeled substances. Isotopic compounds have similar chemical properties, but different physical properties in the case of those depending on mass. (35) There is direct proof that the cell does not differentiate between isotopic isomers. (35) If living cells possessed the ability thusly to differentiate, isotopic abundances would vary from tissue to tissue, and the isotopic concentrations of the elements would change from source to source; but normal abundance holds for all known isotopes regardless of their origin. (48)

When a food substance, labeled by the substitution of an abnormal isotope for the normal, is fed to a living animal, the utilization of the food compound can be traced by the rate of appearance of the heavy isotope in the products of metabolism. (35) The earliest experiment of this type was performed by von Hevesy, (44) who used the radioactive isotope, RaD (lead). The metabolism was followed by means of Gieger counters placed near various parts of the body. Von Hevesy later used radioactive phosphorus in similar experiments

and was able to show that the isotope thus introduced went through all the organs, bones and teeth; thereby indicating that these structural elements are continuously and actively involved in salt metabolism. (35)

The most common food elements are C, N, H, and O. Because there are no radioactive isotopes of these elements of a satisfactory half life, use is made of their stable isotopes, C^{13}, N^{15}, D, and O^{18}. (44) Of these, the most valuable appears to be deuterium. (35, 49) The advantages to its use are that it can readily be prepared in a pure form, (44) and is easily detectable in small samples in concentrations of 0.02% with a precision of 0.001%. (44) Aside from this, the use of all other isotopes requires a mass spectrometer for the isotopic analysis. (45) Although the mass spectrometric method is the best available for deuterium, it is possible to analyse for this isotope, with the same accuracy as found mass spectrometrically, using other techniques more readily available; e.g. the falling drop method. (35)

In the use of the isotopic tracer method, the labeled compound must not change its isotopic distribution by mere exchange with normal isotopes present in the animal material. (35, 44, 45) In the use of deuterium, the chief source of normal hydrogen for exchange reactions is the water present in the body fluids. A knowledge of the reactions of organic compounds predicts which hydrogen configurations are likely to exchange with a water solvent. (44)

It has been found experimentally that the hydrogens of carboxyl, hydroxyl, amino, and other polar groups are labile and exchange in an aqueous medium. (44, 45, 50, 106) For this reason, it is impossible to use compounds containing deuterium in these positions as the labeled compounds. The label would be lost by exchange as soon as the compounds were taken in by the organism, and so would not indicate the fate of the food after subsequent chemical reactions. Deuterium may be stably bound directly to the carbon atoms along the chain of the organic compound. (44, 45, 51)

Although deuterium bound to a carbon atom is not exchangeable, there are chemical reactions taking place in the animal body which could cause a loss of the label. (35, 44, 45) This apparent drawback can be turned to our advantage. If deuterium can be lost by chemical reactions in the animal body, it is conceivable that compounds synthesized in the presence of deuterium enriched body fluids would themselves be deuterium enriched. This leads to a second powerful method of following metabolic processes. The injection of deuterium oxide into the body fluids of animals permits the study of numerous reactions by a determination of the subsequent rate of deuterium uptake by the various parts of the body. (35) (35, 44)

The isotope tracer method has found wide use in studying four types of biochemical problems: (43)

1. The conversion of one compound to another
2. The mechanism of biochemical reactions

3. The rate of reactions.

4. The amounts of a constituent in a mixture.

In the solution of all these widely varied problems, the isotope method is very valuable and has a particular advantage in that the experiments can be carried out on the intact animal. (36)

2. Technical Problems Encountered in Deuterium Isotope Tracer Experiments

a) The Source of Deuterium Gas

This gas is available commercially with a purity of 99.9%. (28) If it is desirable to prepare the gas in the laboratory from 99.9% deuterium oxide, two methods are available.

Rittenberg and Schoenheimer recommend the following procedure. (52) A deuterium oxide solution of deuteriosulfuric acid may be electrolysed to obtain deuterium gas. The gas is then dried by passage through a trap cooled with dry ice and stored at reduced pressure in a bulb closed by a mercury trap. A toeppler pump is used to transfer the deuterium back and forth between this storage bulb and the reaction chamber.

Recently deuterium has been prepared by the action of pure calcium metal on deuterium oxide in an evacuated system. (41) This is a more convenient method, and overcomes the hazards of electrolysis.

b) Combustion of Deuteric Organic Compounds

The deuterium content of organic materials is determined by an isotopic analysis of the water of combustion of these substances, as it is impossible to determine the deuterium content of organic compounds directly. (52, 53)

The combustion is carried out in a vitreosil quartz combustion tube in a stream of purified oxygen. (52, 53) Electrolytic oxygen is purified in the combustion train by passing over a bed of CuO, (52) or through sulfuric acid. (53) It is then dried by passage through a trap cooled by dry ice before passing over the organic substance placed in a quartz boat. The products of combustion are passed over a bed of heated CuO, and the water is collected in a trap cooled with dry ice.

c) Purification of the Water Sample

This step is very important if the isotopic analysis is being done by some method depending on the different physical characteristics of hydrogen and deuterium, or their oxides; for example, the falling drop method. (52, 53) (Chapter 3). If a mass spectrometric determination is being made purity of sample is not nearly so important, as the only contaminant that would alter the analysis is normal water.

The trap containing the frozen water of combustion is connected to a distillation train and barium carbonate is added to remove any nitric acid formed during

combustion. It is important to carry out the purification in a room free of vapours of all kinds, as they dissolve in the water and alter any physical properties. (53) Under vacuum the water is repeatedly warmed, and distilled to the next trap where it is again frozen out; successive traps containing, CrO_3, KOH and $KMnO_4$, (Both of these may be replaced by one trap containing CaO, which eliminates the possibility of replaceable hydrogen being introduced. (31)), and nothing. The water is finally collected in a tube, (from which it can be removed for analysis) by surrounding the tube with dry ice.

d) Preparation of Deuterio Compounds for Feeding Experiments

There are four general methods of preparation of these compounds: (23, 42, 44)

I Hydrogenation, using deuterium, of unsaturated compounds

II Exchange of stable hydrogen in a solution of D_2SO_4

III Catalytic exchange with D_2O in the presence of platinum or palladium

IV Biological synthesis

I Hydrogenation of unsaturated compounds

This method is used when it is possible to prepare the required compound by saturating an unsaturated compound. Instead of using hydrogen gas, deuterium gas is used, forming a deuterio compound. (43, 44, 50)

II Exchange in a Solution of D_2SO_4

Deuteriosulfuric acid labilizes the alpha-hydrogen atoms of saturated fatty acids permitting an exchange reaction. (43, 54, 55, 56) This method is restricted to saturated fatty acids as they are not attacked by the concentrated sulfuric acid used. It is also limited in that only two atoms of deuterium are introduced per molecule, and these two on the alpha-carbon. The deuterium introduced by this method is stably bound; treatment with boiling 80% sulfuric acid or boiling 7% aqueous potassium hydroxide does not remove it.

III Catalytic Exchange in D_2O

Active platinum possesses the ability to labilize carbon bound hydrogen toward exchange in a deuterium oxide solution. (43, 54) In the case of fatty acids, the reaction takes place more smoothly and completely in the presence of alkali, probably due to the fact that their salts are more soluble than the original fatty acids. This method leads to the introduction of deuterium at many, if not all, the carbon atoms along the chain. The concentration of deuterium reached depends on the concentration of D_2O used. This is an economical method as the unused D_2O may be recovered. The deuterium introduced in this manner is also stable toward treatment with hot alkali and acid.

IV Biological Synthesis

In some experiments, the original materials may

be prepared from the products isolated from previous feeding experiments.[57] For example, unsaturated deuteric fatty acids, isolated from mice fed saturated deuteric fatty acids, were used to feed mice in a later experiment. This is an expensive method because large quantities of D_2O are required for a little product.

These general methods cannot be used for all compounds, and reference should be made to the literature when it is desired to prepare any specific deuteric-organic compounds.[43, 58]

Before these compounds can be used in feeding experiments it is necessary to determine the stability of the deuterium label. If the deuterium should have been introduced in such a position as to be completely labile, it would be rapidly lost by exchange with the normal hydrogen in the body fluids. As the fate of the carbon atom to which the deuterium is attached is in general the data sought, removal of the deuterium from the carbon before the compound enters any chemical reaction invalidates the results. The stability of the introduced deuterium is determined by running an isotopic analysis of the compound, and then boiling it in dilute sulfuric acid,[54] hydrochloric acid,[59] alcoholic alkali,[54] or even water.[59] As all these treatments are more drastic than any which the compound will undergo during the metabolic process, the loss of deuterium shown by a subsequent isotopic analysis indicates the stability of our isotopic marker.

There is one other consideration concerning the stability determined in this manner. It is possible that there are present in the animal organism, certain enzymes which might conceivably have a labilizing effect on the carbon-bound deuterium. These enzymes would permit a loss of the isotopic marker not detectable by test tube, or "in vitro" experiments. The presence of such an enzyme might be detected by studying an organism ingesting no food but living for a length of time on food already present.[60] Fertilized hen's eggs were injected with a deuterium oxide saline solution. The eggs were allowed to develop normally and cut open just before the chicks hatched. Had there been labilizing enzymes present, the organic constituents of the chicks should have contained deuterium, introduced by exchange, as no synthesis took place. An analysis showed that the fatty acids and cholesterol of the chicks contained no deuterium. As the chick embryos contain all the enzymes of the adult, it seems fairly certain that no labilizing enzymes are present in mammalian bodies.[60] A further proof is offered by compounds such as lysine, known not to be synthesizable by the animal body, which pass through metabolic processes in an animal, whose body fluids are D_2O enriched, without picking up any traces of deuterium.[61] Added to evidence of this sort, the fact that such enzymes have never been found, leads to the assumption that they do not exist.

(62, 63, 64, 65, 66, 50, 107)
3. The Isotope Dilution Technique

This technique is based on the fact that the chemical reactions of compounds with abnormal isotopic distribution are the same as those of the normal compounds. If a small amount of an isotopically enriched compound is mixed with some of the same compound having normal isotopic concentration, the final mixture will have a uniform isotopic enrichment somewhere between the two concentrations, and the enriched component will be inseparable from the non-enriched by ordinary chemical methods. (62, 66)

To clarify this statement, consider the following example, in which the isotope dilution method is used for the purification of compounds in a separation procedure. Starting with 100 g. of saturated fatty acid, A, 5 g. will be left in the filtrate after precipitation with lead acetate and filtration. Let A be deuterium enriched. In the determination of the deuterium content of an unsaturated fatty acid, B, also present in the filtrate, (Unsaturated fatty acids are not precipitated by lead acetate.), interference is offered by the deuterium content of the unprecipitated A. However, if 95 g. of A', (the same saturated fatty acid as A, but without the deuterium enrichment) were added to the filtrate, and lead acetate were again used as a precipitating agent, 5 g. of saturated fatty acid would again be left in solution. As the only difference in A and A' is the deuterium enrichment of the former, these two compounds would react toward the lead

acetate in precisely the same manner. The precipitate would this time contain 95% of the A, and 95% of the A' in the solution before precipitation. In the second filtrate there would only be 5% of 5 g. of the saturated deuteric fatty acid originally present. This would lower the interference of the deuterium arising from this source to a negligible size.

It is important to note that this technique is essentially a dilution one. There is no isotopic exchange taking place. The results can be expressed in the form of an equation:

$$y = \left(\frac{c_0}{c} - 1\right)(x)$$

x - amount of A' added
c_0 - deuterium content of A'
y - amount of A originally present
c - deuterium content of A originally present.

The isotope dilution method has wide application. If any three of the variables are known, the fourth may also be determined. The technique can be used in the determination of:

a) The amount of a compound present in a mixture, or even in the animal body. (63, 67, 68) (66, 69) An example of this is an experiment used to determine total body water. D_2O was injected into rabbits and samples of the serum were taken after time had been allowed for mixing and equilibration. From the decreased D_2O content of these samples, it was possible to calculate the amount of water with which the original D_2O had been diluted. This gave the amount of

body water in the rabbits.[70]

b) To separate an isotopic compound completely from a normal compound for purposes of analysis.[62, 64, 71, 72] An example of this was illustrated on the preceeding page.[62] Only by the use of isotopes is the complete separation of deuterio saturated fatty acids and deuterio-unsaturated fatty acids possible. The process may be repeated as many times as is desired to obtain any required purity.

c) To overcome difficulties inherent in certain analyses.[63, 67] This method is adaptable to the direct determination of the amount of oxygen in a compound, a determination that had formerly been possible only by the most roundabout calculations.[63, 67]

The chief advantages to this technique is that only a small amount of the material need be isolated, as the yield is unimportant.[66] This permits better purification, often prohibited by the accompanying loss of product. Its use requires isotopic compounds, but has solved many problems related to the use of these materials.

4. Discussion of Experimental Data

a) Use of Deuterium as an Isotopic Label

I Fatty Acid Metabolism

By feeding deuterio fatty acids to several mice, investigators determined that a large fraction of the dietary fat was deposited in body fat depots before

being utilized.(50) That part of the fat was burned directly, was shown by the fact that the body fluids were enriched in deuterium oxide.(50) Since the fat in the depots was found to have the same constituents regardless of the type of deuterio-fatty acid fed, it appeared that the dietary fat is rapidly converted to the special fat of the animal.(50)

Experiments in which deuterium labeled saturated fatty acids were fed to mice, and unsaturated deuterio-fatty acids were isolated from their bodies, demonstrated that the possible desaturation of fatty acids in the animal organism actually occured.(62) The reverse experiment, illustrating the saturation of unsaturated fatty acids by the animal organism, was also successfully performed; and it is interesting to note that the deuterio-unsaturated acids used as food in this experiment were those isolated from the bodies of the mice in the preceeding experiment.(57)

When the body fluids of mice were enriched with deuterium, the synthetic reactions occuring in the body resulted in the incorporation of deuterium into the body tissues in a stably bound form. Therefore, the synthetic processes utilize small organic molecules present in the body fluid.(60) The amount of deuterium in the body fatty acids was determined over a period of several days immediately following the enrichment of the body fluids with D_2O. During this period the diet consisted chiefly of carbohydrates and very little fat. The

deuterium content[F] of the fatty acids increased for 6 - 8 days, then stayed constant, indicating that a deuterium equilibrium between the body fluids and the fatty acids had been reached. At equilibrium, the deuterium content of the fatty acids was 1/7 that of the body fluids.

The decrease in the deuterium content of the fatty acids when the mice were no longer given D_2O to drink, gave an indication of the duration of a fatty acid in the animal body. (60) Results indicated that the rate of deuterium uptake by the body was roughly equal to the rate of removal of deuterium. Together, the two experiments illustrated a very rapid turnover of fatty acids in the living organism. Moreover, as the diet consisted of carbohydrates and was free of fat, it appeared that fatty acids can be synthesized by animals from carbohydrate material.

Some authorities have expressed doubt as to the validity of these conclusions. Schoenheimer and Rittenberg were able to show, from the position of the deuterium on the carbon chains of the fatty acids involved, that the chief possible alternate explanation, the repeated hydrogenation and dehydrogenation of acids already present, is impossible. (60) These men admitted that there was a possibility of labilizing enzymes being present, which could

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[F] The concentrations of deuterium used in all this work are expressed in terms of atom percents; and in all cases represent an enrichment over the normal deuterium concentration of 1 part deuterium per 5000 parts hydrogen.

account for the results on the basis of pure exchange. This possibility has been disproved elsewhere. (P. 70) With these two possibilities ruled out, the experiments were assumed to represent the synthetic and destructive reactions taking place in the animal body. (72)

Experiments in which animals, fed certain deuterio-fatty acids, were subsequently found to have other deuterio-fatty acids present in their body fats, led to the conclusion that there was an interconversion among the fatty acids normally present in the animal body. (72, 72) Even when large quantities of fatty acids, having a normal hydrogen content, are present in the diet, the conversion of other fatty acids, (deuterio-labeled), to these same fatty acids occurs. This is shown by the fact that these fatty acids, containing normal hydrogen when fed, are deuterio enriched when isolated from the body. Because the deuterium content of the fats isolated was considerably higher than that of the body fluids, the fats could not have been synthesized from material present in the body fluids. Products synthesized from the body fluids would have had a lower deuterium content than these fluids as only part of the hydrogen synthetically incorporated would arise from this source. (72) Similarly, the high deuterium content of the isolated fatty acids ruled out an intermediate breakdown of the dietary deuterio-fatty acids. (73) The conclusion based on these factors was that mammalian fatty acids must be interconvertable.

The above experiments concerned only the long chain fatty acids. In a normal diet there is also a certain content of short chain fatty acids, chiefly butyric and caproic. By feeding these acids, labeled with deuterium, it has been determined that both are immediately oxidized in the body metabolic process, and are not stored in any form. (74) Fats, synthesized in a D_2O medium when normal butyric acid was fed, showed no deuterium enrichment, substantiating the above experimental results. (74)

In another experiment mice, given D_2O to drink, were kept on a diet free of highly unsaturated fatty acids. (75) The highly unsaturated body fats were isolated after a few days, and analysis indicated that they were not deuterium enriched. This proved that these fatty acids are not synthesized in the body; they are essential fatty acids.

From the previous experiments it had been shown that the average half life of a fatty acid molecule in the organism was 5 - 9 days. (60) In the last experiment the half life of liver fat was found to be about 1 day indicating a very rapid turnover. (75)

Another experiment indicated that mice could partly absorb deuterio-n-hexadecane, and convert it to fatty acids. (76) The result was surprising as the animal body had been thought incapable of utilizing any of the paraffins for food. The isotope technique also led to the conclusion that certain B vitamins were essential to the rapid turnover of fatty acids in the body. (77, 78) In the case of fatty liver in

mice, the source of the liver fat was found to be the body fat depots.[77, 109] In this experiment the depot fat had been previously labeled by the feeding of deuterio-fatty acids.

Deuterio-fatty acids were fed to congenitally obese mice until they had a sufficiently high deuterium concentration in their body fat. A study of the rate of decrease of the deuterium content of the body fatty acids indicated that the obesity of the mice was not due to excess retention of dietary fat, but to a restriction in the rate of fatty acid oxidation.[79]

The relationship of fatty aldehydes to the fatty acid metabolism was studied both by enriching the body water and by feeding deuterio-fatty acids.[80] Results indicated that fatty aldehydes are not an intermediate in the fatty acid synthesis, but might be formed by the reduction of the corresponding fatty acids.

Survey articles on the fatty acid metabolism in the brain[81] and in tissue have been published by the chief workers in the field.[35, 61, 82]

II Steroid Metabolism

When cholesterol was fed to animals, coprosterol was excreted in the feces, and it seemed probable that the former was a precursor of the latter.[51] Deuterio-coprostanone and deuterio-cholestenone, possible intermediates in cholesterol metabolism, were fed to dogs and humans, and deuterio-coprosterol was subsequently isolated from the feces. The isotope method thus illustrated the conversions:

cholestenone ⟶ cholesterol ⟶ coprosterol

coprostanone ⟶ coprosterol

in the animal organism.[51] Deuterio-coprostanone was also injected into dogs with a bile-fistula.[83] The resulting cholic acid was found, on isotopic analysis, to contain no deuterium.[83] This illustrated that coprostanone was not an intermediate in the conversion of cholesterol to bile.

Experiments based on the feeding of deuterio-acetic acid and the enrichment of the body fluids with D_2O indicated that acetic acid was a possible precursor in the formation of cholesterol.[84] Studies concerning a large number of related compounds, labeled with deuterium, gave further proof of the utilization of acetic acid, or some more active derivative, such as acetaldehyde, in the cholesterol synthesis in the body.[85, 110] The "in vitro" degradation of some of the isolated deuterio-cholesterol indicated that the acetic acid entered into the formation of both the side chain and the steroid nucleus.[85] It was suggested that the formation of cholesterol proceeded by the condensation of numerous small molecules (C_2) with intermediate elimination and uptake of water groups. It was noted that the formation of deuterio-cholesterol from deuterio-acetic acid was not accompanied by a perceptible uptake of deuterium into the fatty acids, which suggested that the latter were not involved in cholesterol synthesis.[85]

Deuterio-cholesterol was prepared by exchange in a D_2O-DAc medium using a platinum catalyst, permitting

further work in the field.[71] The administration of this deuterio product indicated the direct conversion of cholesterol into cholic acid (bile).[71] Enlightening results were obtained from a determination of the deuterium content of the cholesterol of the blood and organs. The dietary cholesterol was found to have been deposited, particularly in the liver and lungs. A lack of deuterium in the cholesterol of the brain and spinal fluids indicated a lack of exchange between the steroids of the central nervous system and the blood steroids.

Deuterio-cholesterol gave proof of the degradation of cholesterol into other steroids necessary to the body by demonstrating the specific conversion:

cholesterol ⟶ pregnandiol

in the human body.[86]

III Acetic Acid Metabolism

Although proof was not obtained that acetic acid was the only precursor in the synthesis of cholesterol, it was suspected that this was the case. If so, in feeding experiments with deuterio-acetic acid, there was a great dilution of the dietary acetic acid somewhere along the metabolic chain.[85, 87] The deuterium concentration of the isolated cholesterol was much lower than had been expected on the basis of the dietary acetic acid being the only source of acetic acid. The actual dilution was determined by removing the acetic acid formed in the body as the acetyl derivative

of some compound (such as phenylamino-alpha-butyric acid) foreign to the animal body, and capable of passing through metabolic processes unutilized. (88, 110) Experiments in which other materials were fed concurrently with this amine indicated that only acetic acid, and none of its derivatives, was capable of acetylating this foreign amine. (88, 110) By an adaptation of the isotope dilution technique, it was possible to show that 15 - 20 mm. acetic acid were synthesized per 100 g. of rat each day, the major portion probably through beta oxidation. A direct modification of this experiment, a substitution of other compounds for acetic acid, led to a discovery of acetic acid precursors in animal metabolism. (89) Of those tested, ethyl alcohol, alanine, and butyric acid were the only ones that gave much acetic acid. (89)

IV Amino Acid and Protein Metabolism

Deuterium has also been used as a tracer for protein metabolism. Ussing determined that the liver was a protein depot and that most of the ingested protein was broken down by the organism. (90) He used deuterio-casein, prepared by exchange with deuterium oxide in strong acids or alkalis, as the experimental nutrient. This reaction introduced the deuterium on the alpha carbons. Stably bound deuterium can also be introduced into protein material by exchange with deuterio-sulfuric acid. (56)

Deuterium contained in the body fluids can also be built into the protein material of the body. (91)

Of 14 natural amino acids isolated from mice whose body fluids were enriched in D_2O, all but lysine were found to have some deuterium incorporated into their structure. The experimental results were in accord with previous data related to the known essential amino acids.[91] The deuterium incorporated into these amino acids was stably bound and not removable by hydrolysis in 20% hydrochloric acid.[91] This proved that the deuterium was introduced into the protein material by a different mechanism to that observed in "in vitro" experiments, as the latter led only to the introduction of semi-labile deuterium.[92]

Other experiments using deuterio-ornithine indicated that it was a precursor in the formation of arginine,[108] proline,[93] and glutamic acid.[93] Dl-phenylalanine was similarly shown to release tyrosine.[94] Thiamine deficiency was investigated by isotope tracer methods, which indicated that the resulting inanition was caused solely through lack of appetite.[80]

V Glycogen Metabolism

Deuterium has been widely used to determine the metabolic processes involving sugar. Early experiments showed that the administration of glucose and lactate to previously fasted rats led to the production of new glycogen.[95] This glycogenesis could occur in two ways:

a - by the direct coupling of dietary glucose molecules.

b - by glycogenesis from fragments smaller than

hexose.

A comparison was made of the effects of feeding glucose and lactate to previously starved rats whose body fluids were D_2O enriched. Under these conditions the rate of glycogen formation was very rapid, up to 38% of the liver glycogen and 18% of the carcass glycogen being newly synthesized in 4 hours. The amount of glycogen formed from molecules smaller than hexose was greater in the case of the lactate than in the case of the glucose as shown by the relative concentrations of deuterium in the products. In the case of lactate, a comparison of the D concentration of the glycogen with that of the body fluids indicated that nearly all the glycogen was derived from small molecules in the body fluids. (It had been calculated that the maximum deuterium concentration from this source would lead to approximately 60% the deuterium concentration of the body fluids.)

In a similar experiment in which galactose was fed, the fraction of the glycogen replaced every day was as great, but smaller amounts were synthesized. Less glycogen was present in the body when the dietary sugar was galactose than when it was glucose or lactate. The deuterium concentration in the synthesized glycogen was lower than in the case of lactate, indicating that a lower percentage of the glycogen was derived from, or in equilibrium with the body fluids during glycogenesis. This eliminated lactate as an intermediate in the metabolism

of galactose. An isotopic analysis of the urinary sugar (galactose in this case) detected no deuterium, proving there was no exchange between the sugar and the body fluids during metabolism; a further proof of the improbability of labilizing enzymes in the body. When glucose was metabolized under similar conditions (body fluids D_2O enriched) large amounts of deuterium were found in the urinary glucose. Glucose was synthesized by the animal during metabolism; and indeed, the rate of appearance of deuterium in the urinary glucose was an indication of the rate of glucose synthesis.[96]

The effects of the administration of adrenaline and insulin were determined by feeding various sugars to previously starved rats, whose body fluids were D_2O enriched, and to which adrenaline and insulin had been administered.[46] Results indicated stimulation by adrenaline on anaerobic glycolysis forming lactic acid. The lactic acid was converted into glycogen. This experiment also confirmed the fact that the extra liver glycogen arising on the administration of adrenaline came from the blood lactate. Insulin was found to favour glycogenesis by direct utilization of hexoses.[46, 47] These experiments also indicated that adrenaline favours lipogenesis, whereas insulin retards it.

Other experiemtns on glycogenesis indicated the metabolic conversion of inositol to glucose in small amounts.[97] The efficacy of various sugars as glycogen

precursors is the following:[98]

glucose - 31% directly converted to glycogen

mannose and fructose - converted to glucose with labilization of hydrogen.

galactose and glyceraldehyde - gave the same deuterium concentration as glucose, but the mechanism of its utilization is not known.

dihydroxyacetone - action similar to that of lactic acid.

The rate of glycogen turnover was determined by the rate of deuterium uptake into the glycogen of an animal with body fluids enriched in deuterium oxide.[99] It was found to be 1 day for liver glycogen and 3 - 6 days for carcass glycogen.

Further experimentation indicated that in a diabetic rabbit about 1/2 of the urinary glucose was of dietary origin, and the other half of synthetic origin.[111] Moreover, at some point in the synthesis, all of the hydrogen was either derived from or in equilibrium with the hydrogen in the body fluids.

VI Transmethylation

Deuteric-methionine was used to study transmethylation in rats.[100] The rate of methylation was found to depend on the amount of methionine fed, but the rate of choline and creatine synthesis were independent of the amount of dietary methionine.

b) The Simultaneous Use of Two Isotopes

Deuterium is not a perfect label because it can be lost by virtue of chemical reactions in the body. Moreover, in many experiments, the fate of the other food elements is of more interest than the fate of the food hydrogen. For example, in fat or carbohydrate metabolism, we are interested in the fate of the carbon chain. As long as the D remains attached to the carbon chain it is a suitable label, but it may be lost by chemical reactions not always detectable. For this reason there is always some doubt as to the validity of the results when the fate of the deuterium label is used as an indication of the fate of the carbon chain. A more dependable label for the carbon chain would be a carbon isotope, and the stable C^{13} is often used. In the case of protein compounds, N^{15} is similarly used to unambiguously label the amino group.

It is possible to prepare compounds containing 2 isotopic labels, and compounds prepared with D and N^{15} or D and C^{13} have been widely used.(43) A compound, doubly labeled in this manner gives more than twice as much information as an experiment dealing with the same compound singly labeled with either isotope.# An amino acid labeled with N^{15} and carbon bound D can give the following information:

I The N^{15} indicates the fate of the amino group, and the D, the fate of the carbon chain.

D. Rittenberg via Dr. H. G. Thode, McMaster University, Hamilton, Ontario.

II If the N^{15}:D ratio in the dietary amino acid is the same as that in the same amino acid in the body, the compound has passed unchanged through metabolic processes. If the N^{15}:D ratio has changed, the amino acid has been degraded and resynthesized during metabolism.

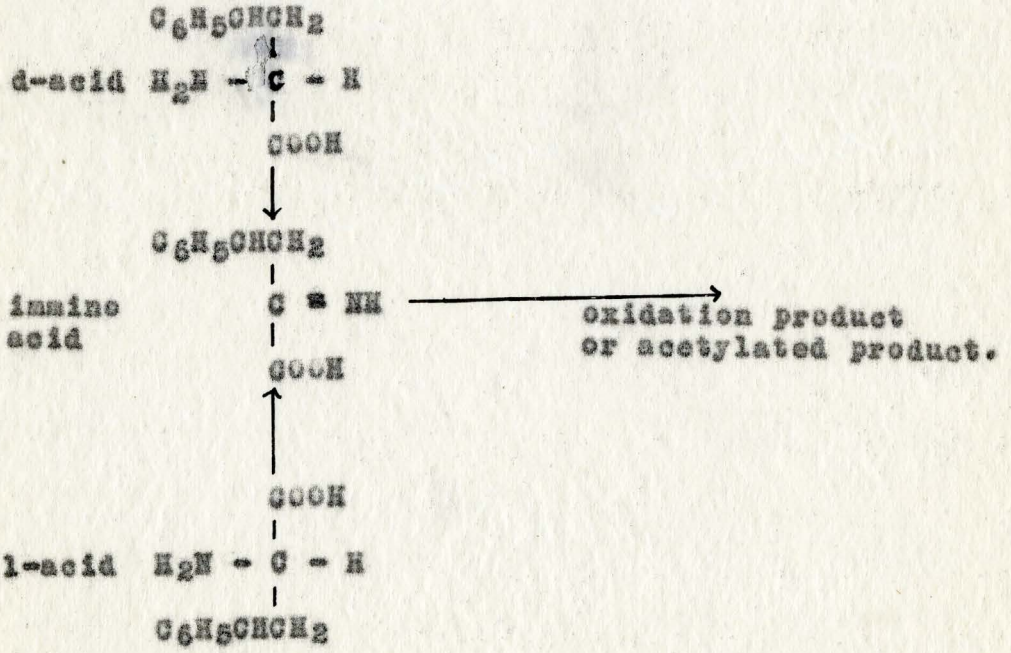
Experiments using the same amino acid, singly labeled with either isotope, would give the information in I only.

Protein metabolism has been widely studied using compounds labeled with N^{15} and D A large number of these compounds are racemic mixtures of optical isomers. In the animal body, there occurs naturally, only one optical isomer, the l-isomer. A number of the following experiments show the different treatment afforded by the body metabolic processes the the l- and d- isomers.

In the use of doubly labeled compounds to study protein metabolism, the amino group is labeled with N^{15} and the carbon chain by D. In rats, l-lysine, labeled with N^{15} and D, was found to be directly incorporated into the protein of the body without any nitrogen exchange, as shown by the constancy of the N^{15}:D ratio before and after metabolism.(101) L-lysine was also found to be utilized in the formation of other amino acids. But, when d-lysine was fed,(102) about 50% was recovered in the excretia. The remainder was found in the protein of the body, but none of it in the form of l-lysine, the natural isomer. This indicated that rats were incapable of inverting(102) d-lysine. A study of the metabolism of l-deuterio-proline by rats

indicated the conversion of proline to glutamic acid,[103] and the interconversion of proline, hydroxyproline, ornithine, and glutamic acid was shown at the same time.[103] D-leucine, labeled with N^{15} and D_2, was shown to be largely excreted, although a small part was converted to other proteins, or inverted by the body to the naturally occuring l-leucine.[104]

A slightly different type of investigation was used to illustrate the inversion of d-phenylaminobutyric acid, and at the same time to clarify its acetylation process.[112] By feeding several rats either pure l- or pure d-phenylaminobutyric acid labeled with N^{15} on the amino group, and at the same time enriching the body fluids with D_2O, it was shown that the acetylation and inversion could proceed by means of an imino acid.



This reaction was indicated by the loss of N^{15} only by the d- or unnatural isomer and not by the l- or natural isomer; and by the fact that the excreted acetyl derivative contained

deuterium on the alpha carbon in such quantities as could be expected from an imino conversion.

The use of C^{13} in conjunction with deuterium has thrown interesting light on the synthesis of fatty acids. Acetic acid, the methyl group labeled with deuterium and the carboxyl group labeled with C^{13}, was used in feeding experiments. The fatty acids subsequently isolated from the animal contained both C^{13} and D indicating that both the carboxyl and methyl groups were utilized in the fatty acid synthesis.(114, 115) It was suggested that the fatty acids were probably formed by successive condensations of C_2 groups from acetic acid or one of its derivatives. Palmitic and stearic acids were the chief products. From the relative concentrations of the fatty acids and the body fluids, it was obvious that the deuterium content of the fatty acids could not have been derived solely from the body fluids.(113, 114)

c) "In Vitro" Determination of Biological Reactions

An entirely different method of attack made use of surviving tissue incubated in satisfactory media. Rat liver slices grown in a Krebs buffer containing deuterio-acetic acid, l-leucine, and l-phenylalanine developed deuterium enriched acetyl-amino acids.(115) This experiment suggested that acetyl-amino acids are normal products of metabolism. The synthesis of cholesterol in surviving rat liver was demonstrated in a similar manner.

The use of either deuterio-acetic acid or deuterium oxide as a substrate led to the incorporation of deuterium into the synthesized cholesterol.(116) This indicated that liver, under aerobic conditions, can itself produce sufficient acetic acid for cholesterol synthesis. This effect was not to be found in studies of kidney, spleen testes, or gastrointestinal tract.

4. Conclusions

The isotope technique has opened the door to extensive study of new fields of scientific research. Earliest attempts to follow the reactions involved in intermediary metabolism failed because of the defects of the balance technique and the inability to label dietary compounds in such a way that the label could be detected by the chemist and not by the organism.(35) In addition, experiments effecting the physiological state of the animal had to be ruled out as they did not offer a true picture of the situation.(172) The use of isotopes overcame these difficulties, thus satisfying the criteria imposed by the problem.

Deuterium is one of the isotopes widely used as a metabolic tracer. It is a constituent of most food compounds and is not only readily available, but also comparatively inexpensive.(28) Another advantage to the use of deuterium is that analytical procedures, other than those requiring mass spectrometers are available.(35)

Although the mass spectrometric method is the best, such equipment is not available in many laboratories; and this fact lends a unique advantage to the use of the hydrogen isotope.

There are two different techniques employed in the use of deuterium as a tracer.[35]

I Organic compounds are prepared containing deuterium stably bound in the molecule. When such deuterio-compounds are fed to animals, their metabolic end products can be readily determined as they too will be deuterium enriched. In this manner it is possible to trace metabolic chains in the animal body.

II When a compound containing hydrogen is synthesized in a D_2O medium, a certain amount of deuterium will be incorporated in the synthetic product. By enriching the body fluids of animals with deuterium it is possible to follow the synthetic processes in the body; and in the presence or absence of certain dietary components, to determine a host of precursors and their products.

A still more powerful attack is made by the simultaneous use of two different isotopes. The use of two isotopic labels on one compound leads to a great deal more information from one experiment.[43] For example, using an amino acid labeled with N^{15} on the amino group and D on the carbon chain, it was possible to determine that l-lysine was directly utilized by the body, but that d-lysine appeared chiefly in the body waste products, or

in protein which was synthesized from the degradation products of d-lysine. (102)

The large number of experiments that have been performed have given much enlightening data on the subject of intermediary metabolism. They have indicated a very rapid turnover of all body tissue, a result which had never been expected. The body is composed of a large number of compounds which are continuously in dynamic equilibrium. They are constantly being synthesized from dietary constituents, and simultaneously being degraded and eliminated as waste. Both reactions proceed at the same rate in the normal healthy animal, and the disbalance of one reaction with respect to the others, in all or part of the body, is a symptom of an unnatural or diseased state.

These studies have further indicated the elaborate mechanisms of the conversion of dietary constituents into the compounds in the body. The fatty acids, both saturated and unsaturated, are (with a few exceptions such as butyric acid, (74) and the highly unsaturated acids) (108) completely interconvertable. (50, 72, 73) Cholesterol, a very complex organic compound, has been found to have acetic acid, a very simple unit, as its only precursor. (85) By feeding isotopically labeled possible intermediates, it has been possible to determine the path followed in the synthesis of certain compounds from their known precursors. For example, the relative deuterium contents of glycogen

synthesized from lactate and galactose in a mouse whose body fluids were D_2O enriched, indicated that the former was not an intermediate in the metabolism of the latter. (96)

This method has given proof that certain compounds cannot be synthesized by the body; for example necessary proteins such as lysine, (91) and the highly unsaturated fatty acids. (106) The absence of labilising enzymes in the body was shown by experiments with hatching eggs. (60) The amount of acetic acid formed by an animal every day was illustrated by an adaptation of the isotope dilution technique. (87, 88)

All these phenomena and many others have been disclosed and studied using isotopic tracers. The complex nature of the problem is shown by the large amount of work that has been done, and the much larger amount of work still to be done before the whole picture is clear. The isotopic method is a very elegant one, and at present is being extensively used, although some hindrance to still wider use is offered by the expense of the isotopic material, the difficulties encountered in the preparation of some of the complex compounds required, (57) and the fact that micro or semi-micro techniques must be employed. The method is remarkable for its scope, exactness, and uniqueness; and as future research overcomes the above hindrances, will doubtless be utilized to its full extent.

Chapter 5

The Physiological Effects of Deuterium Oxide

1. Introduction

Because of the 100% mass difference in the two hydrogen isotopes, their physical properties were expected to differ greatly.[106,117] Deuterium, in fact, was described as a new element, and its substitution for hydrogen in various organisms was expected to have far-reaching effects.[106] The increased melting point, boiling point,[117] viscosity, specific gravity, and osmotic pressure;[118] and the decreased vapour pressure shown by deuterium oxide, as well as the decreased solubility of salts in deuterium oxide, lead to changes in living organisms when considerable parts of the body water (H_2O) are replaced by deuterium oxide.[117] Effects noted vary from organism to organism; in some cases retarding,[117, 119, 120] and in others favouring,[117, 119] certain reactions. In general, a concentration of D_2O increased above the usually safe infusion of 20%,[119] retards growth,[119, 121] but "it is never" (?) "toxic to any high degree and is tolerated in high concentrations by lower organisms".[120] (Lewis). Harvey states, "D_2O affords a generally unfavourable environment for biological activity and changes the rate of biochemical reactions with respect to each other".[117] Lewis believes it will be found that no organism will grow in pure deuterium oxide.[122]

2. Experimental Data

a) Effect on Algae and Protazoa

The effects of D_2O on green algae have been widely studied. These plants are capable of living in relatively high concentration ($\rightarrow$50%) D_2O (119, 124), but with a further increase in the D_2O concentration, the growth decreases until at 85% D_2O it ceases completely and the plant bleaches (106, 124). Surprisingly, the plants survive for several days in a concentrated D_2O nutrient, and later revive if replaced in normal water (124). In lower concentrations of D_2O, the algae grow as well as in an H_2O control (125).

The deuterium of the medium is synthetically incorporated into the body of the algae (117).

Protazoa react toward D_2O in a similar manner. In low concentrations, D_2O has no effect on their growth (15 - 20 % D_2O), but in pure D_2O they are killed in about 24 hours (120). These animals showed more resistance to the toxic effects of D_2O than more highly organized animals.

b) Effect on Yeast, Bacteria, Fungi, Microscopic Cells of Various Types

Yeast cultures were nurtured on D_2O - sugar solutions containing 1 - 99% heavy water. Richards claims the observable growth change of yeast in heavy water containing one part of deuterium per two thousand parts of hydrogen

is caused by an increased volume rather than an increase in the number of cells.(126) The effect of D_2O on the respiration is more striking, the consumption of oxygen being decreased up to 50% by D_2O concentrations ranging from 20 - 97%.(117, 119) The rate of carbon dioxide evolution by yeast in normal water is 10 times that in heavy water.(122, 127) This effect seems to be caused by a "decreased activity of the zymase complex occasioned by an irreversible, partially destructive action of D_2O on the enzyme" (Pascu).(127) It is interesting to note that deuterium is not synthetically built into yeast, but may be found incorporated in the yeast cells through exchange reactions.(117)

In the case of salt-water luminous bacteria, the respiration was decreased, (60% by 86% D_2O, and 12% by 36% D_2O); this phenomenon being accompanied by a marked decrease in the luminescence.(117) Attempts were made to use D_2O as an antiseptic, but its bacterial effects were chiefly negative, while an increased growth of tubercle bacillus in 2 - 24% D_2O has been observed.(117) In 0.54% D_2O, both bacterium coli and bacterium pyocyaneous showed decreased ability to withstand the lethal effect of silver nitrate.(106)

Fungi grown in solutions of sugar and acetic acid at various concentrations of D_2O showed a decreased velocity of growth.(128) The deuterium was built into the tissue of the fungi, the concentration at equilibrium being 1/2 that of the solution.(128) Some molds and fungi, however, thrive

in pure deuterium oxide.(119) In tubes of yeast cultures, the growth of molds initiated by inoculation with common dust, is inhibited if not prevented by D_2O.(122)

Studies on the melanophores of detached fish scales indicated that D_2O causes a reversible contraction similar to that caused by epinephrine.(117, 129, 130, 131) This effect could be counteracted, for D_2O concentrations up to 25%, by ergotoxine. D_2O seemed to potentiate the effect of epinephrine, which led to the hypothesis that D_2O was favourable to the activity of the sympathetic nervous system.(117) This effect is considered more fully in the section dealing with the effects on mammals. D_2O tends to protect the sympathetic hormones by interference with the normal removal of their influence by oxidation, decomposition, etc. A further illustration of this effect is offered by the potentiation of D_2O toward the action of acetylcholine and adrenaline on the melanophores.(132)

Attempts to use D_2O to stop tumor growth gave negative results, and in some cases tumor growth was accelerated.(117) Fischer did some research on the growth, in D_2O of heart fibroblast of chick embryos and carcinoma cells of mice. In the case of the fibroblasts, the initial arrest in growth occured at 20-25% D_2O, and growth was stopped completely in 100% D_2O. In carcinoma, the growth was completely depressed by 50% D_2O. Experiments on the sarcoma of hens were not very successful, but they did indicate that growth was reduced.(121) The decrease in growth of these tumors in D_2O did not seem to be caused by a decreased rate of mitosis, as expected,

but by a restriction in the formation of a growth zone of optimal thickness.(119, 121) The mobility of the cells was decreased. The growth of these cells, carcinoma and lymphsarcoma, in mice, given 40% D_2O to drink (mice 1/5 saturated in D_2O) was decreased to 0.5 normal.(133) This decrease in tumour growth did not enhance the longevity of the mice as the increased concentration of D_2O in their bodies proved to be somewhat toxic.(133, 134)

The growth of microscopic animals in D_2O has been extensively studied. The gastropod mollusks and ostracods are uninjured by 99.6% D_2O.(151, 152) Animals of the groups Tardigrada, and Rotifera permit themselves to be practically dried, but can be revivified subsequently by moistening with water.(106) When D_2O up to 57% for the Tardigrada, and 18% for the Rotifera, was used to moisten them, the animals reacted normally. As the concentration of D_2O increased, the time of revivification increased, and the lifetime decreased greatly.(106) Moreover, following revivification with D_2O, the animals could not again be dried and further revivified.(106) Paramecia survived 2 days in 92% D_2O, although the rate of vacuole contraction was reduced.(148) They were unharmed by a D_2O concentration of 30%.(117, 135) The growth of obelia geniculata is also retarded by concentrations of deuterium oxide up to 10%.(134, 135) These results were explained on the basis of D_2O exerting a blocking effect on anabolism, or by a decreased catabolic rate.(134) This led to longevity in these

animals, but the same is not true of more complex organisms.[155]

In connection with the effect of D_2O on cells, Brooks observed a transitory plasmolysis of plant cells and hemolysis or erythrocytes in D_2O. This he explained on the basis of the higher osmotic pressure of D_2O.[118] The rate of entrance of D_2O into the red blood cells is slower than that of H_2O.[117]

c) Effect on Enzymes

The catalytic effect of D^+ on the reactions of the enzymes, α-d-glucose, (Steacie),[117] pancreatic amylase and zymin, (Barnes and Larson),[117] is much slighter than that of H^+. D_2O retardation does not hold for all enzyme reactions. Macht and Bryan found D_2O increased the reaction rate of oxidases from muscle and brain, and accelerated the action of bean catalase,[117] although Fox obtained negative results for D_2O concentrations from 1 - 99% when studying similar reactions.[117] The enzyme reaction of malic dehydrogenase was speeded up by D_2O,[138, 139] while dehydrogenase from Pisum sativum decolored methylene blue more slowly in D_2O than in H_2O.[137] The reactions of phosphoric ester-phosphoretase, fumarice acid-fumarico-hydratase, and l-aspartate fumarico-aminase were retarded.[139] Many other enzymes have been studied; the reactions in some cases were accelerated, and in some cases were retarded.

The conclusions drawn were that the exact effect of D_2O depended on the affinity of the enzyme for

(117, 119, 140)
the substrate. In any case, it appeared that D_2O had not
(119)
any great effect on enzyme hydrolysis. A review of this
(141)
field has been given by K. F. Bonhoeffer.

d) Permeability of Animal Membranes

The passage of D_2O through animal membranes has been studied using goldfish, (v. Hevesy and Hofer),
(117, 119)
and frogs. Results indicated that the permeability was the same in both directions, and that the diffusion of D_2O through these membranes was essentially the same as that
(117)
of H_2O. Experiments have also shown that D_2O is readily absorbed from the mammalian tract. By drinking D_2O and observing the D_2O content of the urine over a period of time, von Hevesy and Hofer determined that the average
(117)
stay of a water molecule in the human body was 14 days.

e) Effects on More Complex Organisms

I Development of Frogs' and Toads' Eggs

Pure D_2O proved immediately lethal to frogs' eggs; and even 10% D_2O had a noticeable effect on the cell division. In 30% D_2O, cell division never proceeded beyond
(106, 117)
the third or fourth stage. In 25% the eggs developed
(106)
without damage. Similar results were obtained with the
(106)
eggs of the green toad. A secondary effect, the extensively decreased use of acid in the development of the eggs,
(106)
was noticed.

The tadpoles of the green frog, when placed

in 92% D_2O, died in an hour, but were undamaged in 30% D_2O.(120)

II Flatworms and Aquarium Fish

Ninety-two percent D_2O killed flatworms and common small aquarium fish in a few hours, but 32% had no effect.(117, 120, 123)(149, 150) Flatworms that had apparently died in D_2O were removed after four hours, washed, and placed in H_2O. After some time about 1/2 revived and appeared normal.(122, 135, 150)

III Detached Animal Organs

A decreased rate of pulsation of an excised frog heart was observed by Barnes and Warren in D_2O, and an inhibition of the rate and amplitude, and a decreased irritability was noted by Verzan and Hoffter.(117) The study, by Barnes and Warren, of the isolated auricles of a turtle heart indicated that the effect of 75% D_2O on the auricular beat was similar to a temperature decrease of 5° C., and faster standstill was observed in D_2O.(117, 153) The effect was thought due to decreased energy, as a striking recovery was observed on returning the heart to ordinary water.(117)

f) Effect of D_2O on Mammals

Mice were given large doses of D_2O by subcutaneous injection or by mouth.(117) In low concentrations, a general pilometer effect and exophthalmos were observable.(117, 154) Both were removable by ergotoxine, again indicating

sympathomimetric influence. (117) As the D_2O concentration of the body increased from 10 - 20%, the total metabolism increased to 20% above normal, where it stayed as long as the D_2O was administered. (117, 134, 155) Even decreased metabolism caused by a thyroidectomy did not hinder this effect. (117, 155) The body temperature rose simultaneously. (117) Where ergotoxine was used to block the sympathetic nervous system, the calorigenic effect of D_2O was practically eliminated, (117, 155, 156) further proof of the sympathomimetric influence of D_2O. This effect is believed to be caused by a protective action of D_2O delaying the disappearance of sympathetic hormones from the body. (134, 157) The catabolic stimulation caused in mice by D_2O was rather interesting in view of the decreased catabolism of lower forms of animal life. This changed effect was probably caused by the checks and balances acquired in the animal's phylogenetic development. (134)

When concentrated D_2O was given the effects were somewhat different. As the body fluids of the mice became 1/3 D_2O saturated, the mice showed hyperexcitability. (117, 158) and jumping reflexes resembling strychinine poisoning but without convulsions. (117, 122, 157) Here the body temperature and metabolic rate dropped to as low as 20% normal metabolism. (117, 157, 158, 159) Death occured at about 50% saturation of the body fluids with D_2O and was accompanied by degeneration of liver and spleen. (117, 123, 157) (160) By raising the room temperature, the drop in metabolism and body temperature was delayed, but death

occured perhaps even earlier, indicating that the latter (117) was not primarily caused by the former. (123) At 1/2 D_2O saturation, mice were more sensitive to the convulsive action of ergotoxine, and succumbed to 1/2 the usual minimal lethal dose. (117, 154, 155)

Studies were made of the effect of D_2O on the water balance of mice. On the first and second days of D_2O administration an increased retention of water, corresponding to a rise in body weight, was observed. (117, 156, 161) The mice required excess water to keep their body weight normal, (117) and for low concentrations of D_2O showed great thirst and signs of intoxication. (122, 123) (122) During this period there was a decrease in the ratio: (156)

$$H_2O \text{ (insensibly lost)} : CO_2$$

similar to the decreased ratio found in fevers. (117) When D_2O was withdrawn from the diet, this ratio was high for the period of most rapid desaturation, then dropped to below normal as the mice began to gain weight. (156) The mice returned completely to normal when the D_2O was withdrawn. (122)

Because of the prohibitive expense of D_2O this experimental technique was not used for larger animals, but small amounts of D_2O were injected directly into the cerebrospinal fluid. When D_2O was injected directly into the cerebrospinal fluid of cats or rats, a marked degree of catatonia occured. (117, 158) In cats, the

local action of a few drops of D_2O caused a depression of the central nervous system.(158) When small amounts were given to cats over a period of time, water intoxication, indicated by rolling movements and muscular twitchings occured.(117, 162) Some rats experienced other effects such as, receeded eyeballs, ataxia, hyperexcitability, and jumping.(158) When the D_2O treatment was discontinued, the animal recovered completely.(158)

g) Effect of Germination and Growth of Plants

The hydrogen of plants can be largely replaced by deuterium, but because of the lower solubility of salts in D_2O, and the lower melting point of this water, this replacement has an unfavourable effect on the resistance of the plants to low temperatures.(106)

Lewis discovered that tobacco seeds, which germinated 100% in H_2O, showed a remarkably lower percent germination in D_2O, although development was otherwise normal.(122) In pure D_2O the seeds did not germinate.(122, 163) When these seeds were later transferred to H_2O, about 1/2 of them germinated slowly, but developed abnormally, and died in a few weeks.(122) Lewis believed that it was impossible for seeds to germinate in 100% D_2O, and that the abnormal development recorded above was caused by a partial germination in pure D_2O (actually 95% D_2O because of exchange with the hydrogen in the seed, air, etc.) interrupted by a lack of sufficient H_2O before this

partial germination was observable.[122] When these seeds were subsequently placed in H_2O this interrupted germination caused an abnormal sickly development of the seeds. Von Brun and Tronstaudshave found similarly that the germination of seed peas was halted by D_2O concentrations greater than 40%,[106] and Bonhoeffer reported a decreased speed of spore germination.[119]

Pratt and Curry studied the growth of wheat seedlings in D_2O and determined that both root and coleoptile growth were reduced but not prevented by 100% D_2O.[164] Pratt also studied the Erysiphi spores, and found that they germinated in D_2O of all concentrations, but that growth was poor and permament injury resulted if they were left in D_2O for more than 24 hours.[181, 182]

Zirpolo found that seeds of the lactua scariola develop better in 1 - 3 % D_2O than in pure H_2O,[167] but development was arrested for higher concentrations.

3. Conclusions

The effects of D_2O on living organism were largely those expected by early workers in the field.[122] Most organisms are very complex structures consisting of many compounds and tissues in dynamic equilibrium. Because of this complex structure, it is conceivable that the equilibrium could be readily disturbed by small amounts of D_2O because of the great difference in the vapour pressures, osmotic pressures, and solvent powers

of D_2O and normal water. (117, 118)

The toxic effects of D_2O vary from organism to organism. In general, 20% D_2O is a safe infusion, but beyond this concentration ill effects are apparent and become more marked as the D_2O concentration increases. (119) (168)

The effects of D_2O are more noticeable in the case of higher organisms; lower organisms being, in general, less affected by high concentrations of D_2O. Some molds and fungi even thrive in concentrated D_2O. (122, 123) (119)

Although the effects of D_2O are not consistent, even for one class of organism, (D_2O may either retard or accelerate enzyme hydrolysis depending on the affinity of the enzyme for the substrate) its general effect, in low concentrations, is to slow down the life processes of the organism; and at high concentrations, to stop these same processes altogether. It is interesting to note that in some cases life processes, stopped by concentrated D_2O, start again when the D_2O is replaced by normal water. (117, 119, 140) (117, 122, 124)

In the case of animals, the chief effects seem to be centered in the sympathetic nervous system. Injections of D_2O into the cerebrospinal fluids of mice, rats and cats caused catatonia, hyperexcitability, twitching, and jumping reflexes. The effects are believed to be caused by the protective action of D_2O on the sympathetic hormones, delaying their disappearance from the body. This is probably related to the general (117, 122, 158) (134, 157)

conclusion derived from these experiments, that systems containing D_2O have a low energy content. (117, 148)

Although these experiments are very interesting, they have not indicated any particularly useful physiological property of D_2O. Several attempts have been made to utilise the toxic effects of D_2O as an antiseptic, and to stop tumour growth; but in the former case, tubercle bacillus grew better in D_2O, and in the latter case, although the growth of tumours in mice has been found to be decreased by D_2O, the growth of the mice was simultaneously retarded, and the lifetime was shortened. It was suggested that a person could live longer if he drank only D_2O; but this is not the case. (117) (117) (155)

Although the body processes are retarded, one would not live more slowly. Mice indeed, live faster when drinking D_2O than H_2O. (155)

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