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THE USE OF DEUTERIUM IN MEDICINE

R E S U M E

Cet article fait le point de l'utilisation du Deutérium pour la mesure de l'eau corporelle totale et de l'eau extravasculaire pulmonaire dans différents hôpitaux parisiens.

THE USE OF DEUTERIUM IN MEDICINE

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ABSTRACT

Whenever a corporal function experiences a disturbance reflected either by changes in metabolic activity or modifications of the importance of pools of certain molecules the possibility exists of making use of isotopes in diagnosis. This paper discusses the use of Deuterium to measure total body water and extravascular water in the lungs, and gives examples of clinical applications.

INTRODUCTION : STABLE VERSUS RADIOACTIVE ISOTOPES

The use of stable isotopes in medicine rests on three possibilities offered by labelling.

- Identification of an element, a molecule, or a fragment of a molecule along its biological pathway.
- Quantification of biological pools by isotopic dilution.
- Measurement of metabolisation rates, and more generally of clearances.

Generally speaking radioactive isotopes lend themselves to the same uses as stable ones, except for establishing molecular structures which requires the use of mass spectrometry or magnetic nuclear resonance, and concentrations necessary for that particular purpose are so large that labelling by stable isotopes is more practical.

However stable isotopes do not, up to now, open the field of direct visualisation techniques, nor do they of radiation therapy. They could nethertheless be used as targets for neutron irradiation and, for example, subsequent measurement, of the induced nuclear reaction.

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Stable isotopes would seem a priori to be of special interest in medicine as neither hydrogen, carbon, oxygen, nor nitrogen, the most abundant elements of organic matter have convenient radioactive isotopes for medical purposes, as opposed to biology, at least for hospitals that can not use accelerator irradiation facilities.

In brief the reasons are that the use of radioactive isotopes requires exercising caution in order not to expose patients, or medical personnel,to unjustified amounts, of radiation.

Even when all possible care is taken a fraction of any labelled element introduced in a living organism is unretrievable and being fixed it may cause a long term hazard.

Tritium is only a weak β^- emitter.

Carbon offers either a long β^- lived isotope, or the short lived ^{11}C, β^+ emitter.

They can not be analysed in situ and administering T or ^{14}C, with half lifes of 12 and 5700 years, simply as diagnostic aids can only be justified in special cases.

Oxygen and nitrogen do not possess isotopes whose half life enables them to be used for the assessment of most physiological functions, in spite of the existence of the 2,1 min half life $^{15}_{6}O$, β^+ emitter, and the 10,1 min ^{13}N which, as ^{11}C, can only be used in the neighbourhood of a cyclotron, and for investigations compatible with such half lifes.

Why then are not stable isotopes more widely used than they are now, in spite of the fact that they served as tools in biological research almost as soon as Deuterium was discovered by UREY in the early thirties ?

The answer is mostly the difficulty of the analysis of stable isotopes and it's comparative lack of sensitivity, compared to that for radioactive isotopes.

For instance Tritium with a half life of 12,3 years provides an easily countable 37 desintegrations per second, per 10^{10} atoms.

Now 10^{10} atoms is the number of hydrogen atoms contained in 1,5 10^{-13} grams of water only. Even approaching an analysis of Deuterium labelled water in a few micrograms of body fluid requires methods which were not available a few years ago.

This lesser sentivity in detection, in the case of stable isotopes, involves that drugs have to be administered in correspondingly larger quantities. In cases where they may be, per se, harmful this may be a drawback to their use. In all cases it makes the cost of the use of labelled molecules greater and therefore less attractive. However molecules labelled with stable isotopes present the advantage of a perfect chemical stability (no radiolysis), which is important for storage in hospitals.

Finally radioactive isotopes in body fluids are very often measured without any chemical separation. On the contrary Deuterium has to be converted to a gaseous form for introduction into a mass spectrometer, and this necessitates a chemical operation that may lengthen the analytical process, if one does not start from water, which can be reduced on the introduction line itself (13).

The development of instruments enabling stable isotope measurements to be effected in hospitals, possibly at the patient's bedside, may open avenues for the medical

use of Deuterium in diagnoses. Similar developments may be foreseen in the field of ^{13}C tracer work.

We will concentrate on the case of water, the most abundant constitutive molecule of human body (60 %).

Most of the initially anticipated difficulties have been overcome in the measurement of total body water, extravascular water in the lungs, and other specific pools. We will consider the following choices :

- tracer
- analytical method
- specific instrument

and then discuss typical cases.

Choice of tracer :

Ruling out Tritium, for medical diagnoses in man, left open the choice between ^{18}O and D as tracers for water. Deuterium ought, seemingly to be a natural tracer for water. However its abundance, and fast diffusion, sometimes makes it difficult to use for the investigation of a specific organ. ^{18}O a priori offers the advantage of being less liable than D to exchange with other chemical species than H_2O.

For example amino groups would interfere with D as tracer as they will exchange their hydrogen with water but, having no oxygen, they would not perturb the use of $H_2{}^{18}O$.

Also if along a metabolic pathway such exchanges nethertheles occur, accompanying equilibrium isotopic effects are usually smaller with ^{18}O than D. Kinetic effects for ^{18}O are also, most of the time, smaller, except when they concern a group of atoms in which ^{18}O or D follow the same fate. Therefore there is no a priori reason to rule out the use of ^{18}O, may be on the contrary. So we studied the use of $H_2{}^{18}O$.

Analytical possibilities are a factor of choice, cost is another.

It has long been said that ^{18}O can be analysed by mass spectrometry with a better precision than Deuterium, but that much larger samples are required. In most laboratories samples of the order of a milliliter of water are required. In addition equilibration between water and CO_2 is frequently used which introduces a time lag in the analytical process.

However in our laboratories we have developped two methods for ^{18}O analysis that overcome those disadvantages. The first one, for determination of ^{18}O in water is the mass spectrometric analysis of ^{18}O directly on the water molecule, in microgram samples (13). Instruments based on this principle have been operated on a routine basis in Saclay for many years with a sensitivity comparable to that of the conventionnal method, i.e. $\delta < 1$ ‰.

Conversion of the oxygen in the sample into water is required.

We had also developped another method which can be used on most biological samples without chemical treatment (14).

It uses a gamma-activation technique depending on two reactions :

$$^{16}O\ (\gamma, n)\ ^{15}O \quad \text{for} \quad ^{16}O$$

$$^{18}O\ (\gamma, p)\ ^{17}N \quad \text{for} \quad ^{18}O\ ^{+}$$

The ratio of signals due to these two isotopes may be measured precisely enough to determine variations of 1 ‰ of natural abundance. The analysis was very fast : it took only one minute to introduce the sample into the accelerator beam, take the readings, extract the sample and print the results.

However a precision of 1 ‰ is not good enough, because to induce a visible change in the ^{18}O measured on total body water, circa 50 kilograms for an average man, one gram of pure $H_2^{18}O$ is needed?and to perform an experiment probably 10 to 20 grams. $H_2^{18}O$, costs about 150 or 200 $ per gram, In addition the use of accelerators implies a centralised analytical facility, and though, as we have seen, the response of the instrument is very rapid, transportation problems more than outweigh this advantage.

Finally the cost of operating the machine, which could be very small if many samples were to be treated, was large as we had few. This egg and hen dilemma was resolved by the success of the Deuterium analysis. Here again a completely automatic mass spectrometer is available is our laboratory. In fact it is the same machine as that used for oxygen.

As precision in both isotopic determinations is comparable : about 1 ‰ of natural abundance, the amount of tracer needed for a Deuterium experiment with a natural abundance of .15 ‰ , is about ten times less that needed for ^{18}O with a natural abundance of circa 2 ‰.

This, added to a ratio of costs of $\sim$ 1000 in favor the use of heavy water versus $H_2^{18}O$ settles the problem of the choice of the tracer when mass spectrometry is the best available technique, except in cases where specific exchanges have to be avoided.

Improvement in analytical techniques, choice of infrared absorption :

Mass spectrometry is easy and straightforward, but converting samples to water is necessary.

As we wanted to analyse body fluids directly we turned to infrared spectrometry, the only other technique that could have a precision comparable to that of mass

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+ ^{17}N is β^- emitter with a 4.13 second half, ^{15}O is a β^+ emitter with a 2.1 minute half life.

spectrometry, at least with comparatively simple machines. R.M.N. usually requires much larger samples than the two previous techniques, its accuracy for isotope ratio measurements is less and instrumentation much more costly. The infrared absorption spectrum of blood presents a "window" around 2500 $cm.^{-1}$ which allows the measurement of the band due to the OD vibrator when D_2O is injected in the blood.

He have therefore developped a method to work at this frequency, that turns out to be able to operate on line on blood which is the fluid investigated for the main medical applications contemplated.(See CAPITINI and alii (5) (6) (7)).

This kind of analysis can be performed at the patients' bed side and results thus obtained are discussed later.

Trials and tests on discrete samples and other fluids, principally on urine, necessitate a treatment of the samples.

When blood is sampled at intervals each sample must be analysed rapidly if not treated because the absorption at 2500 cm^{-1} varies with time, and usually the result sought involves a comparison of the Deuterium content of blood over a few hours. Up to now the techniques involved for analysis of discrete blood samples are the following.

For mass spectrometric analysis : deproteinisation by zinc sulfate dissolved in water of measured Deuterium content, followed by a double distillation in a flow of Helium gas (8).

For infrared analysis the serum is separated by centrifugation and kept in a refrigerator up to the time it is lyophilised, just before analysis (9).

For urine, that can neither be analysed directly nor be kept without purification, because a marked evolution of absorbance at 2500 cm^{-1} takes place under such conditions, filtration on activated carbon or simple vacuum distillation is not good enough, lyophilisation again is the prefered technique (10).

Experimental work

Principle and Instrumentation

Measurement of water in the lung tissue.

The only practical way to inject the tracer is in the blood. Aerial entrance does not provide an easy quantitative means of doing so.

Labelled water gets immediately diluted in the blood, and mixes rapidly with lung water and, later, with total body water.

The technique, to take this first dilution only into account, is to use, simultaneously with D_2O, a tracer that gets diluted only by the blood volume. Cardiogreen (indocyanine-green) is the ususal choice. D_2O is measured in the blood after this fluid has been pumped through the lungs, but before it has had a chance to irrigate other tissues. It has to be sampled in an artery.

The transit times of both tracers are very small, and continous analysis is preferable for that reason in spite of the fact that measurements on discrete samples are still performed in most research units. The extracellular volume of lung water is evaluated from the following equations :

$$v = \dot{Q} \times \Delta \bar{t}$$

where v is the volume irrigated by the diffusible tracer D_2O.

$\dot{Q}$ the flowrate of blood

$\Delta \bar{t}$ is the difference in the transit times, between the injection point and the analytical point, of D_2O and ICG.

For any one tracer :

$$\dot{Q} = q \Big/ \int_0^{\infty} c(t)\, dt$$

$$\bar{t} = \int_0^{\infty} t\ c\ (t)\ \ dt \Big/ \int_0^{\infty} c\ (t)\, dt$$

where : $\bar{t}$ is the transit time.

q is the quantity of tracer injected.

$c\ (t)$ is the excess concentration of the tracer over its natural level as a function of time, in blood at the sampling point.

Units of concentration are choosen to match units in which q is expressed.

Physical assumptions implied in these equations are :

a) that the measured volume v is uniformly swept by the flow of labelled water during the time $\bar{t}$. In other words preferential paths will lead to erroneous results.

b) that, at the point of analysis and time t the flow of blood in the artery is uniform and that the flow of blood derived from the artery is at the same concentration $c\ (t)$.

c) that the tracer is quantitatively recovered. Otherwise if some is lost (e.g. in edema some water is lost by aerial exit), the equation for $\dot{Q}$ does not apply anymore.

Other mathematical treatments of the curves have been proposed that may prove useful also (18).

Total body water

Feeding heavy water to a patient and analysing the blood or urine after three hours when isotopic equilibrium is completed provides a measurement of total body water if one corrects for heavy water eliminated during the time it took to reach equilibrium.

Total body water :

$$\frac{D_2O \text{ fed to the patient} - D_2O \text{ eliminated}}{\text{concentration of } D_2O \text{ in blood, or urine, at equilibrium.}}$$

This equation simply expresses the balance of Deuterium.

Apparatus

1) For total body water

Special modifications of available infrared spectrometers allow 50 ppm excess Deuterium concentration to be detected in waters resulting from lyophilisation, custom built apparatus has much better performances.

2) For lung water

One will find in (4) and (5) a complete description of the apparatus built for simultaneous D_2O and ICG concentration measurements in blood.

It is characterised by a non dispersive selection of the wave lengths (use of filters), the use of a single source for both wave lenghts. The actual sensivity corresponds to a detection limit of 3 mg of D_2O per liter of blood (3 ppm) i.e $\frac{1}{50}$ of natural concentration, and of .08 mg of ICG per liter.

The necessary volume of blood, (in the cell) is a few tens of microliters. The flow of blood through the cell is a few ml per minute. The total measurement takes three or four minutes, but could be reduced as the transit time between the injection point and the analytical point is less them half a minute with a difference between ICG and D_2O transit times of circa 3 seconds.

Of course the instrument described can also be used for measurement of total body water.

Experimental conditions

Total body water

Heavy water fed to the patient is .15 g per kg of corporal weight, precision of the results is about 2 %.

Lung water

For each measurement on man a sterile solution containing 2 g of D_2O and 5 mg of ICG is injected. Three measurements are performed at 15 minute intervals. The flow in the analytical circuit is about 15 ml per minute.

On animals, dogs or rats, tracer amounts are decreased in large proportions depending on toal body weight and specific lung tissue proportion.

RESULTS

Total body water determinations

They provide answers to some questions in a unique way. We will quote case studies.

1) Determination of total body water (Service de Nutrition, Hôpital BICHAT)

Two true twin sisters, for which a study of genetic factors showed that the probability of being monozygote was very high, were examined (15). Both measured 1.53 m, but P... was overweight : 60 kg. C was 52 kg.

At birth P... was 2,4 kg, C 1,7 kg.

Was the difference in weight genetic, or due to the diet?

A first examination showed that the bi-iliac and bi-trochanderian diameters were similar in P... (26 and 53 cm) and C... (25,5 and 53,5 cm) and that the fat mass established by the "pli cutané" method was 21,31 % for P... and 20,6 % for C...

The two sisters were subjected to the same restrictive regime (1400 calories out of which 87 consisting of proteins) during which P.... lost 10 kg and C.... 7 kg. Their weights stabilised, as their arterial pressure (11,5-6),the same for both.

To investigate the origin of the residual weight difference total body water was measured in both cases and found identical : 26,6 liter. It is concluded therefore that neo-natal diet was at the origin of this difference in weight.

2) Total body measurements on children and young adults help to establish diets. In normal young subjects total body water has been estimated to represent 55 ± 4 % of total body weight.

3) At hopital NECKER the use of D_2O for total body water, simultaneously with that of inulin, a tracer for extracellular water only, enables to calculate, by difference, the amount of intracellular water. The "masse maigre", directly proportionnal to the latter, is evaluated in this way. Children undergoing hemodialysis,or treatment involving hemodialysis are frequently examined in this way.

4) From the litterature (16) we can quote a case where total body water changes, in a premature baby with gastro schisis, ileal atresia and secondary short gut syndrome, were measured to monitor the use of peripheral hyperalimentation.

It was shown that over a period of 4 months simultaneous with a 14 g per day increase in weight the percentage of body water decreased from 77,1 % to 60,5 %. This showed conclusively that the mechanism of weight gain was tissue accretion rather than fluid retention.

Extravascular water in the lung (Hôpital TENON - Hôpital LAENNEC)

It has been shown (2) (5) on several dozens of patients and hundred of animals that the technique provides an early and sensitive method of measuring extravascular water. In some cases it revealed developping edema well before other investigation techniques.

Indeed correlations between radiological signs and pulmonary water accumulation show that at the stage of distension of the upper pulmonary vessels extravasular water excess may be 25 % and is 40 to 45 % when radiological signs of interstitial edema become flagrant.

Comparison between such radiological data and quantised measurements of water by D_2O dilution on a certain number of patients has proved in given cases the superiority of the second method.

This is specially the case for some patients suffering from chronic deficiency of the kidneys along with pulmonary and total body water accumulation that showed an increase of 50 % or more in pulmonary water without radiological signs.

The same observation was made on two patients suffering from encephalitis with coma.

The excellent coincidence, necessary to apply the tracer dilution method, between Q measured using ICG and D_2O tends to suggest that D_2O could be a preferred substitute for the former for cardiac flowrate measurements.

Studies are carried out to improve the interpretation of dilution curves, in order, inter alia, to eliminate the influence of recirculation.

Further investigations in this field will be reported later.

INTERIM CONCLUSIONS AND PERSPECTIVE

Total body water measurements provided by D_2O are already part of routine checks in several hospitals in Paris, and specially in the cases of children patients, and of hemodialysis. Such measurements are performed on discrete samples.

Continous D_2O measurements are easy to perform with the special instrument constructed by R.C. (5) (11). In that case :

a) used alone D_2O can compete with ICG in particular for cardiac flowrate measurements, as it necessitates the same injection and sampling techniques and is less costly, less toxic, does not induce colour in patients. Up to now the use of D_2O for measuring cardiac flowrate has been considered mostly as a possible reference method, improving in ICG. Thermodilution is currently preferred as it does not involve an arterial punction. Hovewer one could use D_2O dilution by sampling blood in the pulmonary artery, and analyse it for Deuterium. In such a case a catheter could be installed for as long a time as one uses to place a thermal probe. Blood sampled could eventually be reinjected. The total cost of a determination would be less because one would spare the purchase of thermal probes, and above all the accuracy of the method would be for greater.

b) used in conjunction with ICG, D_2O has provided a tool for the measurement of extravascular water in the lungs that has proved more sensitive than conventional X ray measurement, however, extravascular lung water measurements are still at the research stage.

We will be watching closely the development of those methods in the coming months.

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Mme BOTTER reread the manuscript carefully, and is author of earlier work (3) (4) (17).

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