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A REVIEW OF DEUTERIUM TRACER STUDIES HAVING A BEARING ON THE PROBLEM OF TRITIUM OXIDE METABOLISM

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A REVIEW OF DEUTERIUM TRACER STUDIES HAVING A BEARING ON THE PROBLEM OF TRITIUM OXIDE METABOLISM

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April 4, 1951

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A REVIEW OF DEUTERIUM TRACER STUDIES HAVING A BEARING ON THE PROBLEM OF TRITIUM OXIDE METABOLISM

INTRODUCTION

In any attempt at evaluation of the radiation hazard due to tritium, the importance of tritium oxide bulks large because of the relative certainty that where there is tritium there will be at least small quantities of tritium oxide due to catalized oxidation or exchange. The hazard of tritium is entirely one of internal exposure, and while the elemental gas is fixed in the body to only a very slight extent (1), tritium oxide will be retained practically 100% as part of the body water. The metabolism of water is a much studied subject, but most of the quantitative data pertinent to the tritium oxide hazard problem has been obtained in recent years using deuterium as a tracer.

In the review of these studies which follows, specific references to individual papers will not be given when general conclusions drawn from many investigations are discussed. For such references the reader is referred to a recently issued bibliography of deuterium tracer studies (2) which includes all of the sources employed in compiling this review.

APPLICABILITY OF RESULTS WITH DEUTERIUM TO TRITIUM PROBLEMS

If valid conclusions regarding the behavior of tritium are to be drawn from experiments employing deuterium, it must first be established that the two isotopes in tracer concentrations behave identically, or very nearly so, in biological processes. This question has not been investigated directly by using both tritium and deuterium in the same experiment; however, a great deal of work has been done on the similar problem of the biological fractionation of deuterium and protium (H^1). It would seem most probable that the conclusions from these experiments would also apply to the question of deuterium vs. tritium.

Experiments on biological fractionation of deuterium and protium fall

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into two categories. First, there are the numerous experiments in which pure D_2O, or water containing a relatively large fraction of D_2O, has been compared with H_2O. Gross differences have been observed in such experiments probably due in large part to the marked difference in physical properties. Such experiments are of little interest insofar as the tritium hazard is concerned. Secondly, there are the many experiments designed to detect differences in the protium-deuterium ratio in naturally occurring biological materials or materials of biological origin. It is these experiments involving very low concentrations of deuterium that are pertinent to the tritium hazard problem.

A number of investigators have reported what they consider to be significant deviations in the protium-deuterium ratio of biological materials. A still larger number of investigators have been unable to detect any such differences and have pointed out the many possible sources of error in such determinations. An excellent discussion of the problem is given by Dole (3). In any event, the differences claimed have never exceeded 10 p.p.m. It would seem to be a safe conclusion, therefore, that significant biological frationation of protium and deuterium does not occur.

GROSS DISTRIBUTION OF DEUTERIUM OXIDE IN THE ANIMAL ORGANISM

All experiments indicate a rapid and complete equilibration of administered D_2O with the total body water of the animal organism. For this reason D_2O has been widely and successfully employed in the determination of total body water, by measuring the extent of dilution upon administration. Careful studies of this type on humans have recently been reported by Schloerb, et al (4), who claim an accuracy of ± 2%. London and Rittenberg (5) report the appearance of D_2O in human venous blood within 8 minutes after ingestion and the attainment of equilibrium in 40 minutes. Hevesy and Jacobson (6) injected D_2O into the juglar vein and removed samples periodically from the carotid artery. Thirty seconds after injection, the D_2O was diluted by a volume of water corresponding

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to that occupying extracellular spaces. This rapid dilution was followed by a slower dilution, completed in 30 minutes, in which cellular water presumably participated. Such a double exponential rate of dilution was also observed by Schloerb, et al (4) in similar experiments. Even after subcutaneous injection complete equilibration with body water was obtained within 3 hours (4). Krogh and Ussing (7) studied the D_2O concentration in the free water of various tissues and organs of the same animal following D_2O administration and found it to be the same in all tissues. The body water of goldfish immersed in water containing D_2O equilibrates within a few hours, the velocity of equilibration decreasing with increasing size of fish (8). In dead fish the exchange is slower. Reversal of the process occurs at the same rate.

Other findings illustrating the extreme mobility of the body water are those which indicate that 73% of the water in the blood of the guinea pig is exchanged with extravascular water every minute (9); that one-half of the water of the aqueous humor of the eyeball of the rabbit is replaced in 2.7 minutes (10); and that the water of the amniotic fluid of pregnant women is completely replaced in 2.9 hours (11).

The biological half-life of body water has been determined by following the decrease in D_2O concentration with time. Values of 9-10 days (12), 8-10 days (13), and 9.3 ± 1.5 days (4) have been reported for man. A value of 3.6 days has been reported for the rat (14).

INCORPORATION OF DEUTERIUM FROM BODY WATER INTO TISSUE COMPOUNDS

While most of the body water is lost with a biological half-life (in humans) of about 10 days, a small proportion of the hydrogen from the body water will become incorporated in tissue compounds from which it will be lost with a half-life characteristic of the compound involved. If this half-life is long compared to body water, the retention of even a small fraction of the tritium in such form might be highly significant from the standpoint of the radiation hazard. This might be especially true if the "bound" tritium were concentrated in a particularly radiosensitive tissue.

Of the many biological experiments involving deuterium labeled compounds, the majority are of little interest in the present connection because the deuterium was administered in the form of labeled compounds rather than as deuterium oxide. A considerable number of investigations have been reported, however, in which deuterium was administered as the oxide. In nearly all of these experiments the object was to study the incorporation of deuterium in some specific pre-selected compound or group of compounds, so that, while scattered information is available, there has been no extensive general study of the binding of hydrogen from the body water.

Several investigators have reported fragmentary data on the gross binding of deuterium. Chomse (15) discusses an experiment of von Hansen (no reference given) in which a female mouse was maintained on 10% D_2O for a long period and then mated. The offspring contained 30-40% as much deuterium as the mother, of which two-thirds was tightly bound. The females from this second generation were mated and their offspring were found to contain "several per cent" of deuterium. Smith, et al (16), found that mice drinking 15% D_2O for two months, contain three times as high a concentration of tissue bound deuterium (relative to body water deuterium) as mice receiving a single injection of D_2O; evidence that the animals are able to store significant amounts of deuterium in non-labile form. Krogh and Ussing (17) found the rate of binding of deuterium in muscle to be slow and increased by exercise.

Deuterium Binding in Lipids

Of the specific types of compounds in which the binding of deuterium has been investigated, the various lipid fractions seem to have received the most attention. Only one observation seems to have been made on man, a value of eight days for the biological half-time for serum cholesterol (5). Numerous experiments utilizing rats and mice have established the fact that the fat stores of the body are in a dynamic state, with molecules constantly being synthesized and interconverted. An exception to this general rule is the case of the doubly and triply unsaturated fatty acids, which are not synthesized in the mammalian organism (18,19).

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The rate of binding of deuterium from the body water of mice was found to be most rapid in the liver fat followed in decreasing order by the fat of the intestinal wall, the kidney, and depot fat (20). In both mice and rats the rate and extent of incorporation of deuterium from body water into stearic and palmitic was found to be about the same (20, 21). About 50% of the hydrogen in these saturated fatty acids is derived from body water, while only about 20% of the hydrogen in the singly unsaturated fatty acids is derived from body water (20, 21, 22, 23, 24). This can probably be interpreted as indicating that the unsaturated acids are synthesized starting from larger molecules, or that a greater proportion is derived directly from the diet. About 50% of the hydrogen of cholesterol in the mouse is derived from body water (21).

The liver fatty acids of the rat were found to bind a greater proportion of deuterium from the body water than the fatty acids of the intestine, brain or carcass (25). The unsaponifiable fraction of the carcass, on the other hand, bound a greater proportion of deuterium than the same fraction in either the liver or intestine (25). Of the total unsaponifiable fraction, the alcohol fraction contained the highest concentration of deuterium (26).

The phospholipid fractions from the liver and muscle of rats bound a higher proportion of deuterium than the glyceride fatty acids in the corresponding tissues (27). In the rabbit the phospholipid fatty acids of lung and intestine were also found to be more active than the corresponding glyceride fractions while the two fractions of the liver fatty acids were found to attain rapid equilibrium (28). The binding of deuterium in the muscle fatty acids of the rat was found to be greater than that in the muscle fatty aldehydes (27).

Lipid synthesis has been shown to occur at many sites in the animal organism. Fatty acids and unsaponifiable lipids are synthesized in the rat brain, at least during the early life of the animal (29, 30). In the rat (31) and rabbit (28) fatty acid synthesis occurs along the entire intestinal tract and an especially high rate of synthesis was noted in the

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rabbit lung (28). Cholesterol synthesis has been shown to occur in the intestine (28), ovary (28) and mammary gland (32) of the rabbit. Fetal synthesis of cholesterol and fatty acids has been demonstrated in the rat (33) and rabbit (32). Fatty acid synthesis was reduced to 5% of normal in the alloxan diabetic rat (34, 35), which effect was reversed by the administration of insulin (36). The distribution of deuterium along the fatty acid chain has been found to be approximately uniform (23).

Of primary interest in connection with the tritium hazard are the quantitative data which have been obtained on the rate of regeneration of various lipid fractions. These data are summarized in Table 1. The interesting qualitative observation has been made that congenitally obese mice, while they store a normal proportion of dietary fat, have a much slower turnover rate for body fat (40).

Deuterium Binding in Carbohydrates

Deuterium from the body water is stably bound into glycogen synthesized by the animal. The proportion of deuterium bound varies with the diet, depending on the predominant precursor in the glycogen synthesis. Fasted rats, injected with D_2O and fed large quantities of glucose contained, after 24 hours, liver glycogen containing 60% of the concentration of deuterium in body water (41). Only half of this amount could be due to freely exchangeable hydrogen. Similar results were obtained when fructose, mannose, galactose, glyceraldehyde or glyceric acid were fed (42). When dihydroxyacetone or lactic acid is fed, however, essentially all of the hydrogen in the synthesized glycogen is derived from the body water (42). The appearance of deuterium in the glycogen is much more rapid in the fasted than in the normal rat (14). Glycogen deposited in the liver and muscle of alloxan diabetic rats in response to insulin came chiefly from dietary glucose directly (35, 36); that appearing after adrenalin injection is formed from blood lactate (35). In the alloxan diabetic rat on a 60% carbohydrate diet, 1/4 of the urinary glucose is synthesized *in vivo*, and the proportion of deuterium from body water bound

TABLE I

RATE OF REGENERATION OF LIPID FRACTIONS AS MEASURED WITH DEUTERIUM TRACER

ANIMAL	FRACTION	RATE	REF.
Mouse	Total fatty acids	Biol. half-life 5-9 d.	(21)
Rat	"Deposited" fatty acids	"Regeneration time" 9 d.	(22)
Mouse	Liver fatty acids	Biol. half-life 1 d.	(20)
Rat	Liver fatty acids	Biol. half-life 1.5 d.	(38)
Rat	Liver fatty acids	Biol. half-life 1.9 d.	(39)
Rat	Liver fatty acids	Biol. half-life 2.8 d.	(40)
Rabbit	Liver fatty acids	Biol. half-life 12 d.	(28)
Rat	Depot fatty acids	Biol. half-life 5-6 d.	(40)
Rat	Brain fatty acids	1/5 replaced/week	(25)
Rat	Fetal fatty acids	1/2 amount in 20 d. fetus synthesized in 36 hrs.	(33)
Mouse	Total cholesterol	Biol. half-life 15-25 d.	(21)
Rat	Liver sterols	Biol. half-life 3-4 d.	(38)
Rabbit	Liver cholesterol	Biol. half-life 3 d.	(28)
Rat	Fetal cholesterol	1/2 amount in 20 d. fetus synthesized in 60 hrs.	(33)
Man	Serum cholesterol	Biol. half-life 8 d.	(5)

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in the liver glycogen is above normal, indicating extensive synthesis from fragments smaller than hexose (34).

For rats at constant body weight on a high carbohydrate diet, the biological half-life of liver and carcass glycogen were determined to be approximately 1.0 and 3.6 days, respectively 38).

Deuterium Binding in Proteins

The introduction from body water of non-labile deuterium into various protein fractions has been reported by several investigators (43, 44, 45). All amino acids investigated have also been shown to bind deuterium (45, 46, 47, 48) with the single exception of lysine (47). Kogl et al (48) studied the incorporation of deuterium from body water in D and L glutamic acid of tumorous rats. The rate was slower for the D form but the eventual equilibrium concentration attained was the same for the two forms.

The biological half-life for liver protein has been determined as 2-3 days in the rat (37, 49) and about one day in the mouse (49). Serum protein has a rapid rate of renewal, like liver, while hemoglobin has a very slow rate which may be a measure of red-cell formation (49). Protein from kidney, muscle and skin represents a mixture with varying rates of renewal. In muscle, the myosin fraction shows a very low rate, while myogen formation is quite rapid (49).

CONCLUSION

It may be concluded that deuterium from body water is available for incorporation in a wide variety of tissue compounds. Quantitative data on the extent of this incorporation and the biological half-life of the bound deuterium have been obtained for a number of compounds. In general, the extent of binding is quite large and the biological half-life quite short. The compounds for which data are available, however, were studied for reasons other than their deuterium binding capacity, and the composite information is insufficient to permit conclusions to be

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drawn as to the extent of incorporation and retention of deuterium in the gross organism.

In the absence of direct experimental evidence, it seems quite probable that such data as are available for deuterium, are equally applicable to tritium.

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