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STUDIES OF METABOLIC TURNOVER WITH TRITIUM AS A TRACER
III. COMPARATIVE STUDIES WITH TRITIUM AND DEUTERIUM

By

Roy C. Thompson and John E. Ballou

Metabolism Unit
Biology Section

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ABSTRACT

Thirty-two rats were administered tritium oxide and deuterium oxide in a constant ratio over a five week period. Animals were then sacrificed in groups over a two month period and the ratio of bound tritium to bound deuterium determined in various tissues. Fat, brain and muscle showed a significant preferential incorporation of deuterium. There was no consistent trend of change in ratios for a given tissue over the 60 day period.

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STUDIES OF METABOLIC TURNOVER WITH TRITIUM AS A TRACER
III. COMPARATIVE STUDIES WITH TRITIUM AND DEUTERIUM

INTRODUCTION

In isotopic tracer studies it is usually assumed that the labeling isotope behaves, in the system under study, in a manner indistinguishable from the isotope whose reactions one wishes to follow. From much recent work it is clear that such an assumption is not always justified. In the case of deuterium, which has twice the mass of the more abundant hydrogen isotope, rather large "isotope effects" have been reported for a number of specific reactions[1]. Smaller, but still significant isotope effects have been reported for carbon, nitrogen and oxygen isotopes[2]. Most of these isotope effects were observed in simple reactions and it has been quite generally assumed that for the complex system of equilibria involved in a living organism, no significant isotope effects were to be anticipated. This conclusion is supported by the many studies of the deuterium - protium ratio in living and non-living materials of all sorts which indicate that this ratio is essentially constant[3,4].

The advent of tritium as a tracer for hydrogen in biological studies has re-emphasized the need for further investigation of isotope effects in living organisms, and has also, fortunately, provided a relatively simple method for making such studies. With two easily traced isotopes of hydrogen available, their comparative behavior in the most complex of systems may be simply studied. The first report of such a study was made by Glascock[5] who fed tritium oxide-labeled deuterium oxide to two rats and determined the change in the T/D ratio in various fractions obtained from these animals. Large isotope effects were observed in certain tissues, but results were not consistent. A later brief note by Glascock and Duncombe[6] reported a consistent 20 per cent decrease in the T/D ratio in

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mammary fat from lactating rats fed tritium oxide-labeled deuterium oxide for a period of two weeks prior to sacrifice. A similar effect was observed in the body fat of the young who were suckling during this period. In a study with deuterium and tritium labeled methanols, Verly, et al., observed a greater retention of the carbon-tritium bond in the utilization of the methyl group of methanol in the biosynthesis of the labile methyl group[7].

More recently, Eidinoff, et al., have reported results on four rats administered tritium and deuterium oxides over periods of 1-4 days which indicate preferential incorporation of deuterium by approximately 8 and 18 per cent in liver glycogen and liver fatty acids, respectively[8].

The purpose of the present study was to determine the magnitude of any isotope effect observed in the incorporation of deuterium and tritium from water into various tissue fractions, and also to determine what changes might occur in the T/D ratio over a period of time following incorporation of these isotopes. The results of a brief study preliminary to this investigation were previously published[9].

METHODS

The animals employed were 32 Sprague-Dawley female rats weighing 155 ± 10 g at the start of the experiment. Diet throughout the experiment was Purina dog chow ad libitum.

The tritium oxide-labeled deuterium oxide solution administered contained 98.5 mole per cent D_2O, 0.50 mc tritium (as the oxide)/ml and 0.9 per cent NaCl. This solution was administered by intraperitoneal injection at intervals of approximately 3.5 days. A series of 11 injections were given to each animal. The first injection was of 5.1 ml which was calculated to bring the body water D_2O level to 5 per cent. Subsequent injections were of such a volume as to increase the D_2O content of body water by 2.5 per cent, which, on the assumption of an approximately 3.5

day biological half-time for body water in the rat[10] should have maintained a D_2O level varying between 2.5 and 5.0 per cent over the five week injection period. Actually, the body water half-time observed in the present experiment was about 2.5 days, and the D_2O level maintained in the animals during most of the injection period ranged between 1.5 and 4.5 per cent.

Animals were sacrificed in groups of four at intervals of 1, 3, 7, 11, 17, 28, 42 and 60 days following cessation of D_2O-HTO administration. Samples taken included muscle (adjacent to right and left femurs), pelt (dorsal), fat (genital and perirenal), liver, and brain. The residual carcass was thoroughly ground and a representative sample taken. Individual samples were weighed fresh and pooled for the four rats. This practice was deviated from in the case of the 1 and 3 day groups, where the individual samples of muscle, pelt, liver and residual carcass were divided into equal portions and these combined for the four animals to give duplicate pooled samples of these tissues.

The previously described procedure[11] employed in removal of body water from the samples and combustion of the dry residue was altered in one significant detail. Following removal of the body water by distillation with benzene, water sufficient to cover the tissue was added to the flask and the tissue-water-benzene mixture allowed to stand overnight. This water, was then removed by distillation with benzene. This procedure was designed to remove freely exchangeable tritium from the tissues, and was employed for all samples except the duplicates of the muscle, pelt, liver and residual carcass samples from the 1 and 3 day rats. For these the equilibration procedure was omitted.

The combustion water obtained from the various dried tissue samples and a sample of body water obtained from the residual carcass sample were analyzed for tritium oxide and deuterium oxide content. For

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tritium analysis a diluted water sample was converted to hydrogen with calcium metal and the hydrogen counted in an atmospheric pressure, externally quenched Geiger tube[12]. Analyses were reproducible to ± 5 per cent.

Deuterium analyses were performed on the undiluted combustion water or body water samples. These water samples were equilibrated with tank hydrogen using, essentially, the procedure described by Fischer, Potter and Voskuyl[13]. The mass ratios in the equilibrated gas sample were determined in a Consolidated-Nier isotope ratio mass spectrometer, Model 21-201. Results were calculated by the graphical method suggested by Krishenbaum[14]. All figures reported are the average of at least two determinations differing from the average by not more than 3 per cent for values above 0.1 atom per cent excess deuterium, by not more than 7 per cent for values between 0.075 and 0.1 atom per cent excess, and by not more than 10 per cent for values between 0.05 and 0.75 atom per cent excess. A detailed description of the mass spectrometric determinations has been reported[15].

RESULTS

The results are summarized in Table 1. Tritium oxide concentrations and deuterium oxide concentrations (in excess of normal abundance) are expressed as "retention fractions"; i.e., concentration in combustion water or body water divided by concentration in solution injected. To convert these figures to absolute units, deuterium retention fractions (D_R) would be multiplied by 98.5 mole per cent (the concentration of deuterium oxide in the solution injected) and tritium retention fractions (T_R) would be multiplied by 0.50 mc/ml. Also listed in Table 1 is the "retention ratio," defined as the retention fraction for tritium divided by the retention fraction for deuterium. In the absence of an isotope effect this number, within the limits of experimental error, should be equal to unity.

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Eidinoff, et al[8]. The considerably larger effects observed by these workers may be due to the shorter time period over which the isotopes were administered in their experiments, which would have allowed less opportunity for metabolic equilibration of the isotopes.

The absence of any consistent change in retention ratios with time would seem to indicate that the isotope effects observed are concerned principally with incorporation of the isotope rather than with retention following incorporation. The magnitude of the effects noted in this study are such as to occasion little concern over the use of tritium as a tracer in long-term, gross metabolic studies in the rat. Conclusion drawn from previous studies in this series[10, 11] are certainly not significantly affected by the present findings. However, the fact that even slight differences were observed in the behavior of tritium and deuterium suggests that under different experimental conditions (particularly in short-term experiments) these differences might be magnified. Certainly these results should caution against the indiscriminate use of tritium as a tracer in systems in which its approximate equivalence to protium or deuterium has not been proved.

It should also be emphasized that the similar behavior of tritium and deuterium offers only presumptive evidence of similarity between these isotopes and protium. Recent studies on rapidly growing algae indicate that deuterium and tritium are similarly incorporated but that they both differ markedly from protium.[1]

The previous discussion applies to stably bound tritium and deuterium. Tritium and deuterium bound in labile positions were removed in

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1 Weinberger, D. and J. W. Porter, private communication.

the present study by equilibration with water. This procedure was adopted on the assumption that an isotope effect would be more likely in the reactions involved in the incorporation of hydrogen into stable positions, than in the simple exchange of labile hydrogens. Some data were obtained on total bound tritium and deuterium (stable + labile) and retention ratios calculated from these data are shown in Table 2. The data are not sufficient to justify comparisons concerning isotope effect between firmly bound and freely exchangeable tritium. No unusual effects were noted.

Also shown in Table 2 is the percentage of total bound tritium and deuterium which was present in the freely exchangeable state at time of sacrifice. These figures are obtained as a difference between two larger experimentally measured quantities [total bound tritium (or deuterium) and firmly bound tritium (or deuterium)] and are therefore apt to be considerably less precise than the primary data. It should also be noted that the first determinations were made a day following the last injection and since freely exchangeable tritium is lost from the body with the same half-time as body water, the percentage of labile tritium and deuterium was already considerably reduced when the first group was sacrificed. The percentages observed, particularly in the case of muscle and pelt, are much higher than the percentage of labile hydrogens in the more abundant body compounds. A maximum of 14 per cent labile hydrogen has been estimated for protein[17]. It may be concluded, then, that considerably less than half of the muscle and pelt hydrogen has become equilibrated with body water hydrogen during the five week injection period.

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TABLE 1

Retention of Firmly-Bound Tritium and Deuterium

Tissue		Time of Sacrifice (Days since final injection) 1	3	7	11	17	28	42	60	Statistical Data
Body Water	T_R*	2.38	1.27	0.410	0.200	0.069	0.012	0.0058	0.0035	Avg. T_R/D_R = 1.03
	D_R*	2.29	1.32	0.406	0.173	0.071				Std. dev. of mean = 0.04
	T_R/D_R*	1.04	0.96	1.01	1.16	0.97				Diff. from unity: .4 < P < .5
Carcass	T_R	0.304	0.262	0.199	0.190	0.176	0.126	0.092	0.080	Avg. T_R/D_R = 1.00
	D_R	0.305	0.264	0.203	0.193	0.193	0.114	0.091	0.081	Std. dev. of mean = 0.02
	T_R/D_R	1.00	0.99	0.98	0.99	0.91	1.10	1.01	0.99	Diff. from unity: .8 P < .9
Muscle	T_R	0.306	0.274	0.229	0.220	0.202	0.124	0.100	0.078	Avg. T_R/D_R = 0.94
	D_R	0.325	0.305	0.244	0.234	0.214	0.132	0.102	0.081	Std. dev. of mean = 0.01
	T_R/D_R	0.94	0.90	0.94	0.94	0.94	0.94	0.98	0.96	Diff. from unity: P < .001
Pelt	T_R	0.220	0.224	0.192	0.186	0.155	0.107	0.100	0.080	Avg. T_R/D_R = 1.02
	D_R	0.203	0.224	0.193	0.183	0.163	0.102	0.096	0.081	Std. dev. of mean = 0.015
	T_R/D_R	1.08	1.00	0.99	1.02	0.95	1.05	1.04	0.99	Diff. from unity: .3 < P < .4
Fat	T_R	0.246	0.251	0.171	0.222	0.206	0.132	0.104	0.092	Avg. T_R/D_R = 0.93
	D_R	0.274	0.284	0.193	0.244	0.218	0.137	0.107	0.096	Std. dev. of mean = 0.01
	T_R/D_R	0.90	0.89	0.89	0.91	0.95	0.96	0.97	0.95	Diff. from unity: P < .001
Brain	T_R	0.400	0.394	0.296	0.304	0.222	0.172	0.140	0.112	Avg. T_R/D_R = 0.94
	D_R		0.406	0.314	0.310	0.244	0.183	0.152	0.121	Std. dev. of mean = 0.01
	T_R/D_R		0.97	0.94	0.98	0.91	0.94	0.92	0.93	Diff. from unity: P < .001
Liver	T_R	0.524	0.393	0.246	0.170	0.108	0.045	0.022	0.013	Avg. T_R/D_R = 1.04
	D_R	0.488	0.376	0.244	0.173	0.101				Std. dev. of mean = 0.02
	T_R/D_R	1.07	1.05	1.01	0.98	1.07				Diff. from unity: .1 < P < .2

*T_R = tritium retention fraction.

D_R = deuterium retention fraction.

T_R/D_R = retention ratio. All terms defined in text.

TABLE 2

Retention of Total Bound Tritium and Deuterium

	Group 1*			Group 2		
Tissue	Retention Ratio**	% Labile ***		Retention Ratio	% Labile	
		T	D		T	D
Carcass	0.99	12	14	0.97	7	10
Muscle	0.99	28	26	0.94	12	12
Pelt	0.95	29	39	0.96	29	31
Liver	0.98	16	22	0.99	13	17

* Group 1 sacrificed 1 day following final injection
Group 2 sacrificed 3 days following final injection

** Defined in text

*** $\frac{\text{Total T (or D)} - \text{firmly bound T (or D)}}{\text{Total T (or D)}} \times 100$

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