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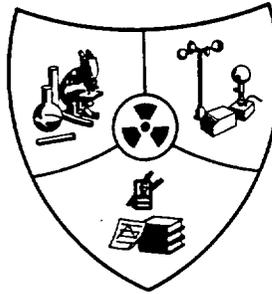
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BIOLOGY SECTION
RADIOLOGICAL SCIENCES DEPARTMENT

STUDIES OF METABOLIC TURNOVER WITH TRITIUM AS
A TRACER. IV. METABOLICALLY INERT LIPID AND
PROTEIN FRACTIONS FROM THE RAT

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STUDIES OF METABOLIC TURNOVER WITH TRITIUM AS
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PROTEIN FRACTIONS FROM THE RAT

by

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Biology Section
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November 24, 1953

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ABSTRACT

Protein and lipid fractions were separated from various tissues of rats sacrificed four months and eight months after administration of tritium oxide. The concentration of bound tritium in these compound fractions was determined, and the biological half-life of the tritium-labeled components of the fraction estimated from the decrease in tritium content between the two sacrifice periods. Collagen fractions exhibited half-lives of 300 days or longer and lipid fractions were obtained with half-lives as long as 300 days. The highest concentration of bound tritium, four months following tritium oxide administration, was found in brain lipids.

STUDIES OF METABOLIC TURNOVER WITH TRITIUM AS
A TRACER. IV. METABOLICALLY INERT LIPID AND
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INTRODUCTION

Previous papers in this series have dealt with the gross metabolic turnover of tritium-labeled compounds in whole mice⁽¹⁾ and in various organs and tissues of rats⁽²⁾. The present study extends these findings to specific compound fractions isolated from rat tissues. Attention is directed toward the compounds of very slow turnover rate which retain appreciable tritium label from four to eight months following tritium oxide administration. The results reported are of a screening nature and do not represent a thorough study of any particular compound. They do indicate, however, an apparent wide-spread distribution of relatively inert compounds amongst a variety of different compound types. They also indicate that the same type of compound may exhibit widely different turnover rates in different tissues.

METHODS

The animals employed were 40 Sprague-Dawley female rats averaging 230 g at the start of the experiment. Diet throughout the experiment was Purina dog chow fed ad libitum. A group of 20 female rats of approximately the same average weight were maintained as controls throughout the experiment.

Each of the experimental animals received a total of 100 millicuries of tritium administered intraperitoneally as the oxide, in five equal, weekly injections of 1 ml volume. The animals were sacrificed in two

groups. The first group of 15 rats was sacrificed 138 days after the initial injection of tritium oxide (109 days after the final injection). The second group of 19 rats was sacrificed 258 days after the initial injection, the interval between sacrifice of the two groups being 120 days.

Animals were sacrificed by exsanguination. Samples taken included: livers, brains, skins (hair removed by clipping), fat (from abdominal cavity), muscle (from hind legs), and residual carcass (minus gastrointestinal tract and viscera). Samples of a given tissue were pooled for each group, thoroughly ground, and stored in the frozen state until separations could be performed.

The results reported in this paper were obtained on fractions separated by two procedures. Protein fractions were separated by the method of Lowry, et al⁽³⁾ as modified by Robertson⁽⁴⁾. From this procedure, water soluble, 0.1 N NaOH soluble, and collagen fractions were obtained.

Lipid fractions were separated using methods described by Bloor⁽⁵⁾. The ground tissues were homogenized in a blender with 5 volumes of 95 per cent ethanol and refluxed for 30 minutes. This procedure was repeated twice on the insoluble residue and followed by two ether extractions of the residue. The combined extracts were evaporated in vacuo to a moist residue which was dissolved in a small volume of ether. Phospholipids were precipitated with acetone. The acetone soluble material was evaporated in vacuo and refluxed for two hours with 5 per cent ethanolic KOH. This solution was diluted with an equal volume of water and the non-saponifiable fraction extracted with petroleum ether. The ethanolic KOH solution was then acidified with H₂SO₄ and extracted with petroleum ether to remove fatty acids. Saturated and unsaturated fatty acids were separated by the lead salt method of Twitchell⁽⁶⁾.

The separated fractions were dried, combusted, and the combustion water analyzed for tritium content by methods previously described^(1, 7).

RESULTS

Table I shows the concentration of tritium in the various fractions at four months and eight months following the cessation of tritium oxide administration. For brain and liver samples the acetone soluble, non-phospholipid fraction was not further separated and the analyses apply to the combined fatty acids and non-saponifiable lipids.

The decrease in tritium concentration over the four-month period between sacrifice of the two groups of rats is due not only to breakdown of labeled compounds, but also to dilution as a consequence of growth. This latter effect was particularly prominent in the case of fatty acids, since the fat content of the animals increased considerable during the interval between sacrificings. To eliminate this effect of growth, tritium concentrations in the various fractions were calculated on a "per rat" basis, and using these corrected concentrations, the average biological half-life of the compounds in each fraction was determined graphically, assuming that loss of tritium proceeded exponentially during the interval between sacrifice of the two groups. The apparent biological half-lives, so calculated, are listed in Table II.

TABLE I

Concentration of Tritium in Compound Fractions from the Rat
($\mu\text{c/g}$ dry wt.)

(Upper figure, 4 mos., lower figure, 8 mos., following administration of HTO)

Compound Fraction	Tissue of Origin					
	Residual Carcass	Muscle	Skin	Abdominal Fat	Brain	Liver
Water soluble	0.38 0.08	0.35 0.08	*			
0.1 N NaOH Soluble	*	*				
Collagen	0.92 0.78	0.78 0.74	1.3 1.0			
Phospholipid	0.85 0.38		0.31 0.08		2.2 0.91	0.10 0.02
Non-saponifiable lipids	1.0 0.76		0.35 0.12			
Sat'd. fatty acids	0.68 0.19		0.52 0.11	0.66 0.10		
Unsat'd. fatty acids	0.52 0.20		0.34 0.08	0.37 0.07		
"Acetone soluble lipids" **					2.8 1.5	0.15 0.03

* Dry wt. of fraction was not obtained.

** Includes non-saponifiable lipids, saturated and unsaturated fatty acids.

TABLE II

Apparent Biological Half-Lives of Long-Lived Components of
Compound Fractions from the Rat (Days)

Compound Fraction	Tissue of Origin					
	Residual Carcass	Muscle	Skin	Abdominal Fat	Brain	Liver
Water soluble	65	50	130			
0.1 N NaOH soluble	65	55				
Collagen	300	∞	1000			
Phospholipid	100		60		90	60
Non-saponifiable lipids	300		100			
Sat'd fatty acids	200		130	60		
Unsat'd fatty acids	300		250	70		
"Acetone soluble lipids"*					120	55

* Includes non-saponifiable lipids, saturated and unsaturated fatty acids.

DISCUSSION

The level of tritium administration in this experiment was higher than is generally employed in tracer studies. This was necessary in order to insure adequate levels of tritium in the various compound fractions at long intervals following exposure. The irradiation dose rate immediately following each 20 mc injection was approximately 30 rads¹/day, assuming uniform distribution of the tritium oxide in the animal. With a body water half-life in the rat of 2.5 - 3.5 days^(2, 7) the dose rate was reduced to about 5 rads/day before the succeeding injection was given. The total absorbed dose received from body water tritium during and following the injection period is estimated at 550 rads². Despite this high irradiation level there was no significant effect on weight gain as compared with the control animals, and no gross evidence of radiation effects was observed at sacrifice. The single injection dose of 20 mc/230 g rat was less than one-tenth of the 30-day median lethal dose for mice⁽⁸⁾.

The data of Table I give a general indication of the extent to which tritium is retained in various compound fractions. The highest tritium concentration was found in the brain lipids. The liver lipids are notably lower in tritium than other fractions studied. All fractions, however,

¹ New terminology agreed upon at International Congress of Radiology, Copenhagen, 1953. 1 rad = 100 ergs/g.

² Absorbed dose rates are calculated from the formula, rads/day = $\frac{51EQ}{W}$. Total absorbed dose is approximately $\frac{51EQ}{\lambda W}$. E is the average energy of the radiation in Mev (0.006 Mev for tritium), Q is the quantity of radioisotope in μc , W is the weight of tissue, in g, containing the Q μc , and λ is the decay constant in day⁻¹. The decay constant is equal to 0.693/biological half-life in days. The term "biological half-life" as used in this paper is in actuality the "effective half-life," combining the effects of biological turnover and radioactive decay. However, since the effective half-lives observed are small compared to the radioactive half-life of tritium, negligible error is introduced by this simplification.

show significant retention of tritium, and with the exception of brain and liver, the tritium concentrations in different fractions vary over a range of only about three-fold. This uniformity of tritium retention makes it difficult to attribute activity in one fraction to contamination with small amounts of materials incompletely separated with other fractions.

It is of interest to compare the bound tritium concentrations shown in Table I with the tritium concentrations in body water during the course of this experiment. The maximum concentration attained following the last injection was approximately 180 $\mu\text{c}/\text{ml}$ body water. This concentration was not measured, but is calculated from the known amounts injected, the weights of the animals, and the assumption of 65 per cent of body weight as body water. When the first group of rats was sacrificed, four months after the final injection, the measured body water tritium content was 0.013 $\mu\text{c}/\text{ml}$. Eight months after the final injection the body water tritium content was 0.003 $\mu\text{c}/\text{ml}$. While the tissue-bound tritium concentrations are small compared with the initial body water tritium concentrations, they constitute the great majority of all tritium which remains in the animals from four to eight months after injection. It is evident from these figures that the tissue-bound tritium is "firmly bound" and is not being continually replaced from a tritium reservoir in the body water.

The apparent biological half-lives listed in Table II must be taken as approximations only, since they are based on only two points on a retention curve. It is known from previous studies, however, that the loss of tritium from tissues over the time interval from four to eight months following exposure is very nearly a single exponential function of time⁽²⁾.

The biological half-lives observed are consistent with those previously obtained for whole tissues and organs⁽²⁾ and confirm the presence of metabolically inert compounds of considerable chemical variety. In view of the recently reported similarity in long-term retention of tritium

and deuterium in the rat⁽⁷⁾, it is unlikely that the observed effects are due to metabolic discrimination between tritium and protium. Evidence for the metabolic inertia of collagen has recently been reported by several workers using isotopic tracer methods^(9, 10, 11). To our knowledge, however, there have not been previous reports indicating the presence of inert lipid fractions.

It should be emphasized that the biological half-lives determined in this study are not representative of the total material isolated in each fraction. For example, all unsaturated fatty acids from the skin will not exhibit a biological half-life of 250 days. As shown previously⁽²⁾ the great majority of tritium incorporated into tissue compounds turns over at a much faster rate. The present study merely focuses attention on those components of each chemical fraction which turn over most slowly. Whether these relatively inert components represent chemical species distinct from the more rapidly catabolized components, or whether the same compounds may exhibit different turnover rates dependent upon their functional involvement within the organism, remains to be determined.

It is of interest to note that the biological half-life for a given compound fraction may vary significantly within the same animal depending upon the tissue of origin. Thus, while for any given tissue, the unsaturated fatty acids exhibited a longer half-life than the saturated fatty acids, the half-life of either of these fractions may vary by a factor of 3-4 between different tissues of origin. It is evident that a given compound or group of compounds within the body may not be considered independently of its location or function in the organism.

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