Radiochlorine as a Tracer in Fat Deposition[1]

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Abstract

Radiochlorine^{38} was evaluated and found to be suitable for use as a tracer in studying the deposition of fat in porcine *panniculus adiposus* tissue. Its short half-life makes its preparation from natural chlorine simple and inexpensive, and also minimizes long-term contamination problems, and its energetic beta and gamma rays makes its radioassay simple and rapid. For swine, the threshhold level for a statistical counting accuracy of 1% was found to be approx 8×10^{-2} mc/lb of live wt. The time required for the digestion, absorption, transport and deposition of statistically sufficient detectable radioactive material was 3½ hr. The incorporation of radiochlorine^{38} into lipid molecules does not appear to alter their normal metabolic pathways.

Introduction

FOLLOWING THE PIONEERING work of Hevesy (9,10), the use of radioactive isotopes was greatly expanded, and in vivo studies (18) with large animals became possible. Although the tracer technique is an extremely popular research tool, the literature (4–6) shows only limited use of the radioactive chlorine isotopes. When the long-lived radiochlorine^{36} and the short-lived radiochlorine^{38} became available, they were used primarily in membrane transport studies (3,11) and in studies concerned with the physiological functions of this element (2,7,19). The preparation of chlorine-labeled organic compounds, except for indicator analysis studies (12,16,17), and the use of chlorine tracers in following the metabolism of other body constituents, particularly lipids, has not been attempted.

Other tracers (14) have been used to label lipid molecules and to follow their fate during the processes of metabolism. However, these labels have presented long-term contamination problems (C^{14}), been too unstable (H^2), have limited application (P^{32}), and/or might present a large mass difference effect (I^{131}). Thus, the importance of developing more suitable radioactive tracers for studying the physiology and biochemistry of adipose tissue is quite obvious. Consequently, the purpose of this research was to prepare an organic compound labeled with the short-lived chlorine^{38} isotope and to evaluate the use of this tracer in studying the deposition of fat in swine.

Chlorine^{38} is available from the neutron activation of natural chlorine^{37} (8). Radiochlorine^{38} emits three distinct beta particles, with maximum energies of 1.16, 2.80 and 4.99 Mev. Gamma radiations with energies of 1.64 and 2.19 Mev are also found. The 4.99 Mev beta rays are among the most energetic found in artificial radioactivity (13). Radiochlorine^{38}, with a half-life of 37.5 min, decays to stable argon^{38}.

Experimental Procedure

The addition reaction, as the method of organic synthesis, and the neutron activation reaction, as the method of inducing radioactivity, were used in the preparation of the tracer material.

Twenty milliliters of glycerol mono-oleate were added to a 25-ml test tube and chlorinated under a chemical fume hood for 30 min. The procedure involved bubbling chlorine gas (30 ml/min) that had been washed in water and dried in conc H_2SO_4 and was designed to produce a simple addition of chlorine atoms to the double bond of the acyl group. To prevent any catalysis of oxidation by light, the reaction was conducted in the dark by wrapping the test tube with black electrical tape and placing it at the back of an unilluminated fume hood. After chlorinating the mono-oleate, nitrogen gas dried by bubbling through conc H_2SO_4 was permitted to bubble (30 ml/min) through the material overnight. This step was incorporated into the preparation to remove any hydrochloric acid contamination by utilizing the volatile property of this compound. Litmus paper was used as an indicator of the termination of this step.

Gelatin veterinary capsules, containing 0.75 ml of the chlorinated mono-oleate per capsule, were made up and individually placed in polyethylene tubes (3 in. long, ½ in. inside diam), which were sealed by heat. These tubes were then delivered to the Penn State Research Reactor and exposed to a thermal neutron flux of 2×10^{12} neutrons/sq cm/sec for 37.5 min (1 half-life or 50% saturation). This neutron activation proceeded according to the Cl^{37} (n, γ) Cl^{38} reaction. After removal from the pile, the amount of induced activity was measured at 3 mc per capsule, a unit amount which was suitable for the purposes of the experiment and within safe tolerance limits of the pigs used as experimental animals (20).

As a check on the purity of the activated mono-oleate, several capsules were analyzed in the laboratory for radiochlorine not incorporated into the mono-oleate molecule. The radiochlorine preparation was removed from the capsule, dissolved in petroleum ether and chloroform, washed with distilled water, and centrifuged. The water layer was then removed, sodium hydroxide added to it to prevent the loss of the chrloride ions, the mixture evaporated, placed on aluminum planchets and counted using a Nuclear-Chicago Model 182A Scaling unit equipped with a pancake-type Geiger counting apparatus. The remaining petroleum ether-chloroform extract was also plated and counted. This analysis showed that the preparation was satisfactory since the petroleum ether-chloroform extract was highly radioactive and no radioactivity above background was noted in the water extract. Also, since no apparent increase in the viscosity of the preparation was observed, it was assumed that little or no chemical modifications (i.e., polymerization) had occurred that would have altered the nature of the radioactive mono-oleate.

The animals were fasted 24 hr before administering the radioactive capsules, not only as an aid to slaughtering but also to facilitate the digestion, absorption, transport and deposition of the labeled material. The capsules, containing the radiochlorine preparation, were administered with a balling gun to the experimental animals. Three and one-half hours after administration of the capsule, the live animals were assayed using a Nuclear-Chicago, Model 2612, Portable Geiger Counter. A spectrum of the animal's back was made by measuring the activity at the shoulder, loin, and rump. The counting tube was rested directly on the pig's back for these measurements. The ani-

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TABLE I
A Summary Comparison of the Live Animal and Carcass Tissue Radioassay Data

Site of measurement	Live animal assay[a]		Carcass tissue assay[b]	
	Individual site activity	Individual site activity/ total activity (all sites)	Individual site activity	Individual site activity/ total activity (all sites)
	cpm	%	cpm/g	%
75-lb. pigs				
shoulder	1200	34.0	658.7	35.6
loin	1400	40.0	714.8	38.8
rump	900	26.0	469.0	25.6
130-lb pigs				
shoulder	1200	33.0	651.0	33.2
loin	1500	42.0	738.4	37.7
rump	900	25.0	571.4	29.1
190-lb pigs				
shoulder	1900	35.0	1383.3	39.0
loin	2200	41.0	1213.4	34.2
rump	1300	24.0	947.6	26.8

[a] Table of mean values for the 6 pigs used. Since the Model 2612 can only be read to the nearest 50 cpm, the measured values and calculated percentages have been rounded off accordingly.
[b] Table represents combined data from samples of both layers of the *panniculus adiposus*.

mals for tissue analysis were also slaughtered approximately 3½ hr after the administration of the capsule. After the pig was removed from the automatic dehairing machine, three tissue samples were taken from the *panniculus adiposus*. These were removed at the first thoracic (shoulder), last thoracic (loin), and last lumbar (rump) vertebrae areas along the median line of the back. Since the *panniculus adiposus* is composed of two different tissue layers, these were then separated for analysis. The tissue samples were cut as geometrically uniform ($2.5 \times 2.5 \times 1.0$ cm) as equipment would permit, and then mounted on aluminum planchets for counting. Each sample was counted to a statistical accuracy of 1% and the usual corrections for time, background, and counter efficiency (10%) were made. The uniform geometry of the tissue sample gave uniform self-absorption losses (assuming that the labeled material was uniformly distributed in the sample) and thus eliminated the need to correct for self-absorption.

Results and Discussion

Abnormalities in metabolism should not be brought about through the action of the administered isotopic tracer. Since all long-chained fatty acids, regardless of the form in which they are fed, have been shown (1) to be metabolized via the lymphatics, it is possible to investigate this avenue of transport by monitoring the junction of the jugular and subclavian veins in the throat. In this area, radioactivity was detected in large quantities within 15 min after administration of the dose, which disappeared at a rate that might be explained by dilution with the blood but not by radioactive decay. Thus, these observations infer the incorporation of radiochlorine^{38} into lipid molecules with no apparent alteration in the normal pathway of metabolism.

Before the animals could be assayed, digestion, absorption, transport and deposition of the radioactive material must have taken place. After slaughtering animals at 2,3,4 and 5 hr after administration of the tracer, it was observed that in the earlier slaughter times sufficient deposition of the material in the *panniculus adiposus* had not taken place. In the later times the radioactive decay process was responsible for the low counting rate observed in the tissue samples. From these studies it was determined that 3½ hr after administration of the radioactive material was the optimum time for slaughter and assay. Consequently, another pig was slaughtered 3½ hr after being given the dose. A statistically acceptable level of activity was observed in the *panniculus adiposus*, and, in scanning the viscera, most of the activity remaining in the gastrointestinal tract was found in the upper part of the small intestine.

When using any radioisotope in a tracer study, the level of administered radioactivity should be established. This level must be of a magnitude that will satisfy health hazard requirements but still be sufficient to withstand dilution during metabolism and give statistically meaningful results on assay. After numerous studies of levels ranging from 0.01 to 0.1 mc/lb of body wt for the species involved, the threshold dose for a statistical accuracy of 1% was determined as approx 0.08 mc/lb of body wt.

In the final study of this experiment, the radioactive tracer was administered to the pigs on the basis of the predetermined statistical threshold dose and their individual live wts. Tables I and II summarize the data on the nine pigs used. Two pigs in each wt group were assayed live and one was slaughtered for the tissue data. As Table I indicates, generally good agreement was observed between the live animal assay and the carcass tissue radioassay results. With the exception of the carcass tissue assay of the 190-lb wt group, where the shoulder region accounted for the greatest percentage of the total activity (all sites), the loin area appeared to be the most active of the three sites, the shoulder site second, and the rump the least active. The obviously higher amounts of radioactivity noted in the 190-lb pigs, as compared to the 75 and/or 130-lb pigs, can be explained as reflecting the change in the pattern of fat deposition that usually occurs as the pig matures beyond 120 days of age (15).

It can also be observed, from the data presented in Table II, that, of the two tissue layers of the *panniculus adiposus* analyzed, the outside layer generally exhibited the highest amount of radioactivity per gram of tissue. The effect of maturity on the metabolism of the *panniculus adiposus* is quite evident, not only within and between layers but also within and between sites.

TABLE II
A Summary of the Radioassay of the *Panniculus Adiposus*

Site of measurement	Outer tissue layer			Inner tissue layer		
	Individual sample activity	Individual sample activity/ total activity (all sites)	Individual sample activity/ total activity (all layers)	Individual sample activity	Individual sample activity/ total activity (all sites)	Individual sample activity/ total activity (all layers)
	cpm/g	%	%	cpm/g	%	%
75-lb pigs						
shoulder	386.5	36.9	58.7	272.2	34.3	41.3
loin	439.8	41.9	61.5	275.0	34.6	38.5
rump	222.2	21.2	47.8	246.8	31.1	52.2
130-lb pigs						
shoulder	330.7	29.8	50.8	320.3	37.7	49.2
loin	506.6	45.6	68.6	231.8	27.3	31.4
rump	273.2	24.6	47.8	298.2	35.0	52.2
190-lb pigs						
shoulder	714.2	37.0	51.6	669.1	41.5	48.4
loin	657.2	34.0	54.6	556.2	34.5	45.8
rump	559.9	29.0	59.1	387.7	24.0	40.9

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Determination of the Length of Polymethylene Chains in Salts of Saturated and Unsaturated Fatty Acids by Infrared Spectroscopy[1]

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Abstract

The length of polymethylene chains is determined by counting the number of, or measuring the position of, methylene vibration peaks in the 1070–710 cm^{-1} and/or the 1380–1170cm^{-1} regions of the IR spectrum of salts of fatty acids. Plotting this peak position against the phase relationship of the vibration in adjacent methylenes gives a curve which is independent of the chain length. (The *phase relationship*, $\Phi/\pi=k/(m+1)$; where ϕ is the phase difference in radians between adjacent methylenes in a chain; m is the number of methylenes in the chain; $k = 1,2,3,\ldots.m$, with $k = 1$ generally assigned to the in-phase vibration.) Separate curves are obtained for methylene wagging and for two arrays of coupled twisting-rocking vibrations.

Coupled twisting-rocking vibrations give as many as one peak per methylene group in the 1070–710 cm^{-1} region with silver, sodium, potassium and barium salts of saturated acids. Lead salt peaks split. These peaks show the total length of salts of both saturated and *trans*-unsaturated acids, but only the length of the carboxylate segment in salts of *cis*-unsaturated acids. (The carboxylate segment comprises the carbons from the carboxylate carbon to the first unsaturated carbon, inclusive.)

Wagging vibrations in the 1380–1170 cm^{-1} region show the total chain length of saturated salts and the length of the carboxylate segment in unsaturated salts, both *cis* and *trans*. This region also has peaks for twisting-rocking vibrations, and they are most conspicuous in the spectra of silver and barium salts.

Introduction

THE POTENTIAL OF IR spectra for the identification of fatty materials has not been fully realized, since many fatty acids are liquid at room temp and randomly oriented. Differences in the spectra related to the length of methylene chains are mainly quantitative. When free rotation about the single bonds is eliminated by examining the materials in the solid state, qualitative variations have been found in the spectra. Examples in the literature include saturated fatty acids, esters, salts and ketones, and even *trans*-mono-unsaturated acids (1,4–7,12,14,17,18,24,26,27,29). Generally, recognition of all the useful peaks has been quite incomplete, and the assignment of peaks to vibrations in the molecule has been disputed (8,9,11,13,16,20,22,28).

We have obtained the spectra of a number of metal salts of a series of saturated fatty acids. The salts are more easily handled and have fewer interfering peaks than the crystallized acids. The salt spectra are compared with those of saturated hydrocarbons which have recently been studied in some detail (19, 23). We have discussed only the regions where peaks due to methylene wagging, twisting and rocking vibrations are found, and in hydrocarbons this is 1413–1170 cm^{-1} and 1061–721 cm^{-1} (19,20,23). We also investigated the spectra of salts of mono-unsaturated acids for comparable behaviour.

When the carbon skeleton is an extended zigzag chain, as in crystals, the methylenes may vibrate in-phase (phase difference 0°), out-of-phase (phase difference 180°), and with phase differences between 0° and 180°. For m methylenes in a chain there are m possible phase relationships between adjacent groups. As a result, the chain may have m vibration frequencies which are seen as m absorption peaks in the IR spectrum. Each peak is not assigned to a particular methylene group, but to the vibration of the groups relative to each other as a whole.

In this paper we are concerned with three ways in which the methylenes may vibrate; wagging, twisting and rocking. Assuming the absence of coupling between the vibrations, there are m possible phase relationships with m absorption peaks for each way in any chain. Thus a C_6 acid or salt with four methylene groups could show $3 \times 4 = 12$ absorption peaks that might be assigned to the vibrations of interest. The number of peaks predicted by theory was found in the hydrocarbon spectra, although this is less than predicted for the salts because of symmetry in the hydrocarbons. The assignments for hydrocarbons were complicated by coupling of the twisting and rocking vibrations which also overlapped the wagging vibrations (19,23).

Experimental

Acids

Commercially available fatty acids (C_3–C_{22}, except C_{21}) were used for preparation of the salts. Subsequently the saturated acids, C_4 and C_7–C_{20}, were analysed as the methyl esters by gas liquid chroma-

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