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Secretion of Radioactivity in Milk Fat after a Single Oral Dose of (^{3}H)Myristic Acid to a Goat

L. REINIUS and J. HASAN

Department of Physiology and Biochemistry, Veterinary College, Helsinki
and
Institute of Occupational Health, Helsinki, Finland

Tritium-labelled myristic acid was administered orally as free acid to a lactating goat and then the secretion of the radioactivity in the total milk lipids and milk glyceride fatty acids was followed for thirteen days. Measurable activity was found in the milk fat 4 h after administration and the maximum specific activity occurred within 28 h. About 10 % of the radioactivity administered was recovered during the experimental period and about 25 % of the activity collected during 13 days was secreted in the first 28 h. In the beginning the non-volatile fatty acids had a higher specific activity than the volatile fatty acids and there seemed to be a tendency for the specific activities in the short chain acids to increase with time whereas the long-chain acids appeared to be less active later in the experiment.

The relative importance of various lipid constituents in the diet for the formation of characteristic elements of milk fat is a question of great interest both from the quantitative and qualitative points of view. It has been demonstrated that, in the ruminant, acetate is a precursor of short-chain fatty acids of the milk [1]. Of the total acetate present in the body of the goat at any moment, about 80 % is oxidised within 6 h while some 50 % of the remainder will participate in the fat metabolism of the udder [2]. Evidence for the existence in the mammary gland of a system capable of synthetising fatty acids up to $C_{(18)}$ from acetate has been produced by Popják and Tietz [3], Hele and Popjak [4] and Hele, Popjak and Lauryssens [5].

In a study of the role played by dietary long-chain fatty acids in the formation of ruminant milk fat [6] it was shown that after oral administration of (^{3}H)stearic acid, the short-chain acids in the milk fat had a much lower specific activity than the long-chain acids and the tentative conclusion was drawn that the contribution of the long-chain acids of the blood to the short-chain acids of ruminant milk fat was small.

The work quoted above deals with fatty acids of very short ($C_{(2)}$) or extremely long ($C_{(18)}$) chain length. In the experiment to be described, which is

an extension of the work done by Glascook *et al.*[6], (^{3}H)myristic acid — representing a dietary fatty acid of an intermediate chain length — was fed to a lactating goat and the time of appearance, rate of excretion of radioactivity in the milk lipids, and the distribution of radioactivity in various fractions of milk lipid was studied.

Radioactive myristic acid

EXPERIMENTAL

(^{3}H)Myristic acid was prepared by treating inactive myristic acid (Fluka, purum) in an evacuated and sealed ampoule for two days at 98°—100°C with concentrated sulphuric acid (sp.d. 1.84) and tritiated water (200 mC/ml) according to the method of van Heyningen, Rittenberg and Schoenheimer [7]. The reaction product, diluted with water and dissolved in ether, was extracted with a solution of alcoholic potassium hydroxide. The radioactive myristic acid obtained after acidification and extraction with ether was recrystallised from aqueous acetone. The product had a melting point of 57.6°C (published melting point 58°C) and a specific activity of 2.55×10^6 counts/min/mg H_2O as assayed with our apparatus.

To check the radiochemical purity of the product, a sample was run on a paper chromatogram [8] together with chemically pure, inactive myristic acid. After development the strip containing the radioactive sample was cut into bands 1 cm wide which were eluated with toluene and the eluates were assayed for radioactivity in a liquid scintillation counter (Packard Tri-Carb Scintillation Spectrometer). The radioactivity was found to be incorporated into a single, welldefined area on the chromatogram, the location of which was the same as that of the pure inactive myristic acid.

It was expected that as a result of the tritiation process used, the radioactivity would be bound to $C_{(2)}$. This was checked by brominating the (^{3}H)myristic acid (as described by van Heyningen *et al.*[7]) and then reassaying the radioactivity of the product. By this procedure 46 % of the radioactivity was lost, indicating that most (92 %) of the tritium was bound to the $C_{(2)}$ atom.

Animal experiment

The experimental animal was a goat of Finnish breed yielding about 1 l milk/day. The labelled material consisted of 3.82 g (^{3}H)myristic acid prepared as above and containing a total activity of 1.08×10^{10} counts/min/mg. A mixture of the myristic acid, 40 ml arachis oil, 2 g sodium stearate and 0.5 g sodium carbonate was homogenised at 60°C for 65 min. and at a pressure of 2 600—2 700 lbs/sq.in. The mean diameter of the particles in the resultant emulsion was about 1 μ.

After an injection of oxytocin the goat was milked out and the emulsion, warmed to body temperature, was administered in one dose by mouth followed by 50 ml water. Water and hay were provided *ad libitum* and concentrates according to the calculated need. The goat was kept in an isolation pen and milked three times a day for the first two days and then twice daily for the following 11 days of the experiment. The two milkings of the 4th, 5th and 6th experimental days were combined to give three samples and from the 7th day onwards the milkings of two successive days were pooled to one sample. Altogether, 15 samples were obtained for radioactivity measurements of the total milk fat. The fractionation of the milk fat was done on three pooled milk fat samples representing the total milkings of following successive periods of the experiment —1st day; 2nd, 3rd and 4th day; and 5th to 13th days.

Chemical methods

The isolation of milk fat for radioactivity analysis was made following the description given by Glascock *et al.*[6] The fractionation of the milk fat into non-volatile saturated, non-volatile unsaturated, steam-volatile water soluble and steam-volatile water-insoluble

glyceride fatty acids was done by procedures similar to those described by Popják and Beeckmans [9].

Radioactivity measurements

After combustion of the lipid samples in a stream of oxygen, the tritium content of the combustion water was measured in a gas counter with a stainless steel cathode after conversion into butane with the aid of *n*-butyl magnesium bromide [10]. All combustions and measurements were made in duplicate. The specific activities are given as counts/min/mg of combustion water as observed with our equipment.

RESULTS

The specific activities of the total milk lipids in successive samples are plotted semi-logaritmically against time in Fig. 1, the time for each point on the curve being the mid-point of the period during which the milk was secreted. Measurable radioactivity appeared in the total lipids as early as 4 h after administration of the (^{3}H)myristic acid, and the milk fat with the highest specific activity (1.2×10^4 counts/min/mg) was collected 28 h after dosing. The specific activity then declined rapidly, and the specific activities of the milk lipids on the 12th and 13th experimental days were only about 100 c.p.m.

The cumulative secretion as a percentage of the total dose administered is shown in Fig. 2 plotted against time. The radioactivity collected during the whole experimental period of 13 days was about 10 % of the dose administered. It can be seen that of the total activity collected, about 25 % was secreted during the first 28 h. Some secretion, however, was still going on at the end of the experimental period.

The specific activities of the four glyceride fatty acid fractions from milk collected during successive experimental periods are shown in Fig. 3. It can be seen that the specific activity of the non-volatile saturated fatty acids

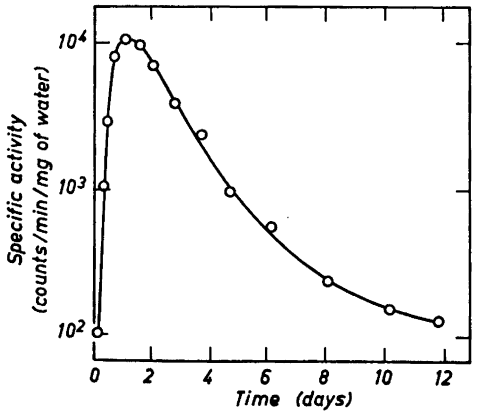

Fig. 1. Specific activity of total lipids in goat milk after ingestion of (^{3}H)myristic acid administered in one dose.

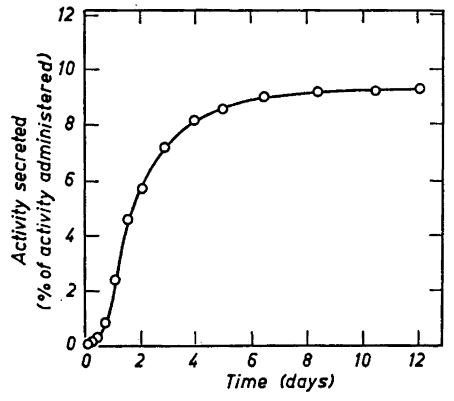

Fig. 2. Cumulative secretion of radioactivity in total milk lipids of goat after ingestion of (^{3}H)myristic acid administered in one dose.

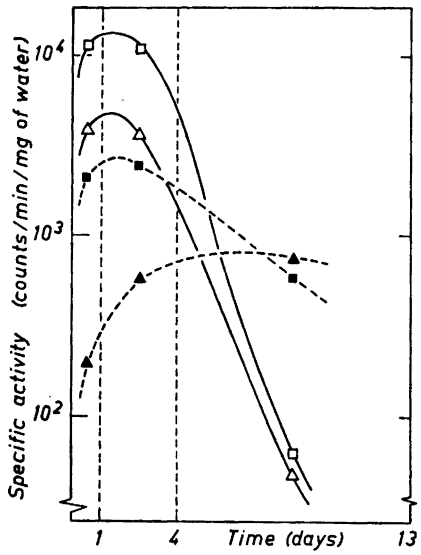

Fig. 3. Mean specific activities of pooled milk glyceride fatty acids on three successive experimental periods after ingestion of (^{3}H)myristic acid administered in one dose. Experimental periods indicated by dotted lines. □ : non-volatile saturated fatty acids; ■ non-volatile unsaturated fatty acids; △ : steamvolatile water-insoluble fatty acids; ▲ : steam-volatile water-soluble fatty acids. A smooth curve drawn arbitrarily through corresponping points.

secreted during the first day is of about the same magnitude as the maximum specific activity of milk fat collected during this period. The radioactivity of this fraction subsequently declined. The other fractions contain considerable less radioactivity, and while the specific activity of long-chain unsaturated fatty acids also declines with time, there seems to be a trend for higher activities in the short-chain fatty acid fractions from samples collected during later periods; the variations, however, are slight and it is difficult to asses their significance. It may be noted that although the short-chain fatty acids contain less radioactivity than the long-chain acids in the beginning, considerable activity is found in these as well.

DISCUSSION

The results described above give information on the role of dietary myristic acid as a precursor of constituents in the milk fat. The appearance, the time curve of secretion, the total amount secreted in the milk lipids, and the distribution of radioactivity between various fractions of the milk fat seem on the whole, to be comparable with corresponding data [6] obtained by using stearic acid in a rather similar experiment. Some divergent points, however, should be noted.

The total amount of secreted radioactivity in the milk was only 10 % of the dose administered. This is a small fraction as compared with the values 25 % and 49 % obtained after 8 and 14 days, respectively, by Glascock *et al.*[3] in the two experiments where free (^{3}H)stearic acid was fed to goats. Since we did not examine the faeces, it is impossible to say whether the results reflect only poor absorption of the (^{3}H)myristic acid or differences in the metabolic

behaviour between myristic and stearic acid of fat in the diet. It seems, however, that in a system including such a multitude of independently variable mechanisms as in the transport of fatty acids from the digestive tract to milk, great differences can exist between different animals as well as considerable short-time variations in the same animal. In both experiments, however, the radioactivity in the milk lipids after administration of the labelled acid was found in the first sample collected 4 h after administration. Maximum activity was reached in the myristic acid experiment after 28 h as compared with 23 h in the stearic acid experiment.

In the experiments of Glascock *et al.*[6] the specific activities of the saturated and unsaturated long-chain fatty acids in the milk were of the same magnitude whereas the unsaturated long-chain acids in our experiment apparently were of significantly lower specific activity than that of the saturated. An explanation for the low specific activity of the unsaturated long-chain fatty acid fraction as compared with the saturated could be the relatively high content of myristic acid in goat milk lipids and the very low content of the corresponding unsaturated acid the myristoleic — 12.3 % for myristic acid (tetradecanoic) and 0.8 % for tetradecenoic. The converse is true for stearic and oleic acids [11]. Our explanation assumes that myristoleic acid is the natural desaturation product of myristic acid in the goat as has been shown [12] to be in the rat and that biological desaturation of myristic acid exclusively labelled at $C_{(2)}$ would not lead to a significant loss of radioactivity. The latter assumption may be valid considering that Hilditch and Longenecker [13] have observed that the double bond in the monoethenoic acids of milk is in the $C_{(9)}$—$C_{(10)}$ position.

Another point of interest in this experiment is the high specific activity in the water-insoluble steam-volatile fraction. From the experiments with (^{3}H)myristic acid, Glascock *et al.*[6] concluded that no significant formation of milk short-chain fatty acids occurred through break-down of dietary long-chain acids. If this also applies for myristic acid, the activity of the short-chain acids in our experiment should originate from radioactive precursors (acetate) formed by degradation of myristic acid. In this case, however, even greater specific activities would be expected in the water-soluble portion of the steam-volatile fatty acids which, in our experiment, actually contain very little activity. Even if small quantities of $C_{(8)}$—$C_{(10)}$ acids are known to pass over into the steam distillate [14,15] it is highly improbable that radioactive contamination from myristic acid $C_{(14)}$ could account for this finding. Considering the specific activity of the long-chain unsaturated fatty acids (highest value about 4 000 counts/min/mg of H_2O), it does not seem possible that the traces of oleic acid, which may also be carried over in the distillation, could cause contamination of the steam-volatile water-insoluble acids (about 2 000 counts/min/mg of H_2O) to the extent observed.

Of interest is the apparent trend for higher specific activities in the short-chain acids during later experimental periods while the radioactivity secreted in the long-chain acids seems to decline with time. This could indicate that a chain-shortening is actually going on in the organism with a fairly slow reaction velocity, but a more probable explanation would be the successively higher radioactivity gained by the metabolic pool of small-molecular entities available for resynthesis from degradation of (^{3}H)myristic acid.

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