# METABOLISM OF $^{14}$C-ERUCIC ACID

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## Introduction

The early findings of ROINE et al. (1960) about lesions in the hearts of rats fed diets containing large amounts of high erucic acid rapeseed oil have been confirmed by ROCQUELIN et al. (1967). Several authors suggested (BEARE et al., 1959; ROINE et al., 1960; ABDELLATIF and VLES, 1970) that erucic acid is the sole cause of the adverse nutritional effects of rapeseed oil. CHRISTOPHERSON and BREMER (1972) suggested that a mitochondrial metabolite of erucic acid inhibits the mitochondrial oxidation of other fatty acids, especially in the heart, and that this causes the accumulation of triglycerides in the hearts of rats fed rapeseed oil. However, ROCQUELIN et al. (1970) observed lesions in the hearts of rats fed a ration containing low erucic type rapeseed oil and concluded that erucic acid was not the only factor responsible for the occurrence of cardiac lesions. This conclusion was supported by recent experiments involving high and low erucic acid type rapeseed oils (KRAMER et al., 1973). These authors found a similar number of cardiac lesions in rats fed either high or low erucic acid type rapeseed oils for 14 weeks.

These reports clearly demonstrated the need for studies of both, the role of non-glyceride constituents of rapeseed oil in nutrition studies and the metabolic fate of erucic acid in different animals and poultry. There is little known on whole animal metabolism of $^{14}$C-erucic acid other than the work of CAROLL (1962) and LAPOUS et al. (1970) with rats and BIOCHOT et al. (1973) with dogs. LECLERCQ (1972) conducted experiments with laying hens using 14-$^{14}$C-erucic and oleic acid and found no difference in the metabolic fate of these two fatty acids. This work did not consider cardiac tissue which is of such obvious importance.

The objective of this study was to determine in vivo the metabolic fate of erucic acid in comparison with other fatty acids in broiler chickens. The comparison of various body tissues was of primary importance, since a difference in the utilization of erucic acid between certain body tissues had been found in previous experiments with broiler chickens (VOGTMANN et al., 1974) and with rats (CHRISTOPHERSON and BREMER, 1972). The influence of sex of the birds and of amount and kind of oil or fat included in the diet consumed on the metabolism of erucic and other fatty acids was examined.

Materials and methods

Male and female crossbred broiler chicks (Hubbard male x White Plymouth Rock female) were housed in electrically heated, thermostatically controlled battery brooders with raised wire screen floors, in a well ventilated, temperature-controlled brooding room. At four weeks of age the chickens were placed in growing batteries. Electric light was provided on a continuous basis. Feed and water were supplied ad libitum.

The chickens were fed a low-fat control ration or diets containing 15 % of either regular rapeseed oil (RSO) originating from B. campestris rapeseed and containing approximately 23 % erucic acid or the low erucic acid Span rapeseed oil (SPO) originating from the Span cultivar of B. campestris rapeseed containing approximately 3 % erucic acid. Soybean oil was included in this study for comparative purposes. The diets were calculated to contain 23 % protein and 3,200 kcal ME/kg diet. The oils used were fully refined and of edible quality. The compositions of the experimental rations are summarized in Table 1.

The experiments were conducted with 10 to 12 week old chickens. The scheme of the system used for the experiments is illustrated in Figure 1. The birds were held in an enclosed chamber wearing a mask.

Figure 1: Experimental outline

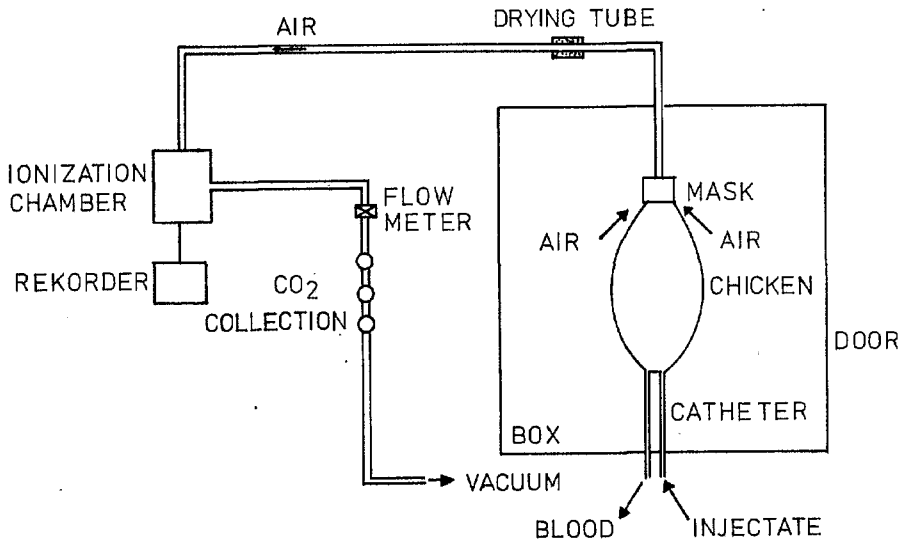


Table 1: Composition of experimental rations

| Components | Control ration, % | Oil-containing ration, % |
|---|---|---|
| Ground wheat | 64.52 | 28.72 |
| Fat, oil [1] | 4.00 | 15.00 |
| Dehydrated alfalfa meal | 1.00 | 1.00 |
| Meat meal | 5.00 | - |
| Herring meal | 3.00 | - |
| Soybean meal (48.5 % protein) | 20.00 | 40.00 |
| Ground limestone | 1.00 | 1.75 |
| Dicalcium phosphate | 0.50 | 1.50 |
| Iodized salt | 0.20 | 0.25 |
| Manganese sulfate monohydrate | 0.02 | 0.02 |
| Zinc oxide | 0.01 | 0.01 |
| Premix [2] | 0.75 | 0.75 |
| Cellulose | - | 11.00 |

[1] Stabilized tallow/lard mixture in control ration. See materials and methods for oils used in 15 % oil-containing rations.

[2] Supplied per kilogram of ration: vitamin A, 4,000 IU; vitamin $D_3$, 400 ICU; vitamin E, 20 IU; vitamin K, 2 mg; riboflavin, 5 mg; calcium pantothenate, 10 mg; niacin, 20 mg; choline chloride, 200 mg; vitamin $B_{12}$, 0,01 mg; DL-methionine, 500 mg.

Air was passed through the mask with a flow rate of 2 l/min. The birds were relatively calm in the chamber already after a short training period. A catheter was established in the brachial vein of the chickens 24 hours before the experiment and a small blood sample (approximately 2 ml) was taken for the preparation of the injectate. The $^{14}C$-fatty acids were bound to the birds own albumin. To the labelled fatty acid 0.01 N KOH in ethanol was added and heated in a water bath for approximately 15 minutes. The soaps were cooled to room temperature and 0.5 ml of sterile saline solution and 0.5 ml of chicken plasma were added. The injectate was well mixed and held at room temperature for at least 30 minutes before injection. The isotope was injected into the brachial vein and, therefore, entered the right part of the heart of the calm bird. The amount of fatty acid injected was either 45 $\mu$Ci in the experiments where only the distribution of label in various tissues was studied or was 100 $\mu$Ci in the experiments where GLC-analysis with splitting of the fatty acid methylesters was necessary. The numbers of replicates per respiration trial were dependent upon the variation of the results between replicates and varied, therefore, between four and ten.

In one experiment the chickens were killed at 5, 10, 20 and 120 minutes postinjection whereas in all other experiments the chickens were killed after 120 minutes. During the 120 minute-runs the radioactivity in respira-

tory $CO_2$ was measured in an ionization-chamber. Immediately after the birds were killed tissue samples were removed and weighed. The samples were then homogenized and an aliquot was oxidized. The developed $CO_2$ was trapped and the activity of the label was measured.

Tissue lipids were extracted and fatty acid methylesters were prepared and measured by GLC-analysis according to VOGTMANN et al. (1974). The amount of label in the individual fatty acids was determined by splitting the sample in a ratio of approximately 50/50 and collecting one half of the sample in a cool-trap. The trap was washed with scintillation liquid directly into a scintillation vial. 1-$^{14}$C-heptadecanoic acid was used as internal standard.

The data for percent of injected label in respired air and for percent of injected label in certain body tissues were analyzed statistically by analysis of variance (STEEL and TORRIE, 1960). Comparison of treatment means was conducted by using Duncon's new multiple range test (STEEL and TORRIE, 1960).

## Results and Discussion

The percentage of injected label appearing in the respired air of the chickens fed the low-fat control ration (Table 1) was significantly different ($P \leqslant 0.01$) between all four labelled fatty acids injected (Table 2). Of the

Table 2: Percent of injected label in respired air during the first two hours postinjection

| Fatty acid injected | Body weight, g | Label in respired air, % of injected label |
|---|---|---|
| 1-$^{14}$C-palmitic | 1680 | 22.9 a[1] |
| 1-$^{14}$C-oleic | 1696 | 31.0 b |
| 1-$^{14}$C-erucic | 1578 | 39.0 c |
| 14-$^{14}$C-erucic | 1831 | 15.2 d |
| SE[2] | | 1.76 |
| Significance | | * |

[1] Numbers with a common letter are not significantly different ($P \leqslant 0.05$)

[2] Standard error

carboxyl labelled fatty acids the saturated palmitic acid (C 16:0) led to lowest amount of label in the respired air as compared to the monounsaturated oleic (C 18 :1) or erucic acid (C 22:1). The two fold greater activity in respired $CO_2$ from the carboxyl position than from the $C_{14}$-position is

what one would expect in the fed rested animal. That the carboxyl group of erucic acid is oxidized to a greater extent than the carboxyl group of oleic acid, fits with the concept that the bird is attempting to convert its dietary erucic acid to oleic acid (VOGTMANN et al., 1974; LECLERCQ, 1972). The amount and the kind of oil or fat included in the diet had no significant influence on the rate of oxidation of 1-$^{14}$C-palmitic or 1-$^{14}$C-erucic acid (Table 3). In a number of test-trials it was shown that the sex of the bird was without significant influence on the results obtained.

Table 3: Percent of injected label in respired air during the first two hours postinjection

| Diet[1] | Fatty acid injected | |
|---|---|---|
| | 1-$^{14}$C-palmitic | 1-$^{14}$C-erucic |
| Control | 23.7 | 38.3 |
| RSO | 21.6 | 43.9 |
| SPO | 21.2 | 43.6 |
| SBO | 23.8 | 43.5 |
| SE[2] | 1.15 | 3.17 |
| Significance | NS | NS |

[1] See materials and methods for meaning of ration abbreviations

[2] Standard error

As indicated by the data in Table 4 there was, generally, a decrease in the specific activity of nearly all body tissues examined with time postinjection of a single dose of labelled fatty acid. However, no extensive changes in the relation specific activities of the tissues occurred. It was, therefore, appropriate to use a 120 minute-run for all further experiments, because that allowed a complete check on each injection by monitoring the curve for the respiratory radioactivity.

The relative uptake of label by the various tissues of the body is indicated by their specific activities, dpm/g tissue per dpm injected/g body weight (Table 5). In general, the highest specific activities in the tissues were found after injection of 14-$^{14}$C-erucic acid. This result was in agreement with the fact that this fatty acid was oxidized to a much lesser extent by the bird than the other fatty acids (Table 2). The liver seemed to be most active in taking up the fatty acids, followed by heart and spleen (Table 5). White skeletal muscle (pectoralis) appeared to be the least active tissue examined in taking up any of the injected fatty acids.

After two hours the heart and the spleen contained very little of the injected $^{14}$C (heart: 0.7 % of 1-$^{14}$C-oleic acid; 0.8 % of 1-$^{14}$C-erucic acid; 1.2 % of 14-$^{14}$C-erucic acid ($P \leq 0.05$); spleen: 0.3 % of 1-$^{14}$C-oleic acid; 0.5 % of 1-$^{14}$C-erucic acid; 0.8 % of 14-$^{14}$C-erucic acid ($P \leq 0.01$) ).

Table 4: Time course change in specific activity of various tissues after injection of 1-$^{14}$C-erucic acid (dpm/g tissue per dpm injected/g body weight)

| Time post-injection, minutes | Heart | Red Muscle[1] | White Muscle[2] | Liver | Blood |
|---|---|---|---|---|---|
| 5 | 3.28 | 0.39 | 0.12 | 14.02 | 1.25 |
| 10 | 2.45 | 0.47 | 0.13 | 12.66 | 0.99 |
| 20 | 1.65 | 0.42 | 0.10 | 10.32 | 0.81 |
| 120 | 1.53 | 0.38 | 0.08 | 7.00 | 0.39 |

| | Spleen | Adrenal | Adipose t. | Lung | Kidney |
|---|---|---|---|---|---|
| 5 | 5.59 | 1.42 | 0.88 | 3.11 | 3.03 |
| 10 | 4.67 | 1.08 | 1.22 | 1.81 | 1.44 |
| 20 | 2.63 | 1.06 | 0.77 | 1.75 | 1.23 |
| 120 | 2.14 | 0.76 | 1.91 | 1.08 | 0.97 |

[1] Semintendinosis [2] Pectoralis

Table 5: Specific activity of various tissues two hours postinjection (dpm/g tissue per dpm injected/g body weight)

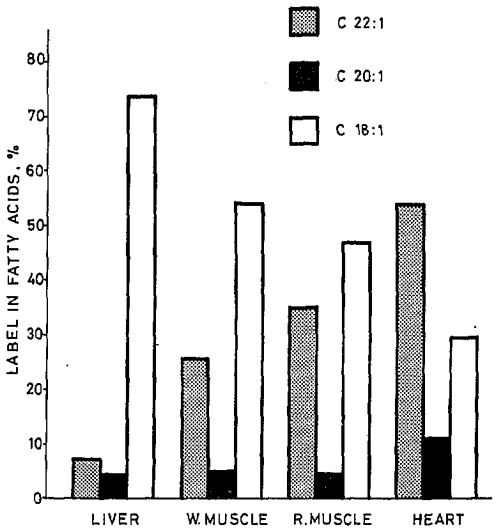
| Tissue | Fatty acid injected: 1-$^{14}$C-oleic | 1-$^{14}$C-erucic | 14-$^{14}$C-erucic | SE[1] | Significance |
|---|---|---|---|---|---|
| Blood | 0.37a[2] | 0.38a | 0.63b | 0.05 | |
| Liver | 5.12c | 7.62d | 10.49e | 0.74 | |
| Heart | 2.18f | 2.07f | 3.54g | 0.28 | |
| Spleen | 1.32h | 2.04i | 3.44k | 0.11 | |
| Adrenal | 1.51 | 0.75 | 1.46 | -[3] | -- |
| Adipose t. | 1.08 | 1.91 | 0.87 | extensive variations | |
| White muscle | 0.12 | 0.08 | 0.14 | - | -- |
| Red muscle | 0.50 | 0.32 | 0.55 | - | -- |
| Kidney | 1.43 | 1.03 | 1.43 | - | -- |
| Lung | 0.95 | 1.31 | 1.59 | - | -- |

[1] Standard error

[2] Within a row numbers with a common letter are not significantly different ($P \leq 0.05$)

[3] Data not analyzed statistically

Figure 2: Distribution of label from 14-$^{14}$C-erucic acid in various tissues (2 hours postinjection)




That label which remained in the heart was largely present as erucic acid (approximately 54 %) whereas the oleic acid contained smaller amounts (approximately 29 %) of the injected label (Figure 2). This observation was in contrast to the liver in which very little label remained as erucic acid (6 to 7 %) but was largely in form of oleic acid (approximately 74 %). This slow metabolism of erucic acid by the heart was also supported by the amounts of label appearing in C 20:1. In the liver there was relatively less label in C 20:1 (approximately 4 %) than in the heart (10 to 12 %) indicating that the overall sequence of reactions C 22:1 to C 20:1 to C 18:1 was indeed slower in the heart than in the liver.

Skeletal muscle in general also appeared to be less capable of metabolizing erucic acid than the liver. While, on the other hand it appeared more capable than cardiac tissue. Though an inadequate number of trials have been performed it appears as though white skeletal muscle (pectoralis) is more capable of oxidizing erucic acid than red skeletal muscle (semitendinosis). This observation cannot be explained at this time. It is of interest that the order of ability to metabolize erucic acid is inversely related to the oxidative capacity of these three muscles. This observation may be a reflection of the relative uptake of erucic acid by these three tissues, i.e. after two hours erucic acid label in cardiac tissue is approximately 6.5

times that found in red muscle and approximately 25 times that found in white muscle.

## Summary

In vivo experiments were conducted to determine the metabolic fate of 1-$^{14}$C-erucic and 14-$^{14}$C-erucic acid in comparison with other long chain saturated and monounsaturated fatty acids in broiler chickens. The results of the experiments were:

1. Erucic acid was extensively catabolized by the growing broiler. 1-$^{14}$C-erucic acid was oxidized to a greater extent than 1-$^{14}$C-oleic acid. After two hours the proportion of the label from 1-$^{14}$C-erucic and from 1-$^{14}$C-oleic acid which appeared in the respired air was larger than that of 14-$^{14}$C-erucic acid (Table 2).
2. The amount of label expired was not influenced by the sex of the birds or the kind and amount of fat or oil included in the diet.
3. In the short term (2 hours) erucic acid seemed to be most extensively taken up by the liver with lesser amounts in all other tissues examined (Table 5).
4. The distribution of label in the fatty acids of liver, heart, red and white muscle after the injection of 14-$^{14}$C-erucic acid revealed that the heart metabolized erucic acid much more slowly than the liver. Skeletal muscle (red and white) ranked between liver and heart (Figure 2).

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