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# The *in vitro* incorporation of acetate-1-$C^{14}$ into individual fatty acids of adipose tissue from young and old rats*

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## SUMMARY

The pattern of *in vitro* incorporation of acetate-1-$C^{14}$ into the individual fatty acids of adipose tissue from young and old rats was determined. It was found that the proportion of total incorporated radioactivity in the monounsaturated acids was significantly higher in tissues taken from young animals than in tissues taken from old animals. When, however, the rats were fed a fat-free diet, the proportion of monoenes increased in both age groups, and the distribution of acetate-1-$C^{14}$ in old animals resembled that of the young. The administration of insulin to old animals also led to an increased proportion of the fatty acid radioactivity in palmitoleic and oleic acids. The possible relationship between monoene synthesis in adipose tissue and glucose metabolism, TPNH generation, and the effects of insulin on adipose tissue, were discussed. Evidence was presented demonstrating that the short-chain fatty acids are not synthesized and stored in adipose tissue.

Recent studies of lipid metabolism in adipose tissue from young and old rats have shown that in the young age group there is a greater rate of incorporation of acetate and palmitate into the total lipids and a higher rate of palmitate oxidation and free fatty acid release under simulated stressful conditions (1). In this paper, data on the incorporation of acetate-1-$C^{14}$ into the individual fatty acids of adipose tissue are reported. It was found that with aging there was a significant decrease in the radioactivity incorporated into the monounsaturated acids, palmitoleic and oleic. The *potentiality* for increased monoene synthesis, as measured by the acetate-1-$C^{14}$ incorporation, was not lost in the adipose tissue from old animals, however, since the pattern of incorporation could be made to resemble that in young adipose tissue when the rats were placed on the fat-free diet or when they received insulin.

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## MATERIALS AND METHODS

Epididymal fat of Wistar rats from a colony maintained for more than 20 years at Columbia University was used. As in the previously reported studies (1), animals from 38 days to 50 days old and weighing from 70 to 120 g were classified as "young"; rats whose body weight ranged from 250 to 430 g and who were from 100 days to more than 1 year old were considered "old." All animals were maintained on Rockland rat pellets[1] ad lib. except those on a fat-free diet. In the latter experiments, the majority of animals, either at the time of weaning or when the rats had attained a weight of 250 g, were given an ad lib. supply of a fat-free diet fortified with minerals and vitamins,[1] with the exception of alpha-tocopherol. In several experiments, alpha-tocopherol was added to the synthetic diet.

In the experiments designed to determine the effect of insulin on the incorporation of acetate into individual fatty acids, rats weighing 300–370 g were injected

[1] Rockland Rat Diet, A. E. Staley Manufacturing Co., Decatur, Ill. "Fat Free" Test Diet, Nutritional Biochemicals Corporation, Cleveland, Ohio.

intraperitoneally with 50–100 units of glucagon-free, amorphous insulin in saline or with saline alone. Five per cent dextrose in water was given ad lib. in the drinking water, and the animals were sacrificed 2 hr after the insulin injections. Blood sugars determined at the time of sacrifice were found to be normal.

The preparation of tissues, incubation procedures, lipid extraction methods, and liquid scintillation counting methods have been previously described (1). The technique for simultaneous detection of the methyl esters of the fatty acids separated by gas-liquid chromatography and their collection for determination of radioactivity has not been previously reported in detail. The Barber Coleman apparatus, Model 10, with a radium-source detector was used for the gas-liquid chromatography. Six- or eight-foot glass columns packed with 17% ethylene glycol succinate on 100-mesh chromosorb W were maintained at 200°. The effluent argon-gas stream was split as shown in Figure 1. The 16-gauge (0.065-in. o.d., 0.009-in. wall) hypodermic tubing inserted in the outlet silicone plug of the column was connected to a stainless steel "T" by a "Swagelock" fitting.[2] The arm of the "T" connected to the detector was a coiled stainless-steel capillary (0.059-in. o.d., 0.01-in. i.d.) with an over-all length of about 15 inches. The coiled tubing was connected to the detector cell by a length of Teflon tubing. The other arm of the "T" was a 1-in. length of stainless-steel capillary joined to Teflon tubing. The latter was led through a heating unit maintained at 275° to the outside of the instrument. By varying the length of the coil between the "T" and the detector, the proportion of the effluent stream available for collection could be changed. In the present experiments, 10–50% of the stream passed through the detector and the remainder was available for collection. The collection was made on cartridges packed with siliconized anthracene (2, 3), which were carried on a rotating table. The resistance to gas flow produced by the anthracene cartridges was negligible when compared to that of the proximal short capillary tubing. When the cartridges were changed, the distribution of the effluent gas between the detector and the collecting side was not perceptibly modified. The heating unit through which the effluent tubing passed could be raised and lowered by an appropriate gear[3] and, when lowered on the cartridges, a tight seal was achieved by a silicone rubber pad. The collection of an individual

[2] Constructed by Mr. Raymond M. Schiff, 630 W. 168th Street, New York, "Swagelock," 1/16 inch stainless steel all tube "T," #100-3-316, R. S. Crum & Co., Mountainside, N. J.

[3] Obtained from Dr. S. R. Lipsky, Yale University, School of Medicine, New Haven, Conn.

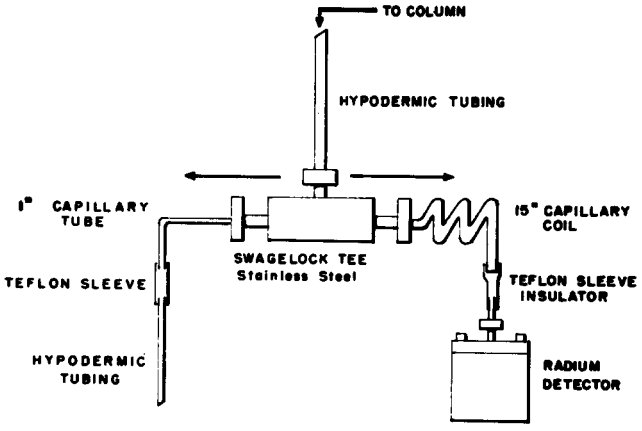


FIG. 1. Diagram of attachment for splitting effluent stream from gas-liquid chromatographic column.

fatty acid was monitored by the simultaneous mass record being made. There was no significant time discrepancy between the mass recording and the sample collection as demonstrated by determinations of radioactivity in samples collected from intervals before and after standard methyl esters of palmitate-1-$C^{14}$ and stearate-1-$C^{14}$ (Table 1). Before removing the anthracene cartridges from the turntable, their sides were washed down with a few drops of liquid phosphor (toluene containing 0.3% 2,5-diphenyloxazole and 0.03% 1,4-bis 2-(5-phenyloxazolyl)-benzene). Paper plugs were then inserted into the open ends, and the cartridges were inverted and inserted into Lucite spheres of the same outside dimensions as the conventional liquid-counting bottle.

## RESULTS

Table 2 shows the quantitative differences in fatty acid composition of the epididymal adipose tissues from rats of different ages. As has been previously noted (1), the major differences are found in the increased proportion of shorter-chain fatty acids (C10, C12, C14) in the younger-age groups. Table 3 shows the rate of incorporation of acetate into the total fatty acids and the percentage distribution of the total radioactivity in the individual fatty acids after *in vitro* incubation of the epididymal fat with acetate-1-$C^{14}$. The results of incorporation of radioactivity into the individual fatty acids are expressed as percentages of the total radioactivity incorporated into all of the fatty acids in order to facilitate comparisons of *de novo* synthesis among the fatty acids. It is possible to express the results as specific activities; however, this has little value for comparative purposes since the size of the nonradioactive fatty acid pools varies so greatly among the

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN FATTY ACID PEAKS ON GAS-LIQUID CHROMATOGRAPHY OF PALMITATE-1-$C^{14}$ AND STEARATE-1-$C^{14}$*†

| | Total cpm† Added | Fatty Acids‡ 12:0‡ | 14:0 | 15:0 | 16:0 | 16:1 | 17:0 | 18:0 | 18:1 | 18:2 | 18:3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Exp't 1 | 872 | 0 | 0 | 0 | 384 | 14 | 8 | 112 | 3 | 0 | 0 |
| Exp't 2 | 855 | 0 | 0 | 0 | 468 | 17 | 12 | 116 | 7 | 0 | 0 |
| Exp't 3 | 858 | 0 | 0 | 0 | 370 | 36 | 2 | 85 | 5 | 0 | 2 |
| Exp't 4 | 890 | 0 | 0 | 0 | 330 | 29 | 7 | 82 | 5 | 0 | 0 |
| Mean and S.D. | 869 ± 13 | | | | 388 ± 50 | 23 ± 2 | 7 ± 0.2 | 99 ± 13 | 5 ± 0.3 | | |

* Palmitic acid-1-$C^{14}$ and stearic acid-1-$C^{14}$ were catalytically hydrogenated to remove any labeled unsaturated fatty acids and were then methylated. The methyl palmitate-1-$C^{14}$ and methyl stearate-1-$C^{14}$ were added to a mixture of methyl esters of fatty acids from adipose tissue. Samples for counting were obtained at intervals of 1 min or less, and no radioactivity was detected between peaks. The incomplete separation of palmitoleic and palmitic acids by gas-liquid chromatography is reflected in the recovery of about 4% of the total counts in palmitoleic acid. No correction was made for this "smearing" since it varied between less than 3% to a maximum of 7%.

† cpm = observed cpm − observed background (18 cpm).

‡ Fatty acids are designated by carbon chain length (e.g., 16:0, palmitic acid) and number of unsaturated bonds (e.g., 16:1, palmitoleic acid).

TABLE 2. THE FATTY ACID COMPOSITION OF THE EPIDIDYMAL FAT PAD FROM RATS OF DIFFERENT AGES*

| | No. of Animals | Fatty Acids† 10:0 | 12:0 | 14:0 | 15:0 | 16:0 | 16:1 | 17:0 | 17:1 | 18:0 | 18:1 | 18:2 | 18:3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| "Suckling", 20 days, 50 g | 3 | 4.5 | 7.5 | 9.0 | 1.0 | 21.6 | 3.7 | 1.1 | 0.7 | 3.6 | 25.9 | 19.1 | 1.5 |
| "Young", 35–48 days, 75–118 g | 7 | 1.2 | 2.4 | 3.7 | 0.6 | 23.5 | 7.1 | 0.4 | 0.5 | 4.5 | 32.0 | 21.9 | 2.0 |
| "Intermediate", 60–90 days, 150–230 g | 6 | 0.3 | 0.6 | 2.5 | 0.7 | 22.2 | 8.1 | 0.5 | 0.5 | 5.3 | 33.0 | 22.3 | 2.8 |
| "Old", 250–400 g | 6 | 0.1 | 0.1 | 1.8 | 0.6 | 21.6 | 7.3 | 0.3 | 0.5 | 4.0 | 34.0 | 27.7 | 1.9 |

* The values are expressed as the mean percentage of the total fatty acids present.

† Fatty acids designed as in Table 1.

fatty acids of adipose tissue. The results of Table 3 show that there is little radioactivity in the shorter-chain fatty acids of either young or old adipose tissue, indicating that these fatty acids are not being actively accumulated at the time studied. Of particular interest is the demonstration that the proportion of the total radioactivity in the monounsaturated acids, palmitoleic and oleic, is significantly greater in the young adipose tissue than in the old, whereas the proportion of total radioactivity incorporated into palmitic acid is significantly greater in the old than the young.

In order to remove the variable of the dietary fat intake and thus to obtain a clearer picture of *de novo* fatty acid synthesis, the animals were placed on a synthetic fat-free diet. Old animals that were fed the synthetic diet continued to gain weight and showed no signs of essential fatty acid deficiency. Animals that were fed the fat-free diet beginning at the time of weaning continued to gain weight (but at a slower rate than the animals on a regular diet) until they weighed approximately 200 g at which time there was no further weight increase. At no time did they lose weight or show any of the clinical signs of essential fatty acid deficiency. When alpha-tocopherol was added to the diet, there was no change in the pattern of weight gain from those rats fed a fat-free diet without alpha-tocopherol. The proportion of extractable lipid from the epididymal fat of old and young animals fed a fat-free diet was not significantly different from that of rats on a regular diet. Table 4 shows the change in fatty acid composition in young and old rats at progressive time intervals on a fat-free diet. The short-chain fatty acids decreased rapidly in the young adipose tissue as did the essential fatty acids — linoleic (18:2) and linolenic (18:3). The per cent monoenes increased strikingly in both age groups when compared to their respective controls. No difference in fatty acid com-

TABLE 3. THE PERCENTAGE DISTRIBUTION OF ACETATE-1-$C^{14}$ IN THE FATTY ACIDS OF RAT EPIDIDYMAL FAT*

| | No. | μmoles Acetate Incorporation per g per hr‡ | Fatty Acids† 12:0 | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 |
|---|---|---|---|---|---|---|---|---|---|---|
| "Young", 40–120 g | 10 | 2.92 ± 2.11 | 0.7 ± 0.4 | 7.8 ± 2.7 | 52.9 ± 4.4 | 11.3 ± 3.4 | 7.6 ± 2.1 | 17.2 ± 3.3 | 0.7 ± 0.6 | 0.5 ± 0.6 |
| "Old", 250–360 g | 11 | 0.21 ± 0.13 | 0.5 ± 0.5 | 7.5 ± 3.1 | 77.1 ± 6.1 | 3.6 ± 2.3 | 6.5 ± 2.1 | 3.4 ± 2.2 | 0.5 ± 0.5 | 0.1 ± 0.1 |

* Tissue incubated for 3 hr in Krebs Ringer bicarbonate with 3% bovine albumin, 50 μmoles glucose, and 15 μmoles acetate containing 10 μc acetate-1-$C^{14}$ in a total volume of 4 ml. The mean percentage of total radioactivity is given together with the standard deviation.

† Fatty acids designated as in Table 1.

‡ The rate of incorporation of acetate was linear over a 6-hr period.

TABLE 4. THE FATTY ACID COMPOSITION OF EPIDIDYMAL FAT FROM RATS ON A FAT-FREE DIET*

| | No. | Days on Diet | Fatty Acids† 10:0 | 12:0 | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| "Young" | 3 | 0 | 4.5 | 7.5 | 9.0 | 21.6 | 3.7 | 3.6 | 25.9 | 19.1 | 1.5 |
| | 2 | 8 | 2.4 | 2.7 | 3.9 | 29.7 | 11.0 | 2.6 | 35.5 | 11.3 | 0.2 |
| | 2 | 20 | tr | tr | 2.5 | 33.9 | 15.0 | 3.0 | 41.4 | 2.5 | tr |
| | 3 | 35 | tr | tr | 2.1 | 26.1 | 14.5 | 3.7 | 50.3 | 1.7 | 0.3 |
| | 3‡ | 85 | tr | tr | 2.2 | 25.2 | 16.8 | 2.8 | 51.5 | 0.6 | tr |
| "Old" | 6 | 0 | tr | tr | 1.8 | 21.6 | 7.3 | 4.0 | 34.0 | 27.7 | 1.9 |
| | 1 | 4 | tr | tr | 1.9 | 24.7 | 5.6 | 6.0 | 34.6 | 22.9 | 2.0 |
| | 1 | 14 | tr | tr | 2.0 | 30.7 | 12.5 | 3.2 | 38.0 | 12.0 | 0.9 |
| | 2 | 25 | tr | tr | 2.0 | 32.8 | 13.9 | 3.9 | 38.2 | 8.0 | tr |
| | 2 | 40 | tr | tr | 1.8 | 28.0 | 13.9 | 2.9 | 41.3 | 9.3 | 0.8 |
| | 1 | 59 | tr | tr | 2.1 | 30.5 | 14.4 | 4.0 | 43.3 | 4.6 | 0.5 |
| | 1 | 120 | tr | tr | 2.0 | 23.3 | 13.8 | 2.3 | 54.7 | 2.6 | tr |

* Values expressed as the mean percentage of total fatty acids present.

† Fatty acids designated as in Table 1.

‡ Two animals were fed the fat-free diet with added alpha-tocopherol.

position was found between animals on the diet with or without alpha-tocopherol.

The rate of incorporation of acetate into the total lipids and the distribution of radioactivity among the individual fatty acids of the epididymal fat from young and old rats kept on the fat-free diet from 25 to 125 days are summarized in Table 5. The rates of incorporation of acetate into the total lipid or into the newly synthesized fatty acids in young tissue were not significantly different from the values observed in normal diet controls. In the fat from old rats on a fat-free diet, however, there was an impressive increase in the per cent of the radioactivity incorporated into the monoenes. Indeed, the pattern of incorporation of acetate-1-$C^{14}$ into the individual fatty acids of old rats on a fat-free diet approached that in the young.

The *in vitro* rate of incorporation of acetate into the mixed lipids and the distribution of labeled carbon among the individual fatty acids of epididymal adipose tissue removed from rats injected with insulin is shown in Table 6. In confirmation of previous experience (4–6), there was a significantly greater incorporation of acetate into the mixed lipids of the adipose tissue from insulin-treated than from saline-injected rats. The new finding is the significant increase in the percentage of the total radioactivity incorporated into the monounsaturated fatty acids.

## DISCUSSION

The present studies have confirmed our previous finding of a decreased rate of acetate incorporation into total lipids of adipose tissue with aging and have demonstrated further changes in lipid metabolism. In adipose tissue from young and old rats on a regular diet, the percentages of the total incorporated radioactivity found in palmitoleic and oleic acids were 3 to 5 times greater in young than in old. The percentage of the

TABLE 5. THE PERCENTAGE DISTRIBUTION OF ACETATE-1-$C^{14}$ IN THE FATTY ACIDS OF RAT EPIDIDYMAL FAT FROM ANIMALS ON A FAT-FREE DIET*

| | | | Fatty Acids† | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. | μmole Acetate Incorporated per g per hr | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 |
| "Young" | 2 | 2.49 | 10.6 | 45.0 | 12.6 | 5.5 | 25.1 | 0.2 | 0.1 |
| "Old" | 5 | 1.00 ± 0.81 | 5.9 ± 3.2 | 53.6 ± 3.6 | 11.2 ± 2.3 | 6.8 ± 2.9 | 17.9 ± 7.2 | 0.9 ± 0.5 | 0.6 ± 0.6 |

* The mean percentage of total radioactivity is given together with the standard deviation.
† Incubation conditions and fatty acid designations as in Table 1.

total radioactivity incorporated into palmitic acid was significantly less in the young than in old tissue. Evidence of Bloomfield and Bloch in cell-free systems has demonstrated that palmitoleic acid can be formed by desaturation of palmitic acid, and oleic acid by desaturation of stearic acid (7). Of particular interest has been the finding of Bloomfield and Bloch (8) in a yeast preparation that the desaturation of palmitic acid is an oxidative reaction requiring oxygen and TPNH. The greater *de novo* monoene synthesis observed in young rat adipose tissue may be correlated with more active metabolism of glucose, via both the hexose monophosphate shunt and glycolytic pathways, in young epididymal fat compared to old (1). Since it has been shown by Milstein (9) and Winegrad (10) that, in adipose tissue, insulin increases glucose utilization via both pathways, it was supposed that insulin administration might also increase the incorporation of acetate-1-$C^{14}$ into the monounsaturated fatty acids. The results presented have indeed demonstrated that the pattern of incorporation of acetate-1-$C^{14}$ into the individual fatty acids of adipose tissue from an old animal can be modified by insulin administration to resemble that of young adipose tissue. The results are consistent with the hypothesis that monoene synthesis in the intact mammalian fat cell is in part dependent upon TPNH. This hypothesis is still speculative and not proved by the present work. Indeed, the observed increase in monoene synthesis may be due to other indirect effects of increased rates of glucose utilization or to the effects of the increased activity of only one of the pathways of glucose metabolism.

Despite the lesser relative synthesis of monounsaturated fatty acids in old adipose tissue as compared to young, the fatty acid composition is not strikingly different between these tissues when the rats are fed a regular diet. This implies that differential fatty acid oxidation, differences in rates of release or entry of fatty acids as well as the dietary fats, may be important in maintaining the characteristic fatty acid pattern. The findings that the over-all rate of acetate incorporation can be increased in old rats on a fat-free diet with a very significant rise in the proportion of total radioactivity incorporated into palmitoleic and oleic acids indicate that the pathway for monoene synthesis can be stimulated. Whether or not the capacity to synthesize monounsaturated fatty acids is maintained with more advanced age, or in diabetes, or when cholesterol is added to the fat-free diet, remains to be determined. At the moment, the lesser *de novo* synthesis of monoenes in old animals compared to young can only be considered another biochemical phenomenon of the aging process. This may assume patho-physiological significance if cells in the tissues that undergo degenerative change in old age, such as arteries, are similar to the fat cell of adipose tissue.

The determination of the distribution of radioactivity among individual fatty acids after incubation of adi-

TABLE 6. THE EFFECT OF INSULIN ON THE INCORPORATION OF $C^{14}$ INTO FATTY ACIDS OF RAT EPIDIDYMAL ADIPOSE TISSUE INCUBATED WITH ACETATE-1-$C^{14}$*

| | | | Fatty Acids† | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | μmoles Acetate Incorporated per g per hr | No. | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 |
| Control | 0.33 ± 0.18 | 7 | 7.3 ± 0.9 | 79.0 ± 1.5 | 3.2 ± 0.3 | 6.3 ± 0.9 | 2.9 ± 0.2 | tr | tr |
| Insulin | 1.06 ± 0.28 | 4 | 4.3 ± 0.4 | 68.2 ± 2.5 | 7.9 ± 0.5 | 7.5 ± 0.5 | 11.4 ± 1.3 | tr | tr |

* The distribution of $C^{14}$ in the individual fatty acids expressed as per cent of the $C^{14}$ incorporated into the total fatty acids. Tr indicates "trace" (less than 0.5% of the total radioactivity measured).
† Incubation conditions and fatty acid designations as in Table 1.

pose tissue *in vitro* with acetate-1-$C^{14}$ has clarified the question of the significance of the higher proportion of shorter-chain fatty acids in the fat from young animals as compared to old. The trace incorporation of acetate into the short-chain fatty acids indicates that they were not being actively synthesized from this precursor at the age studied. This was further corroborated by the demonstration that the concentrations of these fatty acids rapidly decreased when young rats were placed on a fat-free diet. It has previously been shown that rat milk contains a high proportion of these shorter-chain fatty acids (1), and the present evidence indicates that these are stored and persist for some time after weaning. Unlike the lactating mammary gland, the capacity of adipose tissue to synthesize short-chain fatty acids as a final product does not appear to be present in either young or old rats.

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