Synthesis of Fatty Acids in Animal Tissues

II. THE OCCURRENCE AND BIOSYNTHESIS OF cis-VACCENIC ACID*

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The occurrence of cis-vaccenic acid (cis-11-octadecenoic acid) in animal tissues was first reported by Morton and Todd (1), who were able to isolate this acid from horse brain lipids. The extent to which this acid was present in brain was estimated to be about 0.025% wet weight and was thought to be a minor constituent of horse brain fatty acids. The trans isomer, however, was isolated earlier by Bertram (2) from margarine and butterfats and by Nath, Barki, Elvehjem, and Hart (3) from sheep and beef lipids. The origin of the trans-vaccenic acid was obscure but was thought to be derived from oleic acid by a process of cis-trans isomerization in addition to a selective shift of the 9,10-double bond to the 11,12-position.

A more thorough study of the occurrence of cis-vaccenic acid in nature was reported by Hofmann, Lucas, and Sax (4–6) and Hofmann and Tausig (7), who isolated this acid from various microorganisms and characterized it as cis-vaccenic acid by its melting point, oxidative cleavage to C_{11}-dicarboxylic acid, and by its conversion to various other derivatives. These workers also found that cis-vaccenic acid is a major constituent of the free fatty acids of these organisms and in some cases is the only C_{18}-unsaturated fatty acid present (5). Since intestinal bacteria were found to be a rich source of cis-vaccenic acid, Hofmann and Tausig (5) pointed to them as a likely source of the trans-vaccenic acid in the lipids of higher animals. These workers suggested that the cis isomer synthesized by these organisms may undergo cis-trans isomerization during its absorption or transport to the tissues.

Recently, Harlan and Wakil (8, 9) studied the synthesis of long chain fatty acids in subcellular particles derived from livers of various animals and found that C_{18}-acyl CoA was incorporated into various saturated and unsaturated fatty acids. Addition of avidin to a soluble extract of this system resulted in the inhibition of the de novo process of fatty acid synthesis (yielding C_{16} and C_{18} acids) but not the elongation pathway of fatty acid synthesis (9, 10). In our attempt to identify the various unsaturated fatty acids that were synthesized by this system, we were surprised to discover that cis-vaccenic acid constituted a relatively large proportion of the C_{18} acids present in these preparations. A closer examination of the occurrence of cis-vaccenic acid in animal tissues showed that this acid is a normal constituent of the fatty acids of these tissues and sometimes it existed in amounts equal to oleic acid. The cis-vaccenic acid was isolated by a combination of thin layer and gas chromatography and was identified by its oxidation to n-heptanoic acid and 1,11-undecanedicarboxylic acids. The present communication deals with the occurrence of cis-vaccenic acid and its wide distribution in animal tissues. Evidence from studies both in vivo and in vitro will be presented to show that this acid is synthesized by the elongation of cis-palmitoleoyl coenzyme A by acetyl coenzyme A, a process similar to the elongation of fatty acids (10).

EXPERIMENTAL PROCEDURE

Preparation of Rat Liver Mitochondria—Liver tissue was obtained from male albino rats weighing 160 to 200 g. The tissue was homogenized in cold 0.25 M sucrose in a Potter-Elvehjem type homogenizer with a Teflon pestle. After removal of cellular debris at 900 × g for 10 minutes, the supernatant was centrifuged at 10,000 × g for another 10 minutes. The residue obtained was composed of three distinct layers, an upper pink fluffy layer, a middle tan layer, and a bottom dark tan layer. Of these, all but the middle tan layer were discarded. This layer was suspended in cold 0.25 M sucrose and centrifuged at 5,000 × g for 10 minutes. The middle tan layer (mitochondria) which comprised most of the residue was isolated, resuspended in 0.25 M sucrose, and used for the incubations. The supernatant fluid from the 10,000 × g centrifugation was first centrifuged at 20,000 × g for 10 minutes, and the residual particles were discarded. The resulting supernatant solution was further centrifuged at 104,000 × g for 45 minutes and the particulate fraction obtained (microsomes) was suspended in 0.25 M sucrose and used for the incubations. Protein in all fractions was determined by the biuret method (11).

Preparation of Substrates—Acetyl-CoA was prepared chemically by the thiophenol method (12) and C_labeled acetyl-CoA was prepared enzymatically by using the acetate-activating enzyme (13). Fatty acyl-CoA derivatives were prepared enzymatically by the method of Kornberg and Pricer (14). The cofactors, ATP, TPNH, and DPNH, were obtained from commercial sources.

Synthesis in vitro of cis-Vaccenic Acid—The reaction mixture contained 30 μmoles of potassium phosphate buffer, pH 6.5, 1 μmole of TPNH, 1 μmole of DPNH, 100 μmoles of potassium palmitoleate, 4 μmoles of ATP, 95 μmoles of 1-C-acetyl-CoA (20,000 c.p.m.), 1 mg of mitochondrial protein and water to a final volume of 0.5 ml. A similar reaction mixture was also prepared with 80 μmoles of palmitoleoyl-CoA instead of palmitoleic acid and ATP. The incubation was performed at 38°C for 2 hours with constant shaking.
for 30 minutes in a glass-stoppered tube, flushed with nitrogen. The reaction was stopped by the addition of 0.2 ml of 10% ethanolic potassium hydroxide, and the mixture was heated in a boiling water bath for 20 minutes. The mixture was acidified, and the fatty acids were extracted with pentane. After removal of the pentane, the fatty acids were methylated with diazomethane.

Resolution of Fatty Acid Mixture The methyl esters of the fatty acids were separated into cis-monoenoic and polyenoic acids by thin layer chromatography on silica gel H impregnated with silver nitrate according to the procedure of Morris (15). The cis-monoensaturated fatty acids were recovered from the plate by removing the area corresponding to the standard cis-monoensaturated region (methyl oleate) and extracting with freshly distilled diethyl ether. After removal of the ether under a stream of nitrogen, the esters of the cis-monoensaturated fatty acids were further resolved into their individual components by gas-liquid chromatography. A Barber-Colman model 10 gas chromatograph with a packed radium ionization detector was used. Eight-foot columns were packed with 15% ethylene glycol succinate on Chromsorb W, 60 to 80 mesh, and the column was operated at 170-190° with argon gas flow rates of 100 to 125 ml per minute. The radioactive effluent gas was trapped in glass capsules 5 cm long containing Pyrex wool. A Packard gas chromatograph fraction collector was used to facilitate these collections. The capsules were placed in scintillation vials, 20 ml of scintillation mixture were injected through the capsule, and the vials were counted. Between 75 and 80% of radioactivity injected into the column was recovered with this method of collection.

For further analysis of the fatty acids, the individual ester fractions were collected from the column as described previously followed by washing the Pyrex wool with freshly distilled chloroform. The chloroform extracts of the methyl esters of the fatty acids were then analyzed for the presence of various monounsaturated fatty acids by first hydrogenating the unsaturated fatty acid esters to the corresponding saturated methyl esters. The chloroform extracts of the methyl esters of the fatty acids were then analyzed for the presence of various monounsaturated fatty acids by first hydrogenating the unsaturated methyl esters to yield a monocarboxylic and a dicarboxylic acid. When isolation of the monocarboxylic acid was desired, the following modification of the procedure was made. After excess oxidant had been destroyed by sodium bisulfite, the solution was made alkaline with 1 N NaOH and was evaporated to dryness in a stream of nitrogen. The residue was dissolved in sufficient 1 N HCl to make it acidic, and the solution was extracted three times with equal volumes of ether. For facilitation of the withdrawal of the very small volumes of ether (usually 0.1 ml), the aqueous layer was frozen by placing the flask in a Dry-Ice-acetone bath, and the ether layer was removed and dried by passing it through a short column of sodium chloride. The ether extracts were then cooled to -15° in a brine bath, and the ether was removed under a slow stream of nitrogen. The residue was methylated with diazomethane as usual, and the methyl esters were resolved and identified by gas-liquid chromatography (the column temperature being 100°) as indicated above.

RESULTS

Occurrence of cis-Vaccenic Acid—Samples of liver mitochondria (5 mg) obtained from several rats were sonoponified individually with ethanolic KOH, and the fatty acids were extracted with pentane after acidification of the reaction mixture. The pentane was removed in a stream of nitrogen, and the fatty acids were methylated with diazomethane. The methyl esters were then resolved by thin layer chromatography (as described in "Experimental Procedure") into the esters of saturated, mono-unsaturated, and polyunsaturated fatty acids. The monounsaturated methyl esters were extracted from the plate and subjected to gas-liquid chromatography. The latter analysis indicated the presence of large amounts of the methyl ester of C18:1 together with small amounts of unidentified compounds as shown in Fig. 1. When the C18:1 fraction was collected as described in "Experimental Procedure" and rechromatographed, a single peak was obtained in the same position as methyl oleate as shown in Fig. 2, indicating that the C18:1 fraction collected by the procedure used represented the same fraction in the original sample with very little or no contamination of the other fractions. Catalytic hydrogenation (17) of the C18:1 fraction yielded stearic acid only as was shown by rechromatography of the reduction products (cf. Fig. 2).

Oxidation of the C18:1 fraction with periodate-permanganate according to the procedure of Bloch (16) and with the precautionary measures described in "Experimental Procedure" yielded on gas-liquid chromatography the methyl esters of monocarboxylic acids (heptanoic and nonanoic acids) as well as of the dicarboxylic acids (azelaic and undecanedioic acids) as shown in Fig. 3, a and b. These results indicated that the C18:1 fraction is not homogeneous as has been assumed before but a mixture of two C18:1 isomers, namely oleic acid (C18:1 Δ9) and vaccenic acid (C18:1 Δ11,12). Thus, oleic acid would yield nonanoic and azelaic acids, whereas vaccenic acid would yield heptanoic and undecanedioic acids. Indeed, a known sample of methyl oleate on oxidation with periodate-permanganate in a similar manner to that of the C18:1 fraction yielded a C18 dicarboxylic ester, indicating that there was no isomerization of the double bond during the oxidation of the monounsaturated acids. The lack of isomerization during the oxidation of the oleate enabled us to estimate its concentration in the C18:1 frac-
tion by measuring the amounts of the C19 and C11-dicarboxylic esters produced as reflected by their corresponding areas on the chromatogram tracing. This type of measurement reflected the approximate concentrations of the two acids in the original tissues. Thus, analyses of 10 samples of rat liver mitochondria indicated the presence of cis-vaccenic acid in concentrations of 20 to 50% (average about 35%) of the C18:1 fatty acid present, the remainder being oleic acid. Vaccenic acid was identified as the cis isomer by its behavior on thin layer chromatography in a solvent system (ether-hexane, 10:90) which widely separated the cis ($R_f$ 0.29) from the trans isomer ($R_f$ 0.47).

**Fig. 4.** Gas-liquid chromatographic analysis of the methyl esters of the dibasic acids (C19 and C11) obtained from the oxidation of the C18:1 acids that were isolated from normal and fat-deficient rat liver microsomes.

Fig. 2. Rechromatography on gas-liquid column of the methyl esters of C18:1 acids (---) and their hydrogenation products (---). The C18:1 acids were separated from the monoenic methyl esters after chromatography on gas-liquid column in a manner similar to that of Fig. 1 (note the “homogeneity” of the sample).

**Fig. 3.** Gas-liquid chromatography of the methyl esters of the periodate-permanganate oxidation products of the methyl esters of the C18:1 acids. The sample used here was prepared from rat liver mitochondria in a manner similar to that used in Fig. 2 (---). a, gas-liquid chromatographic analysis of the methyl esters of the monocarboxylic acids; b, gas-liquid chromatographic analysis of the methyl esters of the dibasic acids. The percentage of cis-vaccenic acid in this sample as measured by the relative areas of C17 and C11 acids are 39% and 46%, respectively.

**Fig. 5.** Gas-liquid chromatographic analysis of the methyl esters of the mono- and dibasic acids obtained from the periodate-permanganate oxidation of the methyl esters of the C18:1 acids that were derived from beef cardiolipins. The percentage of cis-vaccenic acid in the C18:1 acids was estimated from the relative ratios of the peaks of the C9-monobasic and C11-dibasic acids to be 70 and 80%, respectively.

Cis-Vaccenic was found not only in mitochondria but also in microsomal fatty acids of rat liver cells of both normal and fat-deficient animals as shown in Fig. 4. cis-Vaccenic acid was also obtained from two samples of purified beef lipids (lecithin and cardiolipin). The C18:1 fatty acids of lecithin contained about 25% cis-vaccenic acid, whereas the C18:1 fatty acids of cardiolipin contained about 80% cis-vaccenic acid as measured by the relative areas of both the C9 and C11 dicarboxylic esters and the C9 and C11-monocarboxylic esters (cf. Fig. 5).

**Synthesis in vivo of cis-Vaccenic Acid—**In 1940, Schoenheimer, Rittenberg, and Stetten (18, 19) studied the synthesis of oleic acid in animal tissues and showed that feeding mice with deuterium-labeled stearic acid yielded deuterium-labeled oleic acid, presumably by desaturation of the precursor, stearic acid. This conclusion was upheld by the results of many workers with

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the studies both in vivo (20) and in vitro (21-23) of the synthesis of oleic acid. For the study of origin of the cis-vaccenic acid, potassium palmitate-1-14C (0.16 mc, 3.4 μmoles) was injected intraperitoneally into one set of rats and potassium stearate-1-14C (0.16 mc, 24 μmoles) was injected into another set. After 3 hours, the animals were killed, and the hearts, livers, brains, kidneys, and spleens of each set were removed, pooled together, and homogenized in chloroform-methanol according to the procedure of Bligh and Dyer (24) for removal of lipids. The lipids were saponified with potassium hydroxide, and the fatty acids were isolated in the usual manner. A small sample was methylated with diazomethane and separated into saturated, monounsaturated, and polyunsaturated fatty acids by thin layer chromatography, as described in "Experimental Procedure." The methyl esters of cis-monounsaturated fatty acids were extracted from the plate with ether and were further resolved by gas-liquid chromatography into the C₁₈:₁ and C₁₁:₁ fractions. As shown in Table I, the 14C-palmitic acid yielded 14C-labeled C₁₈:₁ and C₁₁:₁, whereas 14C-stearic acid yielded 14C-labeled C₁₁:₁ only. Oxidation of the C₁₁:₁ fractions with periodate-permanganate yielded both azelaic and undecanedioic acids. These acids were extracted from the reaction mixture, methylated, and resolved by gas-liquid chromatography. The 14C content of the C₈ and C₁₄-dicarboxylic esters was measured and the results show (cf. Table I) that the C₁₁ fraction derived from animals treated with injection of 14C-palmitate yielded 14C-labeled azelaic and undecanedioic acids, whereas the rats given 14C-stearate yielded 14C-labeled azelaic only. In other words, 14C-stearic acid appears to be the precursor of oleic acid whereas 14C-palmitic acid can give rise to oleic acid as well as to cis-vaccenic acid. Palmitate conversion to oleic acid was presumably via the stearic acid, a reaction known to occur in animal tissue involving the elongation of palmitate by one C₂ unit (9, 10). Since stearate did not appear to be a precursor of cis-vaccenate in this system, one possibility considered was that palmitate is first desaturated to form palmitoleate and then elongated by the addition of one C₂ unit to form vaccenate.

Synthesis in vitro of cis-Vaccenic Acid—In order to confirm the above notion that cis-vaccenic acid was derived from the elongation of palmitoleic acid, 14C-aceteyl-CoA and unlabeled palmitoleyl-CoA (or palmitoleic acid and ATP) were incubated with rat liver mitochondria in the presence of TPNH and DPNH as described in "Experimental Procedure." The 14C-labeled fatty acids were extracted from the reaction mixture and the methyl esters of the cis-monounsaturated fatty acids were isolated by thin layer chromatography. The radioactivity of the methyl esters was measured as usual. The results show that the incorporation of 1-14C-acetyl-CoA into the monounsaturated fatty acids was dependent on the presence of either palmitoleyl-CoA or palmitoleic acid and ATP (cf. Table II). Gas-liquid chromatography of the methyl esters of the monounsaturated fatty acids followed by simultaneous 14C determination of the effluent gas showed that the C₁₁:₁ fraction contained all of the radioactivity as shown in Fig. 6, together with small amounts of 14C in C₁₈:₁ and C₁₈:₂.

The C₁₁:₁ fraction from the gas-liquid chromatogram was collected as described in "Experimental Procedure" and oxidized by periodate-permanganate in the usual manner. The resultant dicarboxylic acids were extracted from the reaction mixture, methylated with diazomethane, and chromatographed on a gas-liquid column. The effluent gas was collected at regular intervals for 14C determination. The results as depicted in Fig. 6. Gas-liquid chromatographic analysis of the fatty acids synthesized by mitochondria with palmitoleic and acetyl-1-14C-CoA as substrates. The reaction mixture contained 109 μmoles of palmitoleic, 30 μmoles of potassium phosphate, 4.0 μmoles of ATP, 0.5 μmoles of acetyl-1-14C-CoA (20,000 c.p.m.), 1.0 μ mole of DPNH, 1.0 μ mole of TPNH, 1.0 mg of rat liver mitochondrial protein and water to a final volume of 0.5 ml. The mixture was incubated at 38°C for 30 minutes, and the fatty acids were isolated as described in "Experimental Procedure."
hydrolyzed with KOH, and the result, undecanedioic acid (C₁₁₂). These results suggested that palmitoleic acid or palmitoleyl-CoA were elongated by addition of 14C-acetyl-CoA to form cis-vaccenic acid fraction that chromatographed as cis-monounsaturated fatty methyl ester on thin layer and had a retention time on gas-liquid chromatography similar to that of oleic acid is indeed a mixture of two isomers. This conclusion was based on the a mixture of two isomers. Occasionally, hydrogenation to stearic acid and recently retention time on gas-liquid chromatography were quoted as further proof of the designation. All these properties are common to both oleic and cis-vaccenic acids.

In order to identify further the dimethyl undecanedioate by other methods than its retention time on the gas-liquid column, the effluent gas corresponding to the radioactive peak from the gas chromatograph was collected as usual, and the diester was eluted from the collection tube with chloroform. To the chloroform solution were added 100 mg of dimethyl undecanedioate, and the mixture was evaporated to dryness. The residue was hydrolyzed with KOH, and the resultant undecanedioic acid was separated and recrystallized several times from ether-hexane. No radioactivity was lost on recrystallization as shown in Table III.

### DISCUSSION

cis-Vaccenic acid has eluded detection in routine analysis and studies of animal tissue fatty acids because of the close similarity of physical and chemical behavior between oleic and vaccenic acids. The presence of cis-vaccenic acid in bacteria was recognized by Hofmann et al. (4-7) because of its being the major if not the only unsaturated fatty acid of those microorganisms. These authors documented the existence of this acid not only by its melting point and products of oxidation but also by the synthesis of its various derivatives.

The occurrence of cis-vaccenic acid in animal tissues was first noted by Morton and Todd (1) in 1950 as a result of their studies on the hemolytic activity of horse brain extracts. With the hemolytic activity as an assay method, these authors were able to concentrate cis-vaccenic acid in a fraction that melted at 9° and consisted of a 1:1 mixture of cis-vaccenic and oleic acids. No such mixtures are isolated from other tissues or animals.

In our investigation of the types of fatty acids formed during the incubation of 14C-acetyl-CoA with liver mitochondria, we were constantly impressed by the amount of 14C present in the C₁₈:₁ fraction. Initially (8), we referred to this fraction as oleic acid because of its close behavior on thin layer and gas-liquid chromatography to that of authentic oleic acid. The incorporation of the 14C-acetyl-CoA into the C₁₈:₁ fraction was not diluted by nonlabeled stearyl-CoA, and it did not appear to require O₂ for its formation or involve the intermediate formation of malonyl-CoA since avidin did not decrease the amount of 14C incorporated into this fraction. These observations led Harlan and Wakil to postulate a pathway in mitochondria for "oleic" acid formation from 14C-acetyl-CoA independent of malonyl-CoA that did not appear to involve stearyl-CoA as an intermediate. Moll and Mistry (25) studied fatty acid composition of mitochondria from biotin-deficient rats and found them to contain low levels of palmitic and stearic acids, but the same amount of C₁₈:₁ fatty acid (designated as oleic acid) as that present in mitochondria from normal rats. Several workers have referred to the C₁₈:₁ acid as oleic acid, although unequivocal characterization of this acid was not made. The confusion in identifying the C₁₈:₁ acid as oleate instead of cis-vaccenate lies in the criteria used in the identifications. Neutralization equivalent, boiling point, melting point, and iodine number are similar for the two isomers. Occasionally, hydrogenation to stearic acid and recently retention time on gas-liquid chromatography were quoted as further proof of the designation. All these properties are common to both oleic and cis-vaccenic acids. The results presented in this paper indicated that the fatty acid fraction that chromatographed as cis-monounsaturated fatty methyl ester on thin layer and had a retention time on gas-liquid chromatography similar to that of oleic acid is indeed a mixture of two isomers. This conclusion was based on the following observations, first that catalytic reduction of the C₁₈:₁ fraction yields stearic acid only as revealed by gas-liquid chromatography of the reduction products. Second, periodate-permanganate oxidation of the C₁₈:₁ fraction yields a mixture of C₈ and C₇ monocarboxylic acids and C₈ and C₉ dicarboxylic acids. If the C₁₈:₁ fraction contained only oleic acid, the periodate-permanganate oxidation would yield only nonanoate and azelate, especially since the periodate-permanganate procedure did not cause the isomerization of an authentic sample of oleate. Oxidation of vaccenic acid by the same procedure yields only the C₇ monocarboxylic and C₈ dicarboxylic acids, indicating again that no isomerization of the double bond of the octadecenoic acid occurs during the course of the periodate-permanganate oxidation. These results, therefore, suggest that the C₁₈:₁ fraction is...
a mixture of two isomers, the \( \Delta^{9,10} \) octadecenoic acid (oleic acid) and the \( \Delta^{11,12} \) octadecenoic acid (cis-vaccenic acid).

If one assumes that the two isomers in the C18:1 fraction are oxidized by the periodate-permanganate to the same extent and that the amounts of the C16 and C18-dicarboxylic acids obtained are reflected by their respective areas in the tracing of the gas-liquid chromatogram, the relative amounts of the two isomers, oleic and cis-vaccenic, in the C18:1 fraction can be estimated. The amount of cis-vaccenic acid present in the C18:1 fraction was thus estimated from 10 different determinations to be about 20 to 50%. This value may also reflect the relative amounts of this acid in the original rat liver.

The distribution of the cis-vaccenic acid within the various lipid fractions remains to be determined. In two instances at least, the cis-vaccenic acid content appeared to vary. In a sample of beef lecithin, the cis-vaccenic content was estimated to be about 93% of the total C18:1 fatty acid. Analysis of a sample of beef cardiolipin by the same method indicated a value of 80% of the C18:1 as cis-vaccenic. These results indicate that the content of cis-vaccenate may vary in different classes of lipids. It is too early to attach any physiological significance to this variance in fatty acid and more analytical work is necessary on the distribution of this acid in various lipid fractions.

In view of these findings, it would appear that much of the literature values on "oleic" acid content of various animal lipids is in doubt if cis-vaccenic acid proves to be as widespread as these few experiments would seem to indicate.

Further evidence that supported the occurrence of cis-vaccenic acid were derived from studies of its biosynthesis. When rats were injected with stearic-\( ^{14} \)C acid and the fatty acids of the man organs were isolated and analyzed for \( ^{14} \)C content, it was found that oleic acid was the only C16:1 acid labeled. On the other hand, rats treated by injection with palmitic-\( ^{14} \)C acid yielded a C18:1 fraction that contained \( ^{14} \)C in both oleic and cis-vaccenate acids. The introduction of the double bond in cis-vaccenic acid must, therefore, occur in the C16 acid and not in the C18 acid. The monounsaturated C16 acid was then elongated to the C18:1 cis-vaccenic acid presumably via the elongation system of mitochondria (10, 26). This conclusion appears to be compatible with the available evidence and would account for the position of the double bonds between carbon atoms 11 and 12 of the molecule. Furthermore, the results of the synthesis in vitro of cis-vaccenic acid from palmitoleoyl-CoA and \( ^{14} \)C-acetyl-CoA, in the presence of mitochondria, TPNH and TPNH supported this conclusion and provided direct evidence for the presence of this acid. Palmitoyl-CoA can be elongated by the mitochondrial system to yield stearyl-CoA as shown by Wakil and his collaborators (8, 10, 26). Stearoyl-CoA would then be desaturated to oleoyl-CoA by a microsomal system involving TPNH and \( \text{O}_2 \) (21, 23). The relationship, therefore, between palmitoyl, stearyl and the monounsaturated acyl-CoA derivatives can be depicted in the following diagram.

The existence of these two routes for the synthesis of the two C18:1 acids and perhaps operating with different efficiencies depending on various unknown physiological conditions would serve to explain some anomalous results involving the metabolism of \( ^{14} \)C-palmitic acid in animal tissues. Thus the observation made by Dittmer and Hanshan (27) that palmitic acid was converted to "oleic" acid without the intervention of stearoyl could be explained if the radioactive "oleic" acid isolated by these workers was in fact cis-vaccenic acid. This was suggested by the authors but was not investigated further.

**SUMMARY**

Evidence is presented to indicate that cis-vaccenic acid is a normal constituent of animal tissue fatty acids. This conclusion is based on the isolation of the C18:1 fatty acid fraction from rat liver cells and their subcellular fractions by a combination of thin layer and gas-liquid chromatography. Catalytic hydrogenation of the C18:1 fraction yields stearic acid. Periodate-permanganate oxidation of the C18:1 fraction yields C16 and C17-monocarboxylic acids and C18 and C19-dicarboxylic acids indicating that the C18:1 fraction is a mixture of both oleic and cis-vaccenic acids. The cis-vaccenic acid content appears to range between 20 and 50% of the total C18:1 acids. cis-Vaccenic acid can be derived from palmitic acid but not from stearic acid as evidenced by the incorporation of \( ^{14} \)C-palmitic acid, but not \( ^{14} \)C-stearic acid into the cis-vaccenic acid. Furthermore, incubation of palmitoleic acid plus ATP or palmitoleyl-CoA with mitochondria, \( ^{14} \)C-acetyl-CoA, TPNH and DPNH yields cis-vaccenic acid as the only C18:1-labeled acid, indicating that cis-vaccenic acid is derived by the elongation of palmitoleic acid.

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