Formation of Methyl Sterols in Brain Cholesterol Biosynthesis

STEROL FORMATION IN VITRO AND IN VIVO IN ADULT RAT BRAIN*

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SUMMARY

A detailed study was made of methyl sterols involved in cholesterol biosynthesis in adult brain following intracerebral injection of [2-14C]mevalonic acid in adult brain cell-free preparations. High specific activity lanosterol, 4,4-dimethyl-5α-cholesta-8(9)-24-dien-3β-ol, 4α-methyl-5α-cholesta-7,24-dien-3β-ol, and 5α-cholesta-7,24-dien-3β-ol were formed 1 hour after intracerebral injection of the 14C-labeled precursor. Labeled to a lesser extent were 4,14α-trimethyl-5α-cholesta-7-en-3β-ol, 4α-methyl-5α-cholesta-7-en-3β-ol, desmosterol, and cholesterol. Detected by mass only was 4α,14α-dimethyl-5α-cholesta-7-en-3β-ol. Incubation of adult rat brain cell-free preparations with [2-14C]mevalonic acid yielded two highly labeled methyl sterols. Based on gas chromatographic retention data and gas chromatography-mass spectrometry, these methyl sterols have tentatively been identified as 4,4-dimethyl-5α-cholesta-14(15),24-dien-3β-ol and 4α-methyl-5α-cholesta-14(15),24-dien-3β-ol. The results indicate that the primary pathway of sterol biosynthesis in adult brain is through diunsaturated sterols. The sterols formed in vitro suggest a partial disruption or alteration of the biosynthetic pathway as a result of maceration of the tissue.

Recent studies on cholesterol (cholesta-5-en-3β-ol) biosynthesis have dealt extensively with the removal of methyl groups from positions C-4 (1-3) and C-14 (4, 5). The effect of the position of an internal double bond (or bonds) in ring C or D, on cholesterol biosynthesis has also been examined (6, 7). The bulk of these studies have aided in the elucidation of the mechanisms involved in the formation of cholesterol by liver in vitro. Examination of the pathway of cholesterol biosynthesis in other tissues, including brain, is of great importance. It is also important to determine if the mode of cholesterol formation differs from liver, since this may give insight into control points of sterol synthesis in different tissues, including brain.

Several investigators have identified C7 cholesterol precursors in brain (17, 18). Our studies have also indicated the in vivo formation of methyl sterols (20). We wish now to elaborate more fully the sterols formed in vitro and in vivo from adult rat brain in the presence of [2-14C]mevalonic acid.

EXPERIMENTAL PROCEDURE

Materials and Methods—Cholesterol, desmosterol (cholesta-5, 24-dien-3β-ol), dihydrolanosterol (4,4,14α-trimethyl-5α-cholesta-8(9)-en-3β-ol), and lanosterol (4,4,14α-trimethyl-5α-cholesta-8(9),24-dien-3β-ol) were purchased from Sigma Chemical Company, St. Louis, Missouri. Lathosterol (5α-cholesta-7-en-3β-ol) was the gift of Dr. F. D'Hollander. Dr. H. Kircher donated 4α-methyl-5α-cholesta-7-en-3β-ol. Dr. R. Rahaman supplied 4α-methyl-5α-cholesta-7-en-3β-ol as well as 4α-methyl-5α-cholesta-8-en-3β-ol. Dr. J. L. Gaylor kindly donated 4,4-dimethyl-5α-cholesta-7-en-3β-ol and 4,4,14α-trimethyl-5α-cholesta-7-en-3β-ol. 5α-Cholest-8(9)-en-3β-ol, 5α-cholesta-8(14)-en-3β-ol, and 5α-cholesta-8,14-dien-3β-ol were kindly supplied by Professor R. Paoletti.

Gas-liquid chromatography was carried out on a Barber-Colman gas chromatograph model 5000 and a Varian gas chromatograph model 1740-10. Carrier gas on the former was argon at 60 ml per min, on the latter, nitrogen at 55 ml per min. Both instruments were equipped with hydrogen flame detectors. The Barber-Colman gas chromatograph was used with a radioactive monitoring system, model 5190. The above system was equipped with a stream splitter which allowed 10 parts of the effluent to flow to the proportional counter and one part to the mass detector. Before entering the counter, the carrier gas was diluted 10% with quench gas (propane) at a flow rate of 6 ml per min. The mass of the sample and its radioactivity were recorded simultaneously on separate recorders. The columns, 6 feet by 4 mm, were packed either with 3% OV-17, 3% SE-30, or 3% SE-
elute one fraction. Temperature and pressure. Approximately 1 hour was required to fractions) were eluted with gradually increasing percentages of column (100 x 5 cm, inner diameter) as described by Weiss, Galli, and Paolletti (19). The acetylated sterol (6.71 g) was applied to the column (1200 g) in petroleum ether. The sterols (in 250-ml fraction was then acetylated and fractionated on a Silica Gel-Celite-AgNO₃ column.

TABLE I

| Experiment                  | [α]-[2-14C]Mevalonic acid used | Number of animals utilized | Crude neutral fraction (%) | Isopropanol hydrocarbon | Sterol |
|-----------------------------|--------------------------------|---------------------------|---------------------------|-------------------------|--------|
| Intra-cerebral injection    | mcCi                           | 222                       | 10,100                    | 21.7                    | 4.1    | 17.3 |
| Incubation of cell-free preparation | 50                         | 20                        | 900                       | 14.0                    | 2.1    | 11.8 |

Intracerebral injections were performed as described by Nicholas and Thomas (22). Each animal was injected with 1.55 mcCi of [α-2-14C]mevalonic acid dibenzylethylendiamine salt (4.60 mcCi per mmole) in an aqueous solution of 0.1 ml volume. The animals were sacrificed after 1 hour. Brains were removed, freed of meninges, and washed thoroughly in distilled water. It was anticipated that by sacrificing at 1 hour not only cholesterol but its precursors as well would be sufficiently labeled for identification.

To the brains and in vitro preparations was added 30 ml of ethanolic potassium hydroxide (15%); the mixtures were then warmed briefly on a steam bath to digest the tissue. The alkaline mixture was extracted five times with triple volumes of petroleum ether. The petroleum ether extract will be referred to in the text as the neutral lipid fraction.

The neutral lipid from the injected animals was placed on an alumina column (450 g), which was eluted first with petroleum ether (1500 ml) to remove isoprenoid hydrocarbons and then stripped with ether (200 ml) to remove steroids. The sterol fraction was then acetylated and fractionated on a Silica Gel-Celite-AgNO₃ column.

After preliminary examination of the individual column fractions (250 ml each) on gas-liquid chromatography (3% SE-32) the fractions were pooled and saponified by refluxing 1 hour in the presence of ethanolic potassium hydroxide (15%). The sterols were then extracted as before. These fractions were then chromatographed on thin layer into 4,4-dimethyl (Rₚ 0.38), 4α-methyl (Rₚ 0.51) and 4 desmethyl sterol (Rₚ 0.48) regions with the solvent system trimethyl pentane-ethyl acetate-acetic acid (60:30:0.6).

The neutral lipid fraction from the in vitro experiments was fractionated on an alumina column (10 g) as described for the in vitro studies and then further fractionated directly on thin layer into 4,4-dimethyl (Rₚ 0.65), 4α-methyl (Rₚ 0.57), and 4-desmethyl sterol regions (Rₚ 0.49) with the solvent system described by Rahman et al. (2). The solvent system involves the addition of 1 volume of ethyl acetate-hexane (1:3) to 4 volumes of ether-benzene (12:88).

The above sterol regions were examined by means of gas-liquid chromatography, radioactive monitored gas-liquid chromatography, and gas-liquid chromatography-mass spectroscopy. Amounts of sterol present were quantitated from the gas-liquid chromatograms by triangulation.

RESULTS

Quantitative Aspects—The incorporation of mevalonic acid into brain neutral lipid fractions is presented in Table I. Assuming one utilisable enantiomer, mevalonic acid was incorporated into this fraction of the cell-free incubation to a level of 25.2%. The intracerebraally injected brains incorporated 6.5% of the available labeled mevalonic acid. In this type of experiment leakage of the [2-14C]mevalonic acid from the site of injection resulted in a much lower level of incorporation than if all the precursor had remained within the brain. In both instances fractionation by alumina column chromatography indicated that the majority of the label was in the sterol fraction (Table I).

The isolated sterol fraction from the intracerebrally injected brains was chromatographed on a Silica Gel G-Celite-AgNO₃ col-
The isolated sterol fraction from the in vitro incubation was further fractionated on thin layer chromatography. The thin layer distribution and percentage of composition was as follows: 4,4-dimethyl sterol, $4.53 \times 10^4$ dpm (71.5%); 4α-methyl sterol, $7.88 \times 10^4$ dpm (12.4%); and 4-desmethyl sterol, $1.02 \times 10^4$ dpm (16.1%).

**Identification of Diunsaturated Sterols Formed Following Intracerebral Injection of Mevalonic Acid**—After column chromatography (Silica Gel G-Celite-AgNO₃) of the acetylated sterols from the intracerebrally injected brains, the sterol fractions wereaponified. The nonapoainonable fraction was subjected to thin layer chromatography. The fractions recovered from thin layer chromatography were analyzed on 3% OV-17 by gas chromatography-mass spectrometry. The relative retention times of the reference compounds and the various unknown compounds found is indicated in Table II.

The compound representing Fractions 94 through 100 (tR = 4.00; mass and radioactive monitor peak) yielded a mass spectrum identical with that of authentic lanosterol (Table III). The presence of a reduced peak at M - 2 and at 2 units less than other major fragments in the high mass range is indicative of a Δ4 double bond (24). The relative retention time also indicates the presence of a Δ4 rather than a Δ7 double bond. The sterol unknown must therefore be 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol.

The 4α-methyl sterol (tR 3.73; mass and radioactive monitor peak) gave a mass spectrum with a molecular ion at m/e 412 (Table III). The prominence of m/e 69 and 325 (M - 69) supports the presence of a Δ4 double bond (23). The relatively great loss of two hydrogens from the molecular ion has already been shown to be characteristic of a Δ4 double bond (24). The relative retention time also indicates the presence of a Δ7 rather than a Δ8 double bond. The sterol unknown must therefore be 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol.

The 4α-methyl sterol (tR 3.73; mass and radioactive monitor peak) gave a mass spectrum (Table III) with the molecular ion at m/e 698. Again the appearance of intense peaks at 69 and 325 (M - 69 + 18) has been shown to be indicative of a Δ4 double bond (23). The relatively great loss of two hydrogens from the molecular ion has already been shown to be characteristic of a Δ4 double bond (24). The relative retention time also indicates the presence of a Δ7 rather than a Δ8 double bond. The sterol unknown must therefore be 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol.

Fractions 113 through 115 contained a minor amount of the 4α-methyl sterol just described and a 4-desmethyl sterol (tR = 3.34; mass and radioactive monitor peak). The molecular ion was m/e 384 (Table III). The peaks at m/e 69 and 300 indicate the presence of a Δ7 double bond. The lack of 2 hydrogens being lost from the higher mass fragments and the relative retention time indicates a Δ7 double bond. From these data the structure of the unknown sterol should be 5α-cholesta-7,24-dien-3β-ol.

Fractions 116 through 119 contained desmosterol and a portion of the 5α-cholesta-7,24-dien-3β-ol identified in the previous fractions. The mass spectra of the isolated desmosterol (Table III) was identical with a desmosterol standard.

The total activity of each sterol identified and its mass is shown in Table IV.
TABLE III
Mass spectra of diunsaturated sterols isolated from intracerebrally injected rat brain

| Sterol | \( \Delta_7^{(9)} \) | \( \Delta_7^{(10)} \) | \( \Delta_7^{(14)} \) | \( \Delta_7^{(15)} \) | \( \Delta_7^{(17)} \) |
|--------|----------------|----------------|----------------|----------------|----------------|
| m/e    | Relative intensity | m/e | Relative intensity | m/e | Relative intensity | m/e | Relative intensity | m/e | Relative intensity |
| Molecular ion | 426 | 22 | 412 | 52 | 398 | 100 | 384 | 28 | 384 | 24 |
| M - 2H | 424 | 10 | 410 | 16 | 398 | 31 | 380 | 23 | 380 | 37 |
| M - 15 | 411 | 100 | 397 | 28 | 385 | 14 | 351 | 11 | 351 | 20 |
| M - (15 + 2H) | 409 | 4 | | | | | | |
| M - 18 | 393 | 16 | 379 | 17 | 365 | 4 | 329 | 19 | 300 | 16 |
| M - 33 | 393 | 16 | 379 | 17 | 365 | 14 | 351 | 11 | 351 | 20 |
| M - 69 | 393 | 16 | 379 | 17 | 365 | 14 | 351 | 11 | 351 | 20 |
| (m/e 428) | 339 | 12 | 325 | 16 | 311 | 6 | 285 | 19 | 285 | 19 |
| M - (m/e 414) | 329 | 19 | 314 | 6 | 300 | 16 | 300 | 16 | |
| M - (m/e 400) | 329 | 19 | 314 | 6 | 300 | 16 | 300 | 16 | |
| M - (m/e 388) | 319 | 15 | 304 | 9 | 289 | 3 | 265 | 3 | 265 |
| M - (m/e 376) | 319 | 15 | 304 | 9 | 289 | 3 | 265 | 3 | 265 |
| M - (m/e 364) | 319 | 15 | 304 | 9 | 289 | 3 | 265 | 3 | 265 |
| M - (m/e 352) | 319 | 15 | 304 | 9 | 289 | 3 | 265 | 3 | 265 |
| M - (m/e 340) | 319 | 15 | 304 | 9 | 289 | 3 | 265 | 3 | 265 |

* Fragmentation is shown by these values.

TABLE IV
Quantity of labeled diunsaturated sterol produced by intracerebral injection of \( [2-14C] \) mevalonic acid

| Compound | Activity (apm x 10^-6) | Weight (mg) |
|----------|-----------------------|-------------|
| Lanosterol | 40.80 | 7.20 |
| 4,4-Dimethyl-5α-cholesta-8,24-dien-3β-ol | 4.68 | 0.54 |
| 4α-Methyl-5α-cholesta-7,24-dien-3β-ol | 2.21 | 0.37 |
| 5α-Cholesta-7,24-dien-3β-ol | 33.50 | 6.50 |
| Desmosterol | 2.25 | 0.54 |

as possible. The mother liquor of this final crystallization yielded 128 mg of material. The mixture was further fractionated on preparative thin layer chromatography.

The 4,4-dimethyl sterol region from this thin layer chromatography gave two sterols. One of these (tR = 3.73; mass peak) has a molecular ion at m/e 428 (Table V). A metastable peak was present at m/e 378, supporting the ion transition 428 → 395. The loss of 2 hydrogens from the molecular ion does not appear; however, the loss of 2 hydrogens is apparent in the mass spectra of reference dihydrolanosterol (Table V). The magnitude of the tR and the mass spectra data establishes the structure of this sterol as 4,4,14α-trimethyl-5α-cholest-7-en-3β-ol. The second sterol of the region had a parent ion at m/e 414 (Table V).

TABLE V
Mass spectra of monounsaturated sterols isolated from intracerebrally injected rat brain

| Sterol | \( \Delta_7^{(9)} \) | \( \Delta_7^{(10)} \) | \( \Delta_7^{(14)} \) | \( \Delta_7^{(15)} \) |
|--------|----------------|----------------|----------------|----------------|
| m/e    | Relative intensity | m/e | Relative intensity | m/e | Relative intensity | m/e | Relative intensity |
| Molecular ion | 425 | 14 | 425 | 19 | 414 | 10 | 400 | 100 |
| M - 2H | 426 | 10 | 410 | 19 | 399 | 10 | 385 | 25 |
| M - 15 | 435 | 0 | 411 | 9 | | | |
| M - (15 + 2H) | 435 | 0 | 411 | 9 | | | |
| M - 33 | 395 | 18 | 385 | 11 | 381 | 21 | 377 | 12 |
| M - side chain | 395 | 18 | 385 | 11 | 381 | 21 | 377 | 12 |
| M - (se + 1H) | 297 | 6 | 297 | 11 | 293 | 3 | 289 | 7 |
| M - (se + 27) | 288 | 7 | 283 | 3 | 279 | 7 | 275 | 12 |
| M - (se + 42) | 273 | 18 | 273 | 11 | 269 | 7 | 265 | 12 |
| M - (se + 42 + 18) | 255 | 8 | 241 | 4 | 237 | 2 | 233 | 9 |

* Fragmentation is indicated by these values.
TABLE VI
Retention times and mass distribution of sterols isolated from in vitro incubations on gas-liquid chromatography on 3% OV-17 and 3% SE-30 columns

| Compound                          | Mass distribution | Relative retention time* |
|-----------------------------------|-------------------|--------------------------|
|                                  | %                 | OV-17 | SE-30 |
| 4α-Methyl-5α-cholest-7-en-3β-ol   | 1.3               | 3.15  | 2.30  |
| Lanosterol                        | 7.5               | 3.98  | 2.94  |
| Unknown 4α-methyl sterol          | 13.5              | 3.61  | 2.32  |
| Unknown 4,4-dimethyl sterol       | 77.7              | 4.43  | 2.89  |

* Relative to 5α-cholestanote. The retention time of 5α-cholestanote was 3.40 min on OV-17 and 4.80 min on SE-30.

but a very short ($t_R = 3.06$; mass peak) relative retention time for a sterol of this size. A metastable peak was found at m/e 364, giving support to the conversions $414 \rightarrow 399 \rightarrow 361$. In the mass spectral examination of free sterols present in this fraction only those sterols having a 14α-methyl group have yielded metastable ions involved in the transition of M → M - 15 → M - 33. Such a metastable ion was present here, giving support to the presence of a 14α-methyl group. An extremely intense peak at M - 15 has also been found to be present only where a 14α-methyl group is present in the sterol nucleus. The same phenomenon is found in the spectra of the unknown sterol. The lack of a molecular ion minus 2 hydrogens supports the presence of a Δ5 double bond. The extremely short retention time of both Δ5 and Δ7 compounds having this structure (25, 26) makes the relative retention time difficult to assess. We have recently isolated the

Δ4 isomer from plant material (27) and found the plant sterol had a retention time shorter than the sterol described here. The mass spectra of the plant sterol also gave a reduced peak at M - 2 and at 2 units less than other major fragments in the high mass range; these peaks were absent from the spectra of the sterol in question here. The compound can tentatively be identified as 4α,14α-dimethyl-15α-cholest-7-en-3β-ol. Why such a sterol should chromatograph with the 4,4-dimethyl sterols on thin layer chromatography is not known, but the added mobility could be due to the 14α-methyl group.

There was a total of $4.25 \times 10^5$ dpm associated with 4,4-dimethyl fraction. The fraction contained 4.35 mg of the 4,4, 14α-trimethyl-5α-cholest-7-en-3β-ol and 0.77 mg of the 4α, 14α-dimethyl-5α-cholest-7-en-3β-ol.

The 4α-methyl sterol fraction contained a single component 4α-methyl-5α-cholest-7-en-3β-ol. The mass spectrum was iden-

Fig. 2. Mass spectra of A, 4,4-dimethyl-5α-cholesta-14(15),24-dien-3β-ol and B, 4α-methyl-5α-cholesta-14(15),24-dien-3β-ol isolated from incubation of cell-free preparations of adult rat brain with [2-14C]mevalonic acid.
analyzed as an acetate (28) the unknown 4,4-dimethyl sterol was standard having the double bonds in positions 8(9) and 14(15). The tendency for the loss of 2 hydrogens tates of 5cr-cholesta-8,14-dien-3P-ol and 5αL-cholestZen-3P-ol acetylated and the spectra of the acetate compared with the ace-

The sterol would then have the following structure, 4a-methyl-5a-cholesta-14(15),24-dien-3P-01. A similar 4-desmethyl sterol has been detected previously in brain (29).

The 4α-methyl sterol isolated had a molecular ion at m/e 398 (Fig. 2). Prominent peaks were found at m/e 379(M - 15), 379(M - 33), 325(M - [69 + 18]), 299(M - sc), 283(M - [sc - 2H + 18]), and 69. The fragments at 69 and 325(M - [69 + 18]) again indicate the presence of a Δ^4 double bond. The absence of peaks at M - (sc - 2H + 18) and M - (sc - 2H + 60) suggests the presence of double bond in the Δ^4 position. The loss of 2 hydrogens from the higher mass fragments is very intense. The three largest major fragments have peaks two units less which are of greater intensity than the major peaks themselves. A similar fragmentation has already been indicated for a 4-desmethyl sterol having a Δ^4 double bond (28). Our tentative suggestion is that the structure of this unknown is 4α,4-dimethyl-5α-cholesta-14(15),24-dien-3β-ol. A similar 4-desmethyl sterol has been detected previously in brain (29).

The 4α-methyl sterol isolated had a molecular ion at 398 (Fig. 2). Major fragments were at m/e 383(M - 15), 365(M - 33), 311(M - [69 + 18]), 285(M - sc), 269(M - [sc - 2H + 18]), and 69. The arguments put forth for the 4α-methyl sterol also hold true for the 4α-methyl sterol under study here. There would appear to be present a Δ^4 double bond and a Δ^5(13) double bond. The sterol would then have the following structure, 4α-methyl-5α-cholesta-14(15),24-dien-3β-ol.

Since the 5α-cholesta-14(15)-en-3β-ol previously isolated was analyzed as an acetate (28), the unknown 4,4-dimethyl sterol was acetylated and the spectra of the acetate compared with the acetates of 5α-cholesta-8,14-dien-3β-ol and 5α-cholesta-7-en-3β-ol (lathosterol) to show the effect of the position of nuclear double bond(s) (Table VII). The tendency for the loss of 2 hydrogens is most pronounced in the case of the unknown and the diene standard having the double bonds in positions 8(9) and 14(15). A metastable peak at m/e 203 was observed in the 5α-cholesta-8,14-dien-3β-ol acetate spectra, supporting the transition 426 → 311(M - [sc + 2H]) → 251 (M - [sc + 2H + 60]).

**DISCUSSION**

The literature contains several studies on the formation of cholesterol precursors in brain. Some of these were cited earlier. Of prime interest to our present observations are two references in particular (19,20). Weiss et al. (19) studied the in vitro formation of cholesterol and its precursors in adult rat brain. Labeled mevalonic acid was administered intravenously and the animals sacrificed after 1 hour. The bulk of the label was in sterols having double bonds in positions 8(9) and 24 and having 29, 28, or 27 carbons. The monoenoic also were labeled, but to a lesser extent. Lanosterol was not shown to be present in the adult rat brain.

Another study on adult human brain did indicate the presence of minor amounts of lanosterol and dihydrolanosterol (29). Whether either of these sterols was labeled by mevalonic acid, the cholesterol precursor utilized in the work, was not established. This minor or nonexistent concentration for lanosterol is quite different from the results herein presented. One hour after the intracerebral injection of the labeled mevalonic acid, lanosterol was the dominantly labeled sterol. The possibility that the distribution of labeled compounds was affected by the mode of pre-

| Sterol                  | C^A(14)-24 Acetate | C^A-14 Acetate | C^A(13) Acetate | C^A-7 Acetate |
|------------------------|-------------------|---------------|----------------|--------------|
| Molecular ion           |                    |               |                |              |
| M - 2                  | 454               | 64            | 426            | 5            |
| M - 15                 | 452               | 80            | 424            | 14           |
| M - (15 + 2H)          | 439               | 32            | 426            | 4            |
| M - 50                 | 437               | 44            | 413            | 13           |
| M - 60                 | 337               | 50            | 368            | 5            |
| M - (60 + 15)          | 379               | 50            | 353            | 16           |
| M - (60 + 2H + 60)     | 293               | 24            | 271            | 5            |
| M - (sc - 2H + 42)     | 229               | 30            | 229            | 35           |
| M - (sc - 60)          | 213               | 51            | 213            | 59           |
| M - (sc - 60 + 42)     | 200               | 8            | 69             | 26           |
| M - (sc - 2H + 60 + 42)| 43                | 43            | 43             | 71           |

* Fragmentation is indicated by these values.

Gas chromatography-mass spectrometry yielded the following information. The unknown 4,4-dimethyl sterol had a molecular ion at m/e 412 (Fig. 2). Prominent peaks were found at m/e 379(M - 15), 379(M - 33), 325(M - [69 + 18]), 299(M - sc), 283(M - [sc - 2H + 18]), and 69. The arguments put forth for the 4α,4-dimethyl sterol also hold true for the 4α-methyl sterol under study here. There would appear to be present a Δ^4 double bond and a Δ^5(13) double bond. The sterol would then have the following structure, 4α-methyl-5α-cholesta-14(15),24-dien-3β-ol. The sterol would then have the following structure, 4α-methyl-5α-cholesta-14(15),24-dien-3β-ol.
and gas chromatography-mass spectroscopy (in our study) has shown the presence of several compounds with a Δ7 double bond. The Δ7 sterols can easily be differentiated from the Δ9 sterols because the Δ7 compounds characteristically have a longer retention time on gas-liquid chromatography than the corresponding Δ9 compounds.

The monounsaturated sterols from the in vivo study gave quite a different picture. All of the monounsaturated methyl sterols identified had Δ9 double bonds. The only C9 sterol present was 4,4,14α-trimethyl-5α-cholest-7-en-3β-ol. No dihydrolanosterol was detected. Since isomerization is difficult when a 14α-methyl group is present (31), it may be presumed that the Δ9 compound is a minor product of squalene oxide cyclization. Also present as a minor component, and the only monounsaturated sterol with 20 carbons, was 4α,14α-dimethyl-5α-cholest-7-en-3β-ol. The 4α-methyl sterol fraction contained 4α-methyl-5α-cholest-7-en-3β-ol. Part of this material, which was slightly labeled but in the main probably endogenous, could have been derived by demethylation of 4,4,14α-trimethyl-5α-cholest-7-en-3β-ol or by the Δ9 reduction of highly labeled 4α-methyl-5α-cholest-7,24-dien-3β-ol, or both.

The natural occurrence in various tissues of the sterols discussed above has been established by other researchers, with the exception of one. The one exception is the 4,4,14α-trimethyl-5α-cholest-7-en-3β-ol. The diene analogue, 4α,4,14α-trimethyl-5α-cholesta-7,24-dien-3β-ol, has been identified in skin (32, 33), as has the 4α-methyl-5α-cholest-7-en-3β-ol. The rather unusual 4α,14α-dimethyl-5α-cholesta-7-en-3β-ol has been isolated from human meconium (26). The 4α-methyl-5α-cholesta-7-en-3β-ol has been isolated from several tissues (32, 34) and shown to turn over to cholesterol (35). The sterols mentioned above have not previously been shown to be present in brain tissue. The following sterols which we have isolated have also previously been isolated from brain: lanosterol (29), 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol (19), 5α-cholesta-7,24-dien-3β-ol (15), desmosterol (36), and of course, cholesterol.

Previous work from this laboratory involving incubation of labeled mevalonic acid with cell-free preparations of adult rat brain indicated the formation of methyl sterols of unknown structure (20). Labeled lanosterol appeared to be a minor constituent of the methyl sterol fraction. We have now tentatively identified the two major labeled methyl sterols formed by incubation of adult rat brain cell-free preparations with [2-14C]mevalonic acid. The most heavily labeled methyl sterol was presumably 4,4-dimethyl-5α-cholesta-14(15),24-dien-3β-ol, with the second labeled sterol being 4α-methyl-5α-cholesta-14(15),24-dien-3β-ol. The 14(15) double bond can be assumed to be the result of removal of the 14α-methyl group, the 30-carbon precursor presumably being lanosterol. It then follows that the 4α-methyl sterol identified arises as a result of demethylation of 4,4-dimethyl-5α-cholesta-14(15),24-dien-3β-ol. The 4α-methyl-5α-cholesta-14(15),24-dien-3β-ol could then be further metabolized, ultimately to cholesterol. A 4α-demethyl sterol that could plausibly be an intermediate in this sequence has been isolated from adult brain (28, 29). This sterol is 5α-cholesta-14(15)-en-3β-ol. Lutsky and Schroepfer (37) have postulated the existence of monoene intermediates between a Δ14 diene and a Δ14(15) monoene, both of which have been shown to turn over to cholesterol. The conversion of a Δ9 to a Δ7 double bond has been shown not to pass through another intermediate (37). The only strong possibility remaining then would be a Δ14(15) intermediate.
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