Neutral sterols of rat epididymis: high concentrations of dehydrocholesterols in rat caput epididymidis

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Abstract Phospholipids and sterols are known to have multiple functions in reproductive tissue of mammals. High concentrations of the cholesterol precursor desmosterol have been described in testis, epididymis, and spermatozoa of various species. These findings and the recent discovery of some cholesterol precursors as meiosis-activating sterols suggest important functions of cholesterol precursors in fertility. Many sterol intermediates appear from the 19-step conversion of lanosterol, the first sterol synthesized in the cascade of cholesterol synthesis, to cholesterol. The biochemical basis of the genetically inherited Smith-Lemli-Opitz syndrome has been described as a defective conversion of 7-dehydrocholesterol to cholesterol. Since this discovery, interest has focused on this special cholesterol precursor. Here, we report high concentrations of 7- and 8-dehydrocholesterol in caput epididymidis and spermatozoa derived from caput epididymidis of Sprague-Dawley and Wistar rats, which comprised up to 30% of total sterols. In contrast to caput epididymidis, 7- and 8-dehydrocholesterol were barely detected in cauda epididymidis or testis. Desmosterol increased several times from caput to cauda epididymidis. This is the first report of the natural appearance of high concentrations of dehydrocholesterols in mammalian tissue, and it underlines the putative importance of cholesterol precursors in reproductive tissue.— Lindenthal, B., T. A. Aldaghlas, J. K. Kelleher, S. M. Henkel, R. Tolba, G. Haidl, and K. von Bergmann. **Neutral sterols of rat epididymis: high concentrations of dehydrocholesterols in rat caput epididymidis. *J. Lipid Res.* 2001.** 42: **1089–1095.**

Supplementary key words cholesterol • desmosterol • 7-dehydrocholesterol • spermatozoa • testis • Smith-Lemli-Opitz syndrome • gas chromatography • mass spectrometry

Cholesterol synthesis comprises at least 19 steps from the first sterol, lanosterol, to cholesterol (1) (**Fig. 1**). Although the importance of cholesterol for membrane functions (as a precursor for bile salts and steroid hormones) has been studied thoroughly, considerably less is known about the sterol precursors of cholesterol in biological systems. For a long time, they were regarded as intermediates, appearing only in very low amounts compared with cholesterol and without association to major biological functions. However, recent findings gave rise to a more distinct view of this class of sterols. The cholesterol precursor desmosterol has been found in high amounts in monkey sperm and in reproductive tissue of other animal species (2–8). Moreover, Connor et al. (9) showed that desmosterol is nearly exclusively confined to the flagella of monkey spermatozoa, thus proposing a role for this sterol in motility of spermatozoa. Relatively high concentrations of desmosterol were also described in the developing brain (10, 11). In addition to desmosterol, cholesta-7,24-dien-3β-ol has been described in hamster cauda epididymidis (7, 12). Recently, a class of dimethylated cholesterol precursors has been discovered as meiosis-activating sterols (13, 14), revealing for the first time a major biological effect of sterol precursors of cholesterol. These observations of unusually high cholesterol precursors in reproductive tissues suggest possible functions yet to be elucidated.

Increasing interest in cholesterol precursor comes also from the discovery of highly elevated concentrations of 7-dehydrocholesterol in serum and tissues of patients with Smith-Lemli-Opitz (SLO) syndrome (15, 16). In these patients, a defective conversion of 7-dehydrocholesterol by the enzyme 7-dehydrocholesterol reductase to cholesterol causes the accumulation of 7- and 8-dehydrocholesterol found only in trace amounts in healthy subjects (16–19). In addition to the SLO syndrome, elevated concentrations of cholesterol precursors have been reported in desmosterolosis (20, 21) and chondrodysplasia punctata (22–25). The epididymis is an organ adjunctive to the testis. Further maturation of spermatozoa during their passage from caput to cauda epididymidis enables the spermatozoa to fertilize an oocyte. Here, we report for the first time the normal appearance of high concentrations of 7- and

Abbreviations: FID, flame ionization detection; GC, gas chromatography; MS, mass spectrometry; *m/z*, mass to charge ratio; SIM, selected ion monitoring; TMS, trimethylsilyl.

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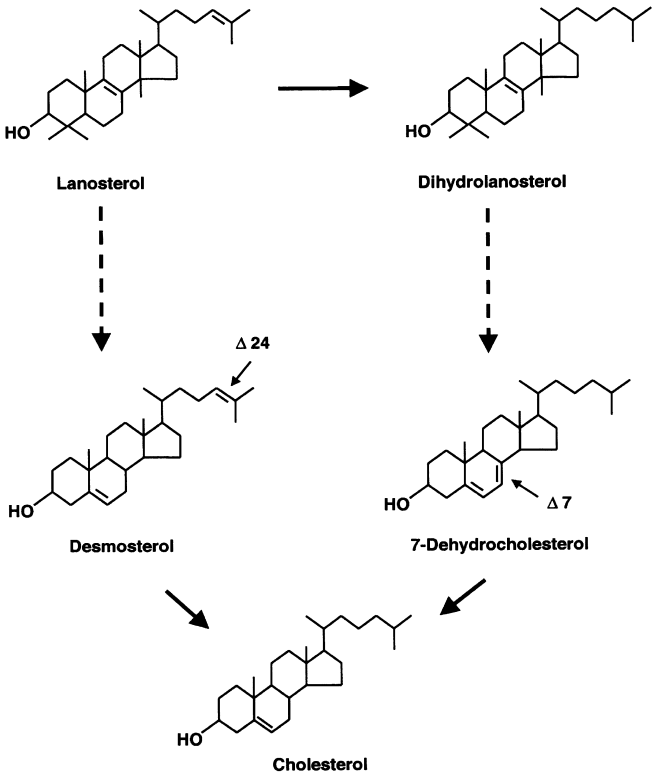

Fig. 1. Late steps of cholesterol synthesis.

8-dehydrocholesterol in a mammalian tissue that is limited to caput epididymidis of rats.

MATERIALS AND METHODS

Unless otherwise specified, chemicals were purchased from Sigma (Deisenhofen, Germany) in the highest available purity.

Sterol extraction and analysis

Caput and cauda epididymidis, as well as testis (0.05–0.15 g wet weight) of Sprague-Dawley and Wistar rats (Charles River Laboratories, Wilmington, MA; adult animals: 179–220 g body weight, prepuberty animals: <60 g body weight), were homogenized manually and diluted 1:1 (wet weight/v) with distilled water. Lipids were extracted in hexane–isopropanol (3:2 v/v; two times with 4 ml) using epicoprostanol (1 μg; stock solution 20 ng/μl in hexane) and 5α-cholestan (50 μg; stock solution 1 μg/μl in hexane) as internal standards. The extracts were deproteinized with acetone (3 ml). After drying under nitrogen, an alkaline hydrolysis was performed with 1ml of 1 N NaOH in 80% ethanol for 1 h at 70°C. After cooling down to room temperature and adding 0.5 ml double-distilled water, the sterols were extracted into cyclohexane (twice with 3 ml) by vortexing for 30 s. Following centrifugation (10 min, 2,500 rpm), the upper layer was transferred to new conical glass vessels. The combined extracts were dried under nitrogen, and the sterols were converted to their trimethylsilyl (TMS) derivatives by adding 100 μl bis-trimethylsilyl-trifluoracetamide–n-decane (1:1 v/v) for 1 h at 70°C. One to 2 ml were used for gas chromatography (GC)/mass spectrometry (MS) or GC/flame ionization detection (FID). The measured sterol levels were normalized to correspond to 0.1 g of tissue (wet weight).

Preparation of spermatozoa

Spermatozoa of caput and cauda epididymidis, as well as testis spermatozoa, were suspended in PBS buffer (pH 7.5) and filtered (150–200 mesh) to remove remaining tissue. PBS buffer containing spermatozoa were centrifuged at 2,000 *g* for 10 min. The remaining pellet was washed twice with PBS buffer, and analyzed for neutral sterols as described, starting with an alkaline hydrolysis.

GC/MS

Analysis was performed on a Hewlett-Packard GC/MS system (5890 series II GC combined with a 5971 mass selective detector) equipped with a DB-XLB (30 m × 0.25 mm id × 0.25 μm film, J&W) in the splitless mode, using helium (1 ml/min) as carrier gas. Temperature program: 150°C (kept for 1 min), followed by 20°C/min up to 260°C, and 10°C/min up to 280°C (kept for 15 min). Mass spectral data were collected either in the full-scan mode (*m/z* 50–550) or by selective ion monitoring (SIM).

Cholesterol precursors and plant sterols were monitored as their TMS derivatives in the SIM mode on desmosterol (*m/z* 456, 441, and 351), lathosterol (*m/z* 458), diunsaturated sterol (*m/z* 456; tentatively identified as Δ7,24 cholesta-dien-3β-ol by comparison with reported mass spectra (7)), triunsaturated sterols (*m/z* 454), campesterol (*m/z* 472), sitosterol (*m/z* 486), and lanosterol (*m/z* 498 and 393). The internal standards epicoprostanol and 5α-cholestane were measured on *m/z* 370 and *m/z* 372, respectively. Peak integration was performed manually, and sterols were quantified from SIM analyses against the internal standards using standard curves for commercially available sterols (cholesterol, lathosterol, lanosterol, desmosterol, 7-dehydrocholesterol, campesterol, and sitosterol).

GS/FID

GS/FID detection was performed on a Hewlett-Packard GC-system (HP 6890 series II plus GC) equipped with a DB-XLB (30 m × 0.25 mm id × 0.25 μm film, J&W) in the splitless mode using hydrogen (1.1 ml/min) as carrier gas. Temperature program: 150°C (kept for 3 min), followed by 30°C/min up to 290°C (kept for 22 min). Sterols were quantified as their TMS derivatives against the peak areas of the internal standards.

Statistics

Results are given as means ± SD. Differences were considered significant at a level of $P < 0.05$, calculated by Student's *t*-test.

RESULTS

The caput epididymidis of Sprague-Dawley rats contained three noncholesterol sterols in considerable concentrations (**Fig. 2**). Two of these sterols were identified as desmosterol (Fig. 2A, compound 3) and 7-dehydrocholesterol (Fig. 2A, compound 4) based on *a*) their mass spectra, *b*) comparing retention times of reference compounds, and *c*) standard addition of reference compounds to biological samples. The mass spectra of 7-dehydrocholesterol is given in **Fig. 3A**. The third major sterol in the caput of the epididymis was identified as 8-dehydrocholesterol based on its mass spectra (Fig. 2A, peak 2; Fig. 3B), revealing a mass spectrum very close to 7-dehydrocholesterol with high abundance of the fragmentation ions on *m/z* 351 and *m/z* 325, typical for C_{27} sterols possessing double bonds on the position Δ7 or Δ8 and on position Δ5 (26). The rela-

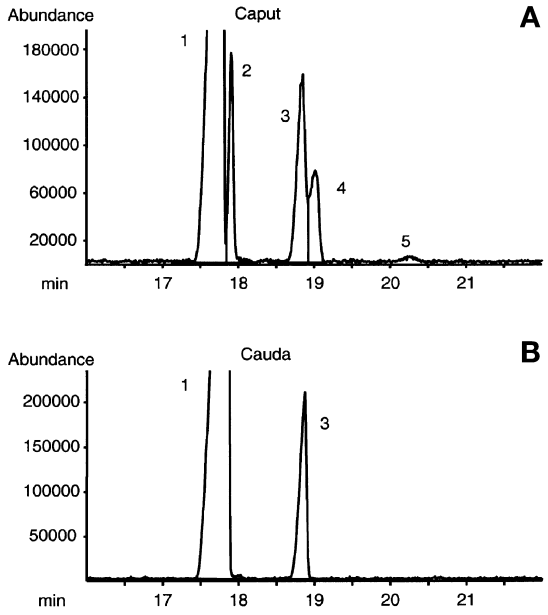

Fig. 2. Neutral sterols of caput (A) and cauda (B) epididymidis of an adult Sprague-Dawley rat. Full-scan chromatogram of a GC/MS analysis; peak 1: cholesterol; peak 2: 8-dehydrocholesterol; peak 3: desmosterol; peak 4: 7-dehydrocholesterol; peak 5: triunsaturated sterol.

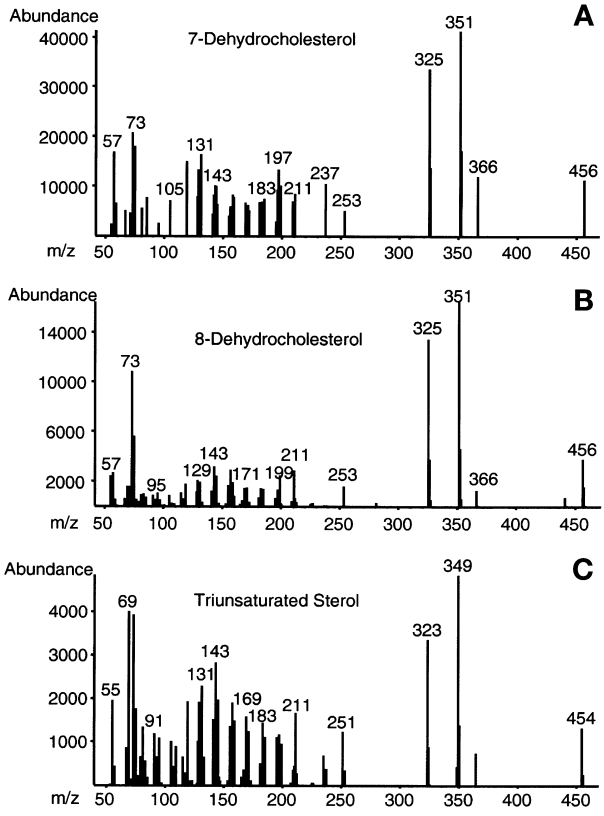

Fig. 3. Mass spectra (m/z 50–500) of 7-dehydrocholesterol, 8-dehydrocholesterol, and triunsaturated sterol in caput epididymidis of an adult Sprague-Dawley rat.

tively lower abundance of the ion m/z 366 compared with the mass spectra of 7-dehydrocholesterol is also typical for 8-dehydrocholesterol (27), and the mass spectra shows a close match to the mass spectra of 8-dehydrocholesterol described by Batta et al. (18). Further evidence for the identity of this sterol with 8-dehydrocholesterol comes from the comparison of retention times and mass spectra with 8-dehydrocholesterol analyzed in serum of patients with SLO syndrome. Another sterol in much lower concentrations was identified as a triunsaturated sterol by its mass spectra, giving a molecular ion of m/z 454 as TMS derivative (Fig. 2A, peak 5; Fig. 3B). The high abundance of the ions m/z 349 and m/z 323 suggests a close structural relationship to 7- or 8-dehydrocholesterol. In addition, small amounts of another triunsaturated sterol with a closely related fragmentation pattern could also be detected. Therefore, it is likely that these triunsaturated sterols correspond to 7-dehydrocholesterol and 8-dehydrocholesterol possessing an additional double bond.

Figure 2 shows that the appearance of 7- and 8-dehydrocholesterol was confined strictly to the caput epididymidis, whereas desmosterol was also present in cauda epididymidis.

Cholesterol precursors and the plant sterols campesterol and sitosterol were quantified in adult Sprague-Dawely rat epididymis using standard curves. However, no quantification for 8-dehydrocholesterol was performed for Sprague-Dawely rats because no authentic reference compound was available for GC-MS analyses and GC-FID was not available for this set of samples; data are given later on for Wistar rats. As shown in **Table 1**, the amount of 7-dehydrocholesterol in the caput epididymidis was 471 ± 58 (μg/g wet weight), and was barely detectable in the cauda of these samples. As judged by full-scan analysis (Fig. 2), 8-dehydrocholesterol was present approximately in the same amount as 7-dehydrocholesterol, and the ratio of 7-dehydrocholesterol to 8-dehydrocholesterol kept constant in all tested samples. Desmosterol was significantly higher in cauda epididymidis than in caput (272 ± 83 μg/g vs. 159 ± 40 μg/g, $P < 0.05$, n = 6). The cholesterol precursor lathosterol was 2.5 times higher in the caput epididymidis compared with cauda (4.93 ± 1.45

TABLE 1. Cholesterol and cholesterol precursors in caput and cauda epididymidis in adult Sprague-Dawley rats

	Caput	Cauda
	μg/g (wet weight)	
Sterol		
Cholesterol	1,498 ± 273	1,853 ± 125[a]
7-Dehydrocholesterol	471 ± 58	n.d.
Desmosterol	159 ± 40	272 ± 83[a]
Lathosterol	4.93 ± 1.45	1.94 ± 0.53[b]
Lanosterol	0.45 ± 0.08	0.28 ± 0.07[b]
	mg/mg	
Ratio to cholesterol		
7-Dehydrocholesterol	0.32 ± 0.05	n.d.
Desmosterol	0.11 ± 0.03	0.147 ± 51
Lathosterol	0.0032 ± 0.00066	0.0011 ± 0.0003[b]
Lanosterol	0.0003 ± 0.00009	0.0002 ± 0.00003[b]

Values given as means ± SD (n = 6). Neutral sterols were measured in adult Sprague Dawley rats (179–220 g body weight) as described under Materials and Methods by GC/MS, n.d., not detected.

[a] $P < 0.05$, Student's *t*-test.

[b] $P < 0.01$, Student's *t*-test.

TABLE 2. Cholesterol and cholesterol precursors in caput and cauda epididymidis in adult Wistar rats

	Caput	Cauda
	μg/g (wet weight)	
Sterol		
Cholesterol	963 ± 131	1,445 ± 110[a]
8-Dehydrocholesterol	152 ± 10	7.5 ± 2.4[b]
7-Dehydrocholesterol	182 ± 19	5.6 ± 1.2[b]
Desmosterol	108 ± 27	481 ± 87[b]
Trienol 1	41 ± 5	4.9 ± 1.2[a]
Trienol 2	43 ± 6	2.1 ± 0.5[b]
Δ7,24-cholesta-dien-3β-ol	7.2 ± 1.9	3.8 ± 0.7
Lathosterol	4.8 ± 1.5	1.6 ± 0.4
Lanosterol	2.2 ± 1.3	0.6 ± 0.1[b]
	mg/mg	
Ratio to cholesterol		
8-Dehydrocholesterol	0.16 ± 0.02	0.0052 ± 0.002[b]
7-Dehydrocholesterol	0.20 ± 0.02	0.0039 ± 0.0005[b]
Desmosterol	0.11 ± 0.02	0.333 ± 0.034[b]
Trienol 1	0.042 ± 0.005	0.0033 ± 0.0006[b]
Trienol 2	0.045 ± 0.006	0.0015 ± 0.0005[b]
Δ7,24-cholesta-dien-3β-ol	0.0074 ± 0.001	0.0027 ± 0.0007[a]
Lathosterol	0.0049 ± 0.001	0.0011 ± 0.0016[a]
Lanosterol	0.0023 ± 0.0002	0.0004 ± 0.00007[b]

Values given as means ± SD (n = 3). Neutral sterols in the caput and cauda epididymidis of adult Wistar rats (200–300 g body weight) were measured as described under Materials and Methods by GC/FID.

[a] $P < 0.05$, Student's *t*-test.

[b] $P < 0.01$, Student's *t*-test.

μg/g vs. 1.94 ± 0.53 μg/g, $P < 0.01$). Lanosterol was also detectable in low amounts in both parts of the epididymis. Again, lanosterol was higher in caput epididymidis than in cauda (0.446 ± 0.081 μg/g vs. 0.276 ± 0.065 μg/g, $P < 0.01$, n = 6).

To evaluate whether the observed sterol pattern is due to physiological changes during puberty, neutral sterols were measured in prepuberty rats (body weight <60 g, n = 5). As for adult rats, 7-dehydrocholesterol was also present in caput epididymidis (206 ± 133 μg/g), but not in cauda epididymidis. Desmosterol was measured in both parts of the epididymis, and was again lower in caput epididymidis (118 ± 33 μg/g vs. 250 ± 29 μg/g, $P < 0.01$). However, lathosterol showed no difference between caput and cauda of the epididymis (5.88 ± 1.86 μg/g vs. 6.70 ± 3.39 μg/g), in contrast to adult rats. Concentrations of lanosterol in these samples were too low for accurate determination.

The content of neutral sterol in caput and cauda epididymidis was also measured in Wistar rats. To obtain further quantitative information, these measurements were performed by GC/FID analysis and MS was used for peak identification. The results given in **Table 2** show the same sterol pattern as seen in Sprague-Dawley rats, with high amounts of 7-dehydrocholestrol, 8-dehydrocholesterol, and desmosterol in the rat caput epididymidis. These three sterols accounted for about 30% of the total sterol content. In addition, the two measured triunsaturated sterols accounted for another 5.6% of the total sterol content. Again, only minor amounts of these sterols were detected in cauda epididymidis. As reported for other species, humans showed no measurable amounts of 7- or 8-dehydrocholesterol in caput or cauda epididymidis. However, cholesterol and desmosterol also increased from caput to cauda epididymidis (unpublished observation).

The two plant sterols, campesterol and sitosterol, showed no marked differences between caput and cauda epididymidis, but were significantly higher in cauda epididymidis compared with cauda in adult Sprague-Dawley rats (7.2 ± 1.6 μg/g vs. 4.2 ± 1.9 μg/g for campesterol, and 7.6 ± 2.1 μg/g vs. 4.6 ± 1.3 μg/g for sitosterol; $P < 0.01$, n = 6). However, this difference was not observed in prepuberty Sprague-Dawley rats and adult Wistar rats (data not shown).

To further localize the appearance of dehydrocholesterols, we measured neutral sterols in spermatozoa obtained from testis, caput, and cauda epididymidis and in the remaining tissue after spermatozoa preparation. The results given in **Table 3** and **Table 4** show that the high concentrations of dehydrocholesterols were present in both sper-

TABLE 3. Cholesterol and cholesterol precursors in spermatozoa obtained from testis, caput, and cauda epididymidis of adult Wistar rats

	Testis	Caput	Cauda
	μg/10⁶ spermatozoa		
Sterol			
Cholesterol	67 ± 23	3.03 ± 1.72[d]	1.53 ± 0.59[d]
8-Dehydrocholesterol	n.d.	0.57 ± 0.39	n.d.
7-Dehydrocholesterol	n.d.	0.66 ± 0.51	n.d.
Desmosterol	0.40 ± 0.10	0.30 ± 0.23	1.17 ± 0.46[a,c]
	mg/mg		
Ratio to cholesterol			
8-Dehydrocholesterol	n.d.	0.19 ± 0.04	n.d.
7-Dehydrocholesterol	n.d.	0.20 ± 0.05	n.d.
Desmosterol	0.006 ± 0.0008	0.11 ± 0.06[c]	0.76 ± 0.08[b,d]

Values given as means ± SD (n = 6). Neutral sterols were measured as described under Materials and Methods in spermatozoa of adult Wistar rats by GC/FID; n.d., not detected.

[a] $P < 0.05$, caput versus cauda, Student's *t*-test.

[b] $P < 0.001$ caput versus cauda, Student's *t*-test.

[c] $P < 0.05$ testis versus caput or testis versus cauda, Student's *t*-test.

[d] $P < 0.01$ testis versus caput or testis versus cauda, Student's *t*-test.

TABLE 4. Cholesterol and cholesterol precursors in the remaining tissue after spermatozoa preparation from testis, caput, and cauda epididymidis of adult Wistar rats

	Testis	Caput	Cauda
	μg/g (wet weight)		
Sterol			
Cholesterol	1,313 ± 423	951 ± 319[a]	809 ± 116[a]
8-Dehydrocholesterol	n.d.	136 ± 63	n.d.
7-Dehydrocholesterol	n.d.	155 ± 68	n.d.
Desmosterol	8.8 ± 4.3	43 ± 22[a]	308 ± 70[b,c]
	mg/mg		
Ratio to cholesterol			
8-Dehydrocholesterol	n.d.	0.14 ± 0.04	n.d.
7-Dehydrocholesterol	n.d.	0.16 ± 0.04	n.d.
Desmosterol	0.007 ± 0.004	0.056 ± 0.05	0.38 ± 0.05[b,c]

Values given as means ± SD (n = 6). Neutral sterols were measured as described under Materials and Methods by GC/FID; n.d., not detected.

[a] $P < 0.05$ testis versus caput or testis versus cauda, Student's *t*-test.

[b] $P < 0.01$ testis versus caput or testis versus cauda, Student's *t*-test.

[c] $P < 0.001$ caput versus cauda, Student's *t*-test.

matozoa and the remaining tissue of caput epididymidis. In spermatozoa of testis, cauda epididymidis, and the remaining tissues, dehydrocholesterols were virtually absent. Interestingly, the cholesterol content of testis spermatozoa was markedly increased compared with spermatozoa obtained from caput and cauda, whereas in the remaining tissue, cholesterol of the testis was only slightly higher compared with caput and cauda epididymidis. The results in Table 3 and Table 4 also show the marked increase of desmosterol from caput to cauda epididymidis, whereas 7- and 8-dehydrocholesterol were not detected in spermatozoa obtained from cauda epididymidis.

DISCUSSION

The conversion of lanosterol, the first sterol intermediate in cholesterol synthesis, to cholesterol is comprised of different steps: *a*) the removal of the methyl groups in position 4α, 4β, and 14, *b*) the saturation of the Δ24 double bond, and *c*) shifting the Δ8 double bond to Δ5 (1). Desmosterol (double bonds at Δ24 and Δ5) and 7-dehydrocholesterol (double bonds at Δ7 and Δ5) are the ultimate precursors of cholesterol in this process. Although both sterols are C_{27} cholesterol precursors, they cannot be converted into each other in the cholesterol biosynthetic pathway, and they originate from two different routes of cholesterol synthesis (Fig. 1).

To our knowledge, this is the first report of high levels of 7- and 8-dehydrocholesterol in a mammalian tissue except in pathophysiological conditions, drug interventions, or elevated concentrations of 7-dehydrocholesterol in human skin, serving as a precursor for vitamin D formation (28). Desmosterol has recently been found to be increased after puberty in rhesus monkeys testis (2). The same authors reported high levels of desmosterol in monkey and human spermatozoa, where desmosterol and docosahexaenoic acid was nearly exclusively confined to the flagella of monkey sperm (3, 9, 29). Their investigations point to a role for desmosterol in sperm motility. However, because 7-dehydrocholesterol and 8-dehydrocholesterol were virtually absent in cauda epididymidis in this study, any possible function of these two sterols in spermatozoa maturation seems to be different from the putative functions of desmosterol. Because 7- and 8-dehydrocholesterol cannot be converted to desmosterol, they cannot serve as a precursor pool for desmosterol synthesis.

Desmosterol is also a known constituent of spermatozoa of human, rhesus monkey, hamster, boar, rabbit, goat, and mouse, and also increases from caput to cauda in some, but not all, species investigated (3, 5, 6, 8, 29, 30). The appearance of desmosterol together with cholesta-7,24-dien-3β-ol has been described (7). However, cholesta-7,24-dien-3β-ol gives a mass spectrum distinct from 7- and 8-dehydrocholesterol due to the fact that one double bond is located in the B ring and one in the side chain, which is in contrast to 7- and 8-dehydrocholesterol possessing both double bonds in ring B (26). Cholesta-7,24-dien-3β-ol can be converted to desmosterol (cholesta-5,24-dien-3β-ol) and might, therefore, be regarded as a desmosterol precursor. Desmosteryl-sulfate has been shown to increase markedly from hamster caput to cauda epididymidis spermatozoa, with a proposed role in membrane stabilization and the capacitation process (8, 31). Therefore, cholesterol precursors such as desmosterol seem to play an important role in spermatozoa maturation and function. However, from the known distribution of desmosterol in the male reproductive tract in mammals, it is difficult to predict a possible role of 7- and 8-dehydrocholesterol because 7- and 8-dehydrocholesterol are *a*) strictly confined to the caput epididymidis and *b*) 7-dehydrocholesterol cannot be converted to desmosterol. Furthermore, 7- and 8-dehydrocholesterol, as well as desmosterol, are present in prepuberty rats in relatively high amounts, which indicates that their appearance is not limited to maturation during puberty. However, this finding does not exclude an important role for these sterols in fertility.

The analysis of spermatozoa from testis, caput, and cauda epididymidis and the remaining tissue revealed that dehydrocholesterols and desmosterol are present in both spermatozoa and remaining tissue of caput epididymidis. However, the ratio of these sterols to cholesterol is higher in the spermatozoa compared with the remaining tissue. It is unlikely that the marked change of the sterol composition of spermatozoa during the epididymal maturation is accomplished by changes of cholesterol synthesis in the spermatozoa alone. Therefore, it might be assumed that other cells of the caput epididymidis have a markedly altered biosynthesis providing dehydrocholesterols and desmosterol for spermatozoa. In addition, cauda epididymidis cells must also possess marked changes in cholesterol biosynthesis compared with other tissues to provide high amounts of desmosterol. In addition to the direct effects of enzymes involved in cholesterol synthesis, accumulation of cholesterol precursors (e.g., desmosterol) might also be due to changes in intracellular transport of sterols (32).

Considerable interest has focused on 7-dehydrocholesterol since the discovery of highly elevated plasma and tissue concentrations of 7-dehydrocholesterol in patients with SLO syndrome (15, 33–37). Based on the association of high levels of 7-dehydrocholesterol and the SLO syndrome, the underlying biochemical defect of this disease has been identified as an impaired conversion of 7-dehydrocholesterol to cholesterol by the sterol Δ7-reductase (19, 38–44). In addition to elevated concentrations of 7-dehydrocholesterol, higher levels of 8-dehydrocholesterol, as well as triunsaturated sterols, have also been described in patients with SLO syndrome (45, 46). Paik et al. (47) demonstrated the reversibility of the Δ8–Δ7-isomerization by partly purified sterol Δ8-Δ7-isomerase. This might explain the simultaneous appearance of 8-dehydrocholesterol together with 7-dehydrocholesterol.

However, elevated concentrations of desmosterol are not found in patients with SLO syndrome or in biochemical models with blocked Δ7-reductase. Therefore, the simultaneous appearance of desmosterol together with 7- and 8-dehydrocholesterol in the caput of rat epididymis is a unique cholesterol precursor pattern not reported previ-

ously; 7- and 8-dehydrocholesterol were not detected in the epididymis of guinea pigs and humans (unpublished results) and other mammalian species. Therefore, the rat caput epididymidis displays a rare or even unique pattern of high concentrations of dehydrocholesterols among mammals whose possible function(s) has yet to be described.

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