Desmosterol in Developing Rat Brain

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Abstract

The brain of the young rat contains appreciable amounts of desmosterol (24-dehydrocholesterol). The peak desmosterol concentration is seen during the first week of life and only traces of this sterol are found at 21 days. The spinal cord also contains some desmosterol. Rat brain desmosterol is distributed in the white matter, gray matter and cerebellum and occurs in the same proportion to cholesterol in each of these brain fractions. Rat brain contains a small amount of sterol ester but no appreciable amounts of desmosterol are present in this fraction.

Studies carried out in intact animals injected either intraperitoneally or intracerebrally with mevalonic acid-2-^{14}C or glucose-U-^{14}C indicate the biosynthetic origin or brain desmosterol. Rat brain slices (1–20 day old) incubated in suitably fortified medium convert sodium acetate-2-^{14}C and glucose-U-^{14}C to desmosterol, whereas brain slices from adult rats yielded no radioactive desmosterol under similar conditions. When labeled desmosterol was incubated with young rat brain slices, it was converted to cholesterol.

When pregnant rats were treated with triparanol (20 mg/kg/day) during the course of their pregnancy, they either resorbed the fetuses or gave birth to small, stillborn litters. The brains of the progeny of triparanol treated mothers contained large amounts of desmosterol as well as another sterol which may be $\Delta^{7,24}$-cholestadiene-3β-ol.

SINCE OUR ORIGINAL communication describing the occurrence of desmosterol (24-dehydrocholesterol) in the brain of the young rat (1), the presence of this sterol in rat brain has been confirmed (2), and it has also been found in fetal human brain (3) and in young mouse brain (4). The presence of this sterol has been associated with the myelination process since no desmosterol has been detected in the brains of adult rats (1–3) or humans (2) nor is any present in the brain of the newborn guinea pig, an animal which is fully myelinated at birth. The question of the origin and localization of the desmosterol present in the brain of the young rat has not been investigated to date, and our experiments in this area are the basis of the present report.

TABLE I

Desmosterol/Cholesterol Ratios in Young Rat Brain

Age (days)	Brain wt. (g)	Desmosterol/cholesterol
2	0.240	0.418
4	0.360	0.463
5	0.360	0.492
6	0.427	0.497
7	0.425	1.052
8	0.568	1.555
9	0.650	1.165
9	0.560	1.100
10	0.930	0.221
11	0.658	0.400
12	1.015	0.282
14	0.988	0.227
14	0.768	0.186
15	0.958	0.074
16	1.050	0.122
17	1.155	0.031

Experimental

The rats used were all of the Wistar strain. For assay of brain sterols, animals were killed by decapitation and the brain removed, dissolved in 15% alcoholic KOH and the nonsaponifiable lipid was extracted into petroleum ether. Gas chromatographic analyses were usually carried out on the crude extract using a Barber-Colman Model 10 apparatus. The liquid phase was either SE-52 or a mixed SE-52 and XE-60 packed on Gas-Chrom P, in a 4 ft column. The column temperature was 230C; an Argon Radium detector was used.

In experiments involving separation of free and ester sterol the brain lipids were extracted into chloroform:methanol (2:1) and subjected either to thin-layer chromatography (TLC) on Silica Gel G (Brinckmann) using petroleum ether:ether:acetic acid (90:10:1) as developing solvent or to column chromatography on Unisil (Clarkson) the ester fraction being eluted with hexane:benzene (8:2) (5). Cholesterol and desmosterol were separated by TLC on Silica Gel H impregnated with 12.5% $AgNO_3$. The developing solvent was chloroform: acetone (95:5). Under these conditions the R_f values for cholesterol and desmosterol are 0.33 and 0.27, respectively (6).

In studies of biosynthesis in intact rats the appropriate substrate (sodium acetate-1-^{14}C, mevalonic acid-2-^{14}C or glucose-U-^{14}C) was injected intraperitoneally or intracerebrally. The labeled substrates were all purchased from the New England Nuclear Corporation, Boston, Massachusetts. In vitro biosynthesis experiments were carried out using 0.5 g of brain slices suspended in 7 ml of Gey's buffer prepared without glucose and containing 0.03M nicotinamide and 0.006M $MgCl_2$. Each flask also contained 1.0 mg each of DPN and ATP. The buffer composition (gram/liter) is: NaCl, 8.28; KCl, 0.40; $CaCl_2 \cdot H_2O$, 0.174; $MgCl_2 \cdot 6H_2O$, 0.21; $Na_2HPO_4 \cdot 7H_2O$, 0.224; KH_2PO_4, 0.025; $NaHCO_3$, 0.250. The solution was brought to pH 7.45 with 1N HCl. Incubations were carried out for 2 hr with constant shaking at 37C in an oxygen atmosphere. At the end of the incubation period 10 ml of 15% alcoholic KOH was added to each flask and the contents were warmed until the tissue had dissolved. The nonsaponifiable lipids were extracted into petroleum ether.

TABLE II

Effects of Gum Tragacanth or Method of Collection on Desmosterol/Cholesterol Ratios in Rat Brain

Age (days)	Study A		Study B	
	Gum tragacanth	Water	Formalin	Methanol
3	....	.....	0.394	0.384
4	0.463	0.479	0.426	0.406
5	0.483	0.530	0.431	0.390
6	0.525	0.514	0.435	0.421
7	0.559	0.493	0.447	0.406
8	0.483	0.435	0.387	0.362
9	....	.....	0.330	0.324
10	0.340	0.370	0.317	0.314
11	0.299	0.291	0.273	0.279
12	0.318	0.264	0.275	0.286
13	0.276	0.239	0.232	0.218
14	0.250	0.205	0.204	0.193
15	0.224	.....	.....	0.151
16	0.177	.....	.....	.....

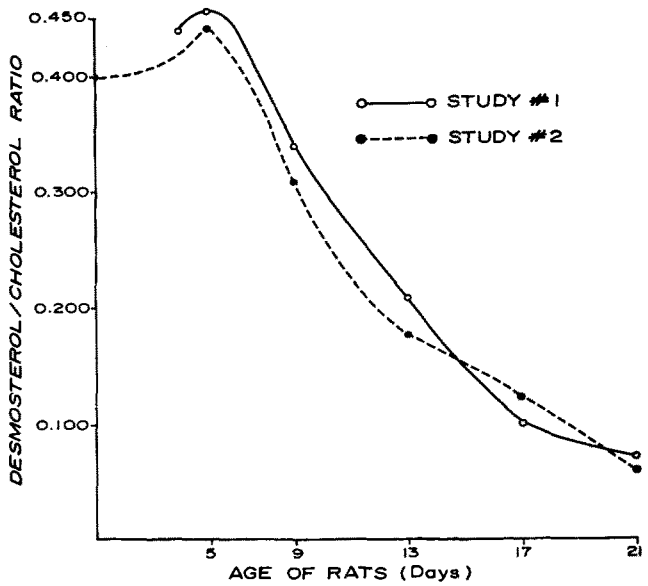

FIG. 1. Desmosterol/cholesterol ratios in brains of young rats.

The cholesterol and desmosterol were separated by TLC on $AgNO_3$-impregnated silica gel H. The bands of silica gel containing each sterol were scraped from the plate, and the sterol eluted with chloroform and radioactivity assayed by liquid scintillation spectroscopy. In some cases the silica gel was scraped directly into a counting vial and assayed with proper corrections for quenching.

In experiments in which pregnant rats were treated with triparanol (MER/29; 1-[(4-diethylaminoethoxy) phenyl]-1-(p-tolyl)-2-(p-chlorophenyl) ethanol), the drug was administered orally as a suspension in 1% gum tragacanth. The rats received 20 mg/kg of triparanol daily.

Results and Discussion

In our earlier work (1) we had reported rather high desmosterol/cholesterol ratios (D/C) in the brains of 11–16 day old rats. These ratios were considerably higher than those reported by subsequent workers (2) and the peak desmosterol content appeared somewhat later than it did in other studies. In a second experiment we again found high desmosterol/cholesterol ratios, but the peak desmosterol content occurred at 7–9 days. These data are presented in Table I. The rats used were not all litter mates, which may explain some of the differences in brain weight and D/C. These discrepancies prompted us to investigate this point further. Our original study involved the administration of triparanol to pregnant rats for the purpose of determining its effect on the desmosterol concentration of fetal tissues. The controls were given a 1% suspension of gum tragacanth. Brains were collected from newborn rats at given days and stored individually in either 10% formalin or methanol prior to saponification and assay for sterols. To determine whether any of these variables may have affected the ratios of desmosterol to cholesterol we carried out two parallel studies. In one, pregnant rats were fed either 1% gum tragacanth or water during the course of their pregnancy; brains of the offspring were collected in formalin. In a second study, brains of offspring from untreated rats were collected in either formalin or methanol. Data are presented in Table II.

It can be seen that neither the treatment nor

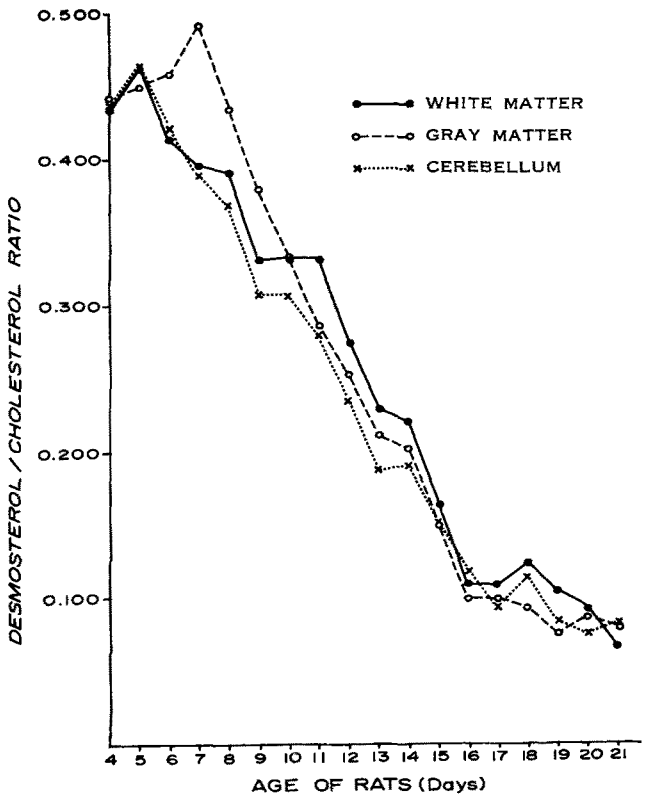

FIG. 2. Desmosterol/cholesterol ratios in white matter, gray matter and cerebellum of young rat brain.

method of collection of brain tissue affected D/C ratios and that peak D/C ratios occurred at the fifth to seventh day. The days of peak D/C ratio and the actual values (0.42–0.56) are within ranges observed by other authors and are similar to those observed by us in subsequent studies. Data from two of the later studies are represented in Figure 1. We have no explanation of our early results.

In an effort to determine whether desmosterol is localized in the nervous system, a series of rat brains (day 4–21) were separated into white and gray matter and cerebellum and desmosterol/cholesterol ratios were determined for each portion. It can be seen (Figure 2) that the D/C ratios were similar for all parts of the brain. A small, but significant, amount of zymosterol was found in the white matter of the 16–21 day old rats. The zymosterol/cholesterol ratios were between 0.038 (day 16) and 0.021 (day 21).

The desmosterol/cholesterol ratios in brain and spinal cord were also determined. Brains and cords were collected from several rats at days 1 to 7 of age. The amount of desmosterol in the cord is considerably less than that in the brain.

There are conflicting reports concerning the presence of cholesterol esters in brain. Mandel et al. (7) and Johnson et al. (8) found some sterol ester in chick embryo and human infant brain, respectively, whereas Brante (9) and Cummings et al. (10) found

TABLE III

Mean Desmosterol/Cholesterol Ratos in Rat Brain and Spinal Cord

Age (days)	Number	Brain	Cord
1	3	0.310	0.078
2	3	0.311	0.082
3	3	0.345	0.086
4	4	0.358	0.112
5	3	0.349	0.119
6	3	0.336	0.107
7	2	0.339	0.118

TABLE IV

Desmosterol/Cholesterol (D/C) Ratios in Free and Ester Sterol Fractions of Rat Brain

Age (Days)	Brain fraction	% Ester	D/C	
			Free	Ester
3	White matter	0.62	0.348	.....
	Gray matter	1.50	0.461	.....
	Cerebellum	N.D.[a]	0.142	.....
4	White matter	1.20	0.317	.....
	Gray matter	0.26	0.301	.....
	Cerebellum	0.80	0.153	0.096

[a] N.D. = none detected.

none. Adams and Davison (11) have reported that 20% of the sterol of neonatal human brain corpus callosum and 6% of the 30-day-old chick brain corpus callosum is present as esterified cholesterol. Clarenburg et al. (12) found that 0.5% of 17–33 day old rabbit brain cholesterol is present as ester and Pritchard (13) found small amounts of cholesterol ester in 7-day-old rat brain. We have separated the various brain portions of 3- and 4-day-old rat brains into free and ester sterol fractions and have found some sterol ester, but in only one case was it possible to detect desmosterol in the ester fraction (Table IV).

It has been shown by a number of authors that when cholesterol and other sterols are injected intraperitoneally or intravenously into rats (14) or rabbits (12,15,16) or into the chick embryo yolk sac (17), these sterols reach the brain of the animal and may persist there for relatively long periods of time. In order to assess the maternal contribution of the brain desmosterol of newborn rats, several pregnant rats were injected intravenously or intraperitoneally with sodium acetate-1-^{14}C and the offspring killed serially and their brains assayed for radioactivity and for sterols. Of the two rats injected intravenously, the brains of the newborn rats aged 4 to 9 days showed very little radioactivity (about 20 counts/min/brain above background) and were not further analyzed. Of the three mothers who were injected intraperitoneally, appreciable radioactivity was recovered in the newborn brain after 4 days and some of the radioactivity was recovered in the brain desmosterol (Table V). In view of Morris and Chaikoff's finding (18) that cholesterol-^{14}C is not transferred to the brain of the offspring when administered orally to a pregnant rat, it is concluded that the brain sterol radioactivity represents synthesis from acetate in utero. Nicholas and Thomas (19) also found very little radioactivity in rat, mouse or guinea pig brain after maternal injection with sodium acetate-2-^{14}C.

The in vivo synthesis of brain cholesterol by young rats was first demonstrated by Waelsch, Sperry and Stoyanoff (20,21) using deuterium as a tracer. Young

TABLE V

Recovery of Labeled Sterol from Brains of Newborn Rats Following Maternal Injection with Sodium Acetate-1-^{14}C (100,000 cpm)

Litter	Brain ^{14}C-(total cpm)	Desmosterol (cpm)	Cholesterol (g)
Litter 1			
Day 1–3	15–25	....	....
4	115	NCO[a]	NCO
5	150	NCO	NCO
6	175	10	108
7	375	88	288
Litter 2			
Day 1–3	10–25	....	....
4	120	NCO	NCO
5	270	80	100
6	160	16	170
7	280	Lost	Lost
Litter 3			
Day 1–4	10–35	....	....
5	160	16	140
6	90	NCO	NCO

[a] Not carried out.

TABLE VI

Recovery of Radioactivity from Brain Sterols After Injection of Newborn Rats with Mevalonic Acid-2-^{14}C (60,000 cpm)

Brain non sap. (cpm)	Desmosterol (cpm)	Cholesterol (cpm)	D/C	
			cpm	Wt.
Intraperitoneal injection				
Day 2	355	36	216	.167	0.31
	335	54	216	.250	0.33
	420	25	205	.122	0.35
Day 6	670	20	400	.05	0.34
Day 8	730	72	432	.167	0.36
	750	80	620	.129	0.32
Intracerebral injection					
Day 2	3150	930	1760	.528	0.32
	1115	324	628	.500	0.33
	2950	864	2376	.366	0.36
Day 6	2145	396	2052	.193	0.35
	2575	250	1790	.140	0.31
Day 8	3745	1296	2196	.590	0.36
	5525	1440	3260	.442	0.36

rats and mice can convert acetate, mevalonate, leucine and glucose into brain cholesterol following intraperitoneal injection of the appropriate precursor (19, 22–24), and intracerebral injection of precursor results in even better incorporation into brain sterol (19–22). Adult dogs (25) and rats (19) show no in vivo synthesis of brain cholesterol regardless of whether the precursor is fed or injected intraperitoneally, but intracerebral injection results in some conversion to brain cholesterol (22,26). We have carried out a series of experiments in which newborn rats were injected intraperitoneally or intracerebrally with mevalonic acid-2-^{14}C or glucose-U-^{14}C. All rats were injected at birth and were killed at various intervals thereafter. The results are presented in Tables VI and VII. It is seen that intraperitoneally administered glucose is a much more efficient precursor of brain sterol than is mevalonate, but that more mevalonate than glucose is converted to brain sterol after intracerebral inoculation. The specific activity ratios suggest a product-precursor relationship for desmosterol and cholesterol when one considers all the data derived from glucose or the data obtained after intracerebral injection of mevalonate. Relatively little of the intraperitoneally administered mevalonic acid was converted to brain sterol and the data thus suggest that much of the labeled cholesterol recovered from the brain may have been derived from sources other than brain biosynthesis. The data definitely establish the biosynthetic origin of brain desmosterol.

Early experiments by Srere et al. (27) showed that infant, but not adult, rat brain slices could convert acetate to cholesterol in vitro. Subsequent work has involved various other cholesterol precursors but the relative efficiency of conversion of these compounds to cholesterol has not been definitely established.

TABLE VII

Recovery of Radioactivity from Brain Sterols After Injection of Newborn Rats with Glucose-U-^{14}C (100,000 cpm)

Brain non sap. (cpm)	Desmosterol (cpm)	Cholesterol (cpm)	D/C	
			cpm	Wt.
Intraperitoneal injection				
Day 2	1155	324	288	1.125	0.35
	2645	1280	1080	1.185	0.28
	1920	360	1044	0.345	0.37
Day 6	6600	1400	4540	0.308	0.33
	3335	612	1548	0.495	0.40
Day 8	1490	584	648	0.901	0.28
	1810	670	680	0.985	0.38
	835	216	252	0.857	0.37
	840	144	234	0.444	0.33
Intracerebral injection					
Day 2	1925	972	972	1.000	0.33
	2280	450	800	0.563	0.35
	1215	628	468	1.385	0.38
Day 6	1835	410	1360	0.301	0.37
Day 8	1235	628	324	2.000	0.30
	1470	450	650	0.692	0.28
	815	396	468	0.846	0.41
	1240	648	684	0.947	0.34

TABLE VIII

Average Incorporation of Acetate-2-^{14}C and Glucose-U-^{14}C into Desmosterol and Cholesterol by Brain Slices from 1, 5, 20 and 90 Day-Old Rats (4 Incubations per Group)

	Brain non sap. (cpm)	Desmosterol (cpm)	Cholesterol (cpm)	D/C (cpm)	D/C Wt.
Day 1					
Acetate	5710 ± 1380 [b]	884 ± 380	5200 ± 855	0.168	0.296
Glucose [a]	457 ± 29	52 ± 9	374 ± 39	0.140	0.287
Day 5					
Acetate	37239 ± 7962	2840 ± 422	13556 ± 2513	0.226	0.315
Glucose [a]	4810 ± 203	490 ± 197	1860 ± 224	0.267	0.318
Day 20					
Acetate	8452 ± 3764	383 ± 215	4726 ± 2600	0.080	Tr. desmosterol
Glucose	1226 ± 408	30 ± 19	456 ± 194	0.066	Tr. desmosterol
Day 90					
Acetate	508 ± 137	.....	NCO [c]	.....	No desmosterol
Glucose [a]	318 ± 144	.....	NCO	.....	No desmosterol

[a] One flask lost.
[b] Std. deviation.
[c] Not carried out.

Grossi et al. (28) compared the conversion of acetate, butyrate, mevalonate and glucose to cholesterol by young (10-day) and older (60-day) rat brain slices and found that acetate was the most efficient precursor at both ages. Garattini et al. (29) also reported that acetate was a better precursor of cholesterol than mevalonate. Nicholas (30) found that mevalonate was more efficiently utilized for cholesterol synthesis than acetate by brain slices from one-year-old rats. In a later experiment, Grossi et al. (31) found acetate to be a better precursor of brain cholesterol than mevalonate when incubated with brain slices from 12-day-old rats, but the reverse was true when 90-day rats were used. At either age, glucose was a poor substrate for cholesterol biosynthesis. Smith (32) has found that acetate and glucose are equally good precursors of rat brain stem and spinal cord cholesterol in vitro.

We incubated slices prepared from brains of 1-, 5-, 20- and 90-day-old rats with both acetate-2-^{14}C or glucose-U-^{14}C. We used 0.5 g of brain tissue per incubation. With the younger animals we pooled tissues so that one flask each of acetate and glucose was incubated with brain tissue from the same source. With the older animals we cut the brain in half and prepared 0.5 g of slices from each hemisphere. The data are summarized in Table VIII. It is seen that in brain slices from young rats acetate is indeed a much more efficient precursor of cholesterol than is glucose. The ratio of radioactivity of desmosterol to cholesterol is lower than is the weight ratio, in contrast to the results obtained in vivo after intracerebral or intraperitoneal inoculation of various precursors. The slice experiments were carried out with tissues which had desmosterol present, and the discrepancy between count and weight D/C ratios may reflect dilution with endogenous sterol as well as differences in the rates of conversion of desmosterol to cholesterol, since the incubation mixtures were augmented with DPN and ATP. The peak biosynthetic activity in the 5-day-old brain is noteworthy. Even at day 20 a slight synthesis of desmosterol is observed, but in the adult animals no desmosterol was present. The slice experiments also show the endogenous synthesis of desmosterol. The discrepancies in glucose utilization between slice and injection are of interest and suggest an area for future investigation.

In an effort to further establish the direct conversion of desmosterol to cholesterol by rat brain, radioactive desmosterol, prepared by TLC isolation from pooled radioactive brain sterols obtained in earlier experiments, was incubated with tissue slices prepared from brains of 6-day-old rats. The desmosterol used (2250 cpm), which was shown by gas chromatography to be uncontaminated by cholesterol, was emulsified in Gey's buffer with Tween 20 and added to the tissue slices suspended in Gey's buffer containing DPN and ATP. After 2 hours of incubation in an oxygen atmosphere at 37C the reaction mixture was treated with 15% alcoholic KOH and the nonsaponifiable material extracted into petroleum ether. The free sterol fraction was subjected to TLC on $AgNO_3$-impregnated Silica Gel H and the desmosterol and cholesterol bands eluted with chloroform-methanol 2:1. The two sterol fractions were subjected to gas chromatography and shown to be pure desmosterol and cholesterol, respectively. Of the radioactive desmosterol incubated, 18% was recovered as desmosterol and 82% as cholesterol. These data demonstrate the conversion of desmosterol to cholesterol by brain slices in vitro.

The genesis of these experiments was an interest in the possible transfer of desmosterol from mother to fetus. We initially administered triparanol (20 mg/kg/day) to six pregnant rats for a period of 20 days. Several of the mothers failed to deliver any young and two gave birth to small, stillborn litters. Gas chromatography of the brains of the stillborn rats yielded very large D/C ratios and gave evidence of another sterol which may be $\Delta^{7,24}$-cholestadiene-3β-ol. This sterol was present in larger amounts than was desmosterol. Three rats from one litter yielded brains weighing an average of 0.191 g with an average D/C ratio of 4.36. Three rats from another litter yielded brains weighing 0.176 g with an average D/C ratio of 5.49.

Roux and Dupuis (33) administered 1 g/kg (half the LD_{50}) of triparanol to 5 pregnant rats on their seventh day of pregnancy with the result that three of the rats aborted and the other two gave birth to malformed young. Wexler, et al. (34) have reported that when rats are injected subcutaneously with 1–2 mg of triparanol three times per week, the results

TABLE IX

Desmosterol/Cholesterol Ratios in Brains of Rats Born to Triparanol-Treated Mothers

Number	Brain wt. (g)	D/C
Group 1 (stillborn) [a]		
1	0.180	4.80
2	0.192	3.12
3	0.200	5.17
Group 2 (stillborn) [a]		
1	0.170	5.31
2	0.175	8.30
3	0.183	2.85
Group 3 (viable) [b]		
Age (days)		
5	0.355	2.01
8	0.385	1.78
13	0.585	0.31
18	0.970	0.10

[a] Mother treated with 20 mg/kg/day for 20 days.
[b] Mother treated with 20 mg/kg/day for first 10 days.

are impaired conception, prolonged gestation, fetal resorbtion and stillbirths. The triparanol effect can be counteracted by administration of ACTH. Scallen et al. (35) injected mice intraperitoneally with triparanol (600 μg every third day for 30 days) and found that brain slices from the treated animals synthesized desmosterol and $\Delta^{7,24}$-cholestadiene-3β-ol.

In one of the pregnant rats which had been treated with triparanol for 10 days, the treatment was suspended for the last half of the pregnancy. This rat gave birth to a litter of four young whose brains were assayed for desmosterol and cholesterol at 5, 8, 13 and 18 days. It can be seen from Table IX that the effect of the drug had begun to dissipate by the 13th day and in the 18-day-old rat brain the D/C ratio was normal.

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