Studies on Brain Sterols in Normal and Pathological Conditions

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Abstract

Desmosterol (24-dehydrocholesterol), a normal constituent of developing brain, rapidly disappears during the prenatal and postnatal development, according to the degree of maturation, in all species considered (chicken, mouse, rat, guinea pig, man).

During brain maturation, desmosterol concentrations are sensitive to treatments with drugs interfering with sterol synthesis (Triparanol) or with nervous tissue differentiation (thyroxine and propylthiouracil).

Desmosterol has been also detected in some experimental and human glial tumors, and the concentra. in the tumor may be specifically increased in the tumoral tissue by the administration of Triparanol.

Introduction

SEVERAL STEROLS OTHER than cholesterol have been identified in brain extracts, but only in traces or in small amounts. The presence of dihydrocholesterol (1), 7-dehydrocholesterol (2,3), 24-hydroxycholesterol, 7β-hydroxycholesterol (4) and lathosterol (5) has been shown, but there has been no demonstration that these compounds are normal constituents of the nervous system.

More recent investigations have shown that desmosterol (24-dehydrocholesterol) is present in considerable amounts as a normal constituent of nervous tissues of the chick embryo (6) newborn rat (7) and human foetus (8) although absent from rat and human adult brain, and, at all ages, in all other tissues and body fluids investigated. The presence of desmosterol therefore appears to be very specific for developing nervous tissues (9).

In the present report, the sterol composition of growing and adult nervous tissues in different animal species was analyzed by gas chromatography. Experimental and human brain tumors were investigated for sterol content in view of the postulated similarities in sterol biosynthesis between normal developing brain and brain tumors (10,11).

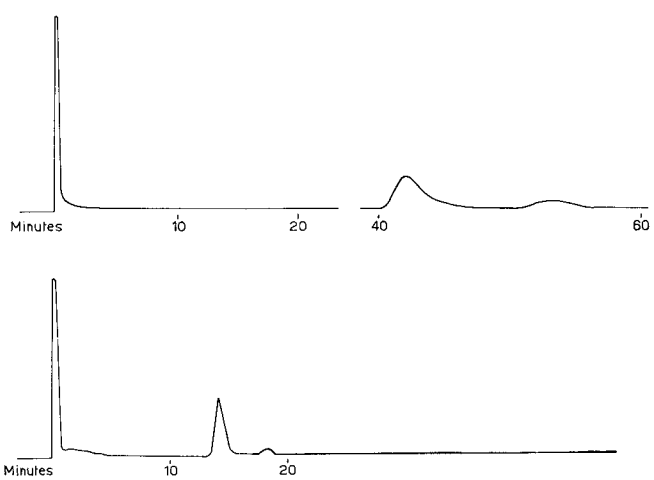

FIG. 1. Gas chromatographic separation of cholesterol and desmosterol and their trimethylsilyl ethers on neopentylglycol succinate. First peak is cholesterol and later peak is desmosterol. Upper tracing H– the free sterols lower tracing H: the silyl derivatives.

Gas Chromatographic Determination of Brain Sterols

Sample Preparation

Samples of normal brain of different animal species and of experimental and human brain tumors have been prepared immediately after animal sacrifice or surgical removal. In the case of human foetuses, frozen immediately after death, the brain samples were removed within 12 hrs. Brain and tumor tissues were washed in saline and necrotic areas discarded. A part of each sample was histologically examined.

Sterol Extraction

The nonsaponifiable fraction was obtained by saponification in 2 N KOH in 95% ethanol for 1 hr at 75C under reflux, and extraction with light petroleum ether (30C–50C) after addition of an equal volume of H_2O. The ether extract was dryed over anhydrous sodium sulfate; the solution filtered, solvent evaporated to dryness under N_2 and the residue dissolved in CS_2 for gas-liquid chromatographic analysis of sterols.

Gas Chromatographic Determinations

A. C. Erba (Milan, Italy) Fractovap Model C gas chromatograph, equipped with a hydrogen flame ionization detection system was used. Columns of 2 mx 4 mm spiral glass tubes were packed according to Horning et al. (11) with PhSi 191-43 (phenylmethylsiloxane polymer containing 40 mole % of phenyl substituent, General Electric Co.) and NGS (neopentyl glycol succinate, Applied Science Laboratories) as stationary phases on Gas-Chrom P 100 140 mesh (Applied Science Laboratories) as solid support.

Nitrogen was used as carrier-gas with flow rates of about 40 ml/min. A vaporizer temp of 260C with column temps of 230C for PhSi and 225C for NGS were used.

Cholesterol and desmosterol are readily separated on both phases (11,12). Separation is obtained on PhSi in about 20 min, but NGS requires a longer time. When the trimethylsilylether derivatives of the sterols prepared according to Luukkainen et al. (13), are used, the retention times of the sterols are considerably reduced on NGS, without modification of area ratio.

Figure 1 illustrates the separation on NGS of sterols

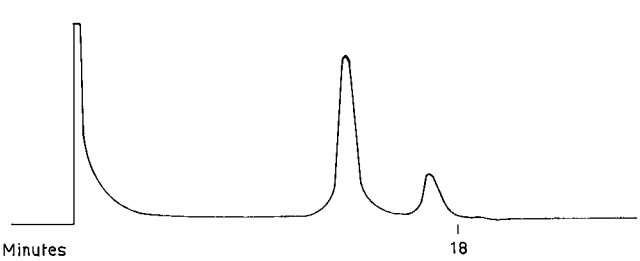

FIG. 2. Gas chromatographic separation of cholesterol and desmosterol on phenylmethyl siloxane polymer. First peak is cholesterol and later peak is desmosterol. Sterols run in the free form.

obtained from the nonsaponifiable fraction of growing rat (12-day-old) brain. In the upper chromatogram the two sterols, cholesterol (early peak) and desmosterol (late leak)are in the free form. The trimethylsilylethers (TMSi) are shown in the lower chromatogram. Figure 2 shows that free sterols are separated quickly on PhSi and there is no advantage in running the TMSi derivatives on this phase. For this reason the PHSi column was selected for routine examination of free sterols in biological samples.

It is to be emphasized that a complete separation of two sterols is a necessary condition for quantitative evaluation of small percentages of one sterol (1–5%) in presence of a large excess of the other. In our hands quantitative separation of desmosterol from cholesterol over a range of ratio (from 4.3% to 90.1% for desmosterol and from 9.9% to 95.7% for cholesterol in a standard mixture) has been obtained as previously shown (13).

Identification of brain sterols was based on the retention time relative to cholestane on PhSi and NGS, and by using standard sterols (Table I).

TABLE I

Retention Times Relative to Cholestane of Brain Sterols on PhSi and NGS

Sterol	PhSi [a]	NGS [b]	
	free sterols	free sterols	TMSi
Cholesterol	2.76	6.22	2.10
Desmosterol	3.46	7.95	2.65

[a] For operating conditions see text. Cholestane time 6.88 min.
[b] For operating conditions see text. Cholestane time 5.44 min.

Brain Sterols at Different Ages in Different Animal Species

The sterol composition of the brain was examined in adult and growing animals of different species. Brains were selected from chicken, mice, rats, and guinea pigs, as well as foetal and adult human brain tissues obtained during necroscopy or neurosurgical operations. Guinea pig was selected particularly because it shows at birth a complete maturation of the nervous system (15). Brain maturation was evaluated according to the classification of McIlwain (16). In the growing brains of the animal species examined, desmosterol was the only sterol, other than cholesterol, present in detectable amts. Figures 3–7 illustrate the pattern of desmosterol concn (expressed in percentage of total sterols) in the brain of the species considered. The brain maturation period is divided into four stages and it is possible to observe that desmosterol disappears during the third stage of maturation after a strong decrease during the last part of the second stage. This occurs in all the selected species and it is independent of the day of birth, although strictly correlated with the degree of brain maturation (as evaluated according to morphological and behavioural patterns) (16).

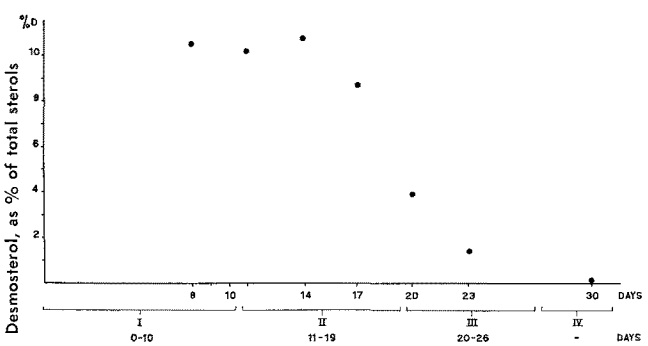

FIG. 3. Desmosterol in growing chicken brain.

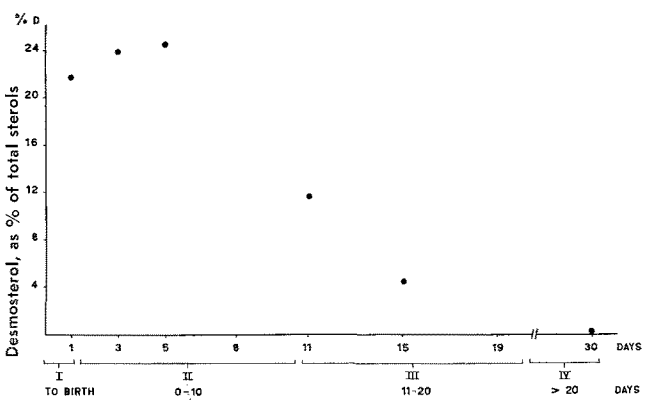

FIG. 4. Desmosterol in growing mouse brain.

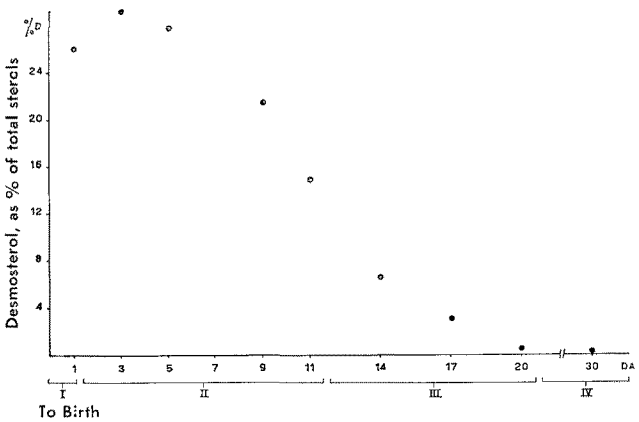

FIG. 5. Desmosterol in growing rat brain.

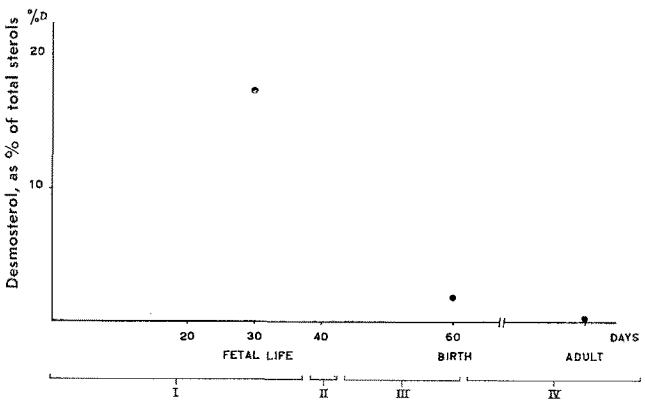

FIG. 6. Desmosterol in growing guinea pig brain.

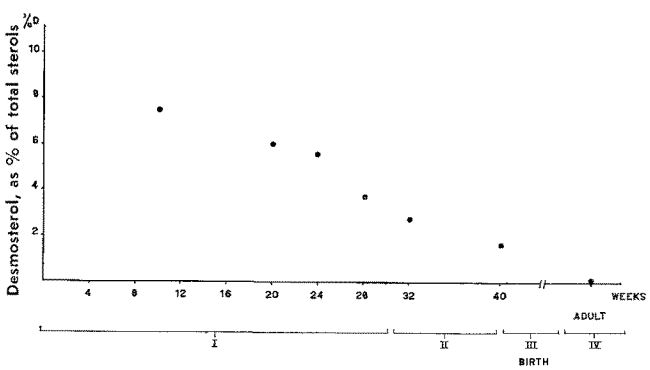

FIG. 7. Desmosterol in growing human brain.

TABLE II
Brain Sterols in Chick Embryos

Incubation days	Total sterols mg/g	Desmosterol % total sterols	Desmosterol μg/g brain
11	3.49	10.2	356
14	3.93	10.7	420
17	5.40	8.7	469
20	8.23	3.9	321
23	9.99	1.4	139

Each figure represents the average of 6 determinations.

TABLE III
Brain Sterols in Growing C 57 Bl/6 Mice

Days	Total sterols mg/g	Desmosterol % total sterols	Desmosterol μg/g brain
3	3.80	23.6	897
	3.67	24.2	888
5	4.39	24.2	1062
	4.61	24.8	1143
11	6.34	11.1	704
	6.07	12.0	728
15	8.68	4.9	422
	8.30	4.2	384

The decrease of desmosterol was not a dilution due to an increase of newly formed cholesterol, but a real disappearance as shown in Table II and III, where calculations of total amts of desmosterol at different ages in chick embryos and mice are shown.

These observations seem to indicate that the presence of large amts of demosterol in brain may play a role during the development of the central nervous system.

Drugs Affecting Brain Sterols

The possible role of desmosterol and other sterols during brain development prompted a test of the effect of drugs acting on animal maturation in order to see if they could change the brain sterol content.

An accumulation of desmosterol in blood and peripheral tissues has been observed after administration of Triparanol (1-[(p-(beta-diethylaminoethoxy) phenyl] 1-(p-tolyl) 2-(p-chlorophenyl)-ethanol). This well-known hypocholesteremic agent has been shown by Steinberg and Avigan (17) to inhibit the reduction of the double-bond in position 24 after cyclization of the squalene chain, and to induce a marked accumulation of desmosterol in blood and peripheral tissues, including brain (18).

Recently, a considerable amt of desmosterol was found by a gas-chromatographic method in the brains of mature rats treated with Triparanol (19). Even larger amts (8% of total sterols) were found after a short period of treatment with 20,24-diazcholesterol (SC 12-937). This suggests either that some cholesterol biosynthesis may still occur in mature brain or that the blood-brain barrier is still permeable to blood desmosterol in adult rats.

It was therefore of interest to attempt to modify the rate of disappearance of brain desmosterol by drug treatment during the brain maturation period. A suitable experimental system for this purpose is the chick embryo.

In chick embryos myelination starts in the cerebellum at the 11th and in cerebral hemispheres at the 14th day of incubation and is almost completed at hatching (21st day of incubation) (20). Desmosterol is constantly present in the nervous tissue during the developing period, but it is found only in traces on the 21st day, and disappears shortly afterwards (21). After treatment with an inhibitor of thyroid function, propyl-thiouracil (PTU), which induces a marked delay in hatching (22), a correlation was found between the prolonged incubation time, the amt of total brain sterols, and the percentage of brain desmosterol. Table IV shows that when chicken embryos were treated with 1 mg PTU, hatching time occurred two days later and the total sterol amt in the brain was lower and the desmosterol concn higher in the treated than in control newly hatched chickens. The mechanism of action of propyl-thiouracil in modifying brain sterol levels and composition is apparently through a thyroid blockade because at the dose used, PTU induces an evident increase of thyroid weight and an alteration of its morphology (22). In addition PTU activity in the embryonic brain becomes evident only after the 14th day of incubation, as soon as thyroid function starts in chick embryos (23). Table V shows that, at the 14th and 17th day of incubation, thyroxine treatment has an opposite effect. These results seem to indicate that brain maturation of chick embryos is under thyroid control.

TABLE IV
Effect of Propylthiouracil on Chick Embryo Development

No. of animals	Treatment	Age (days)	Body weight		Brain (fresh weight)		Total sterols mg/g brain		Desmosterol % of total sterols	
6	—	11	2.779±0.389	n.s.	0.1559±0.0184	n.s.	3.49±0.55	n.s.	10.2±1.10	n.s
6	PTU	11	2.823±0.178		0.1603±0.0100		3.76±0.30		9.1±0.64	
6	—	14	7.851±0.685	n.s.	0.3165±0.0252	n.s.	3.93±0.24	n.s.	10.7±0.59	n.s.
6	PTU	14	7.626±0.616		0.3266±0.0195		3.91±0.17		9.9±0.59	
6	—	17	16.064±1.80	<0.01	0.5321±0.0374	n.s.	5.40±0.35	n.s.	8.7±0.78	n.s.
6	PTU	17	12.130±1.99		0.4845±0.0616		5.47±0.38		9.9±1.02	
6	—	20	24.519±2.52	<0.001	0.6424±0.0692	n.s.	8.23±0.67	<0.05	3.9±0.51	<0.001
6	PTU	20	15.062±1.04		0.6549±0.0529		7.18±0.22		6.1±0.56	
6	—	23[a]	32.916±1.80	<0.05	0.6864±0.0002	n.s.	9.99±0.50	<0.001	1.4±0.38	<0.001
6	PTU	23[b]	28.000±4.25		0.7786±0.0374		8.81±0.45		3.2±0.36	

The treated eggs were injected in the yolk sac with 1 mg propylthyouracil and the controls received the same volume of water at the 8th incubation day.
All the figures ± S.D.
[a] Hatched at the 21st day, killed 2 days after.
[b] Hatching starts at the 23rd day.

TABLE V
Effect of Thyroxine on Chick Embryo Development

No. of cases	Treatment	Age (days)	Body weight		Brain (fresh weight) g		Total sterols mg/g brain		Desmosterol % of total sterols	
6	—	11	3,216±0.338	=0.02	0.2007±0.435	n.s.	3.24±0.47	n.s.	9.7±1.06	n.s.
5	Thyroxine	11	3,735±0.225		0.1868±0.0158		3.49±0.16		9.5±0.81	
6	—	14	9,405±0.893	<0.05	0.3534±0.0173	<0.05	4.22±0.15	<0.01	10.6±0.23	<0.01
6	Thyroxine	14	7,900±1.166		0.3186±0.0345		4.52±0.12		9.5±0.64	
5	—	17	19,254±1.24	n.s.	0.5898±0.016	<0.05	5.51±0.33	<0.01	8.9±0.75	<0.01
6	Thyroxine	17	17,408±3.52		0.5105±0.083		6.24±0.42		6.8±1.10	

All figures ± S.D.
Thyroxine was injected in the yolk sac (μg of L-thyroxine in phosphate buffer at the 8th incubation day).
The controls received the same volume of buffer.
Total sterols were determined according to Zlatkis et al. (25)

TABLE VI
Sterol Composition of Transplantable Mouse Brain Tumors

N.	Type of tumor	Origin	Sterol composition
1	Ependymoma	(1)	Cholesterol only
2	Ependymoma n. 15	(2)	Cholesterol only
3	Ependymoma n. 48	(2)	Cholesterol only
4	Ependymoma ZE	(2)	Cholesterol only
5	Ependymoma Z 131	(3)	Desmosterol 2.5%
6	Astrocytoma G 26A	(2)	Cholesterol only
7	Polymorphic glioblastoma G 26B	(2)	Cholesterol only
8	Spongioblastic glioblastoma G 26 C	(2)	Desmosterol 2.3%
9	Neuroblastoma C 1300	(4)	Cholesterol only

(1) R. A. Stein.
(2) Massachusetts General Hospital.
(3) Albert Einstein College of Medicine.
(4) Roscoe Jackson Memorial Laboratories.

Desmosterol in Experimental Tumors of Nervous Tissue

The relations observed between sterol metabolism in normal growing nervous tissues and brain tumors (10,11) prompted an investigation of sterol composition in experimental brain tumors. Nine different murine transplantable brain tumors were obtained from the sources noted in Table VI.

Among the nine different types of tumors studied, five were ependymomas, two glioblastomas, one astrocytoma, and one neuroblastoma. Table VI shows the presence of amts of a sterol other than cholesterol for only two of these tumors. In both cases this sterol was desmosterol, which represents 2.3% of the total sterol in a spongioblastic glioblastoma and 2.5% in the ependymona Z 131.

Two of these tumors were selected for transplantation; one of them (glioblastoma G 26C) contained desmosterol. The histological constancy of these tumors was regularly checked. Table VII shows the desmosterol content in G 26C tumors at different growth stages after transplantation.

Desmosterol, was found at all stages of growth in the spongioblastic glioblastoma. On the 10th day after transplantation, desmosterol represents 3% of the total sterols, and more or less the same percentage is present in the following days. The degree of development of the tumor does not seem to influence the percentage of desmosterol.

Samples of normal brain, plasma and liver have also been examined for sterol composition and no trace of desmosterol or other sterols different from cholesterol was detected in the normal tissues.

Desmosterol in Human Brain Tumors

Table VIII shows the analyses of 40 human brain tumors which were examined for sterol composition. Fifteen were nonglial tumors and 25 were gliomas of various stages of immaturity. Among the nonglial tumors only a cholesteatoma contained an unidentified sterol different from cholesterol (4%). Sterol composition in gliomas differed in that most of these tumors contained desmosterol and another sterol (probably zymosterol). The presence of these precursors of cholesterol was particularly high in the most immature tumors.

TABLE VII
Desmosterol Content in Murine Transplantable Spongioblastic Glioblastoma (G 26C)

Days after transplantation	Tumor W. weight mg	Desmosterol[a]	Notes
10	129	3.0	.....
12	250	3.0	.....
14	190	2.6	.....
18	832	2.4	large necrotic areas
24	392	2.2	large necrotic areas
30	93	3.3[b]	atrophic tumors with internal bleedings

[a] Desmosterol values are expressed as % of total sterols in tumors. Each value is the mean of two determinations.
[b] Plus 1.5% of unidentified sterol.

TABLE VIII
Sterol Composition of Human Brain Tumors

No. of cases	Type of tumors	Sterols present
	Non-glial tumors:	
5	Meningeomas	Cholesterol only
5	Neurinomas	Cholesterol only
3	Metastatic carcinomas	Cholesterol only
1	Angeoma	Cholesterol only
1	Cholesteatoma	4% of unidentified sterol
	Gliomas:	
5	Astrocytomas	Cholesterol only
1	Ependymoma	Cholesterol only
1	Oligodendroglioma	D 2%; traces of 2 other sterols
1	Ganglioneuroma	D 1%
1	Medulloblastoma	D traces
2	Astrocytic gliomas	Z 1%
14	Glioblastomas	of which 7 containing D (1–4%) 4 Z 1% 3 cholesterol only

D = desmosterol
Z = zymosterol (?)

In a number of patients, with suspected intracranial tumors, triparanol was administered orally at a daily dose of 500 mg, for different periods of time.

The purpose of this treatment was to determine whether the accumulation of desmosterol would be specific for brain tumors. This would be expected because brain tumors actively synthesize cholesterol from simple precursors, in contrast to adult normal brain (10). After a treatment ranging from 7 to 44 days, operations were performed on the patients. Immediately before the operation, samples of blood were withdrawn, and during the operation samples of the tumors and of normal surrounding brain tissues were removed and histologically identified. Quantitative gas chromatographic examination for sterol composition was carried out in plasma, brain and tumor samples, as described before. The results are shown in Table IX. The amt of desmosterol in plasma was significant after a short (7-day) treatment (7.4–12.6% of total sterols) and increased regularly when treatment was prolonged, and a plateau of 15%–20% of total sterols was reached after 2 weeks. Normal brain and normal cerebellum did not show even a trace of desmosterol after 44 days of triparanol treatment. In contrast to this, brain tumors (from the same patients) in all cases contained considerable amts of desmosterol. In one case plasma desmosterol represented 18.7% of the total sterols and no trace of desmosterol was detectable in brain. In one of the patients with a meningeoma a sample of the normal dura was obtained. The percentage of desmosterol in this non-nervous tissue was fairly high in contrast with the total absence of this sterol in normal brain.

TABLE IX
Triparanol Treatment and Sterol Composition of Human Brain Tumors

Case No.	Sex	Age	Days of treatment[a]	Type of tumor	Desmosterol in tissues (% of total sterols)
1	F	29	18	Meningeoma	23.2 plasma 13.7 normal dura 7.6 tumor
2	F	65	12	Meningeoma	13.7 plasma 0 normal brain 4.3 tumor
3	F	47	44	Acoustic neurinoma	19.2 plasma 0 normal cerebellum 13.3 tumor
4	F	22	7	Angioblastic glioma	7.4 plasma tumor traces
5	F	38	6	Fibrillar astrocytoma	11.2 plasma 2.7 tumor
6	F	25	7	Glioblastoma	12.6 plasma 4.2 tumor

[a] Each patient received daily 500 mg of triparanol by oral route.

This finding underlines the difference between the various tissues, as far as sterol synthesis or uptake of plasma circulating sterols is concerned.

Further study is required to determine whether the relevant levels of desmosterol found in human brain tumors after triparanol treatment arise by local synthesis or deposition of desmosterol from the blood. It is known that human brain tumors actively synthesize cholesterol from labeled precursors (10) in contrast to normal adult brain, but it is also known that the blood-brain barrier is less efficient at the level of the tumors (24). However, direct evidence is still lacking as to whether blood sterols can be taken up and accumulated in human normal and tumor nervous tissue.

The present investigations on brain sterols suggest that the desmosterol levels are correlated with the degree of maturation of the brain. Desmosterol is present in brain during maturation in different animal species, and from the data obtained with PTU and thyroxine, the desmosterol concn seems related with the degree of development. On the other hand, the appearance of desmosterol in pathological specimens such as experimental and human brain tumors confirms the significance of this sterol in immature nervous tissue.

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Lipid Class Composition of Normal Human Brain and Variations in Metachromatic Leucodystrophy, Tay-Sachs, Niemann-Pick, Chronic Gaucher's and Alzheimer's Diseases

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Abstract

Procedures suitable for obtaining representative samples of whole brain and of total grey and white matter of brain are presented and discussed. A procedure is described for the quantitative determination of lipid class distribution of human brain specimens utilizing in sequence: a cellulose column to separate gangliosides and nonlipid material from the remaining lipids, diethylaminoethyl (DEAE) cellulose column chromatography to separate the lipid classes into manageable groups, and finally quantitation of the lipid classes by thin-layer chromatography (TLC). TLC is made quantitative by correlating the amt of charring of spots on chromatograms with the amt of lipid present by means of transmission densitometry. The use of two-dimensional TLC for the analysis of brain lipids and its application to the study of pathological brain specimens is also described.

The application of these procedures to the study of metachromatic leucodystrophy, Tay-Sachs, Niemann-Pick, and Alzheimer's diseases and senile cerebral cortical atrophy is described and data are presented. In two cases of Alzheimer's disease, a large reduction in fresh weight and total lipid of brain were found; the lipid class distribution of whole brain in one case and of total grey and total white matter in another were essentially normal. The lipid class distributions of the brain in metachromatic leucodystrophy, Tay-Sachs disease, and Niemann-Pick disease were shown to be similar to that of normal infant brain except that one sphingolipid was greatly increased in each disease (sulfatide in metachromatic leucodystrophy, one ganglioside in Tay-Sachs disease, and sphingomyelin in Niemann-Pick disease).

Introduction

LIPIDS ARE OF SPECIAL importance to brain. About 50% of the dry weight of brain is lipid. A part of this lipid is derived from brain cell membranes, membranes of subcellular particles and other structures but by far the largest part is derived from the myelin sheath, a special structure about the axons of the neurons. For the past several years we have investigated the relationships between lipid composition of the human brain and developmental stages, the aging process, and specific pathological processes.

This report presents procedures for the study of brain lipid class distribution for accurate determination of the composition of whole brain and total grey and total white matter. Results and interpretations for several hereditary metabolic diseases are also presented.

Materials and Methods

Brain Specimens

Fresh (unfixed) specimens obtained as soon as possible were frozen in liquid nitrogen or over dry ice, transported over dry ice, and kept in the frozen state (−20C) until extracted. Each brain was cut longitudinally into equal halves. One-half was used