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Effects of Chronic Alcohol Abuse on the Structural Lipids in the Human Brain

Hepatocerebral Degeneration, I.

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Eight human brains with clinical signs of "hepatocerebral degeneration" (6 alcoholic and 2 post hepatitic in aetiology) were examined in 4 different regions for their content of cerebroside, sphingomyelin, phosphatidyl choline, phosphatidyl ethanolamine, and cholesterol. Compared with normal brains the lipid content was reduced in all these regions. The grey matter showed mainly a reduction of cerebroside, whereas significant loss of cerebroside, phosphatidyl choline, phosphatidyl ethanolamine, and cholesterol was found in the myelin rich areas. Apart from parenchymal oedema, morphological changes were not apparent in the brains examined. These findings suggest that in "hepatocerebral degeneration" there is a pre-morphological, generalised cerebral degeneration involving the structural lipids which cannot be attributed to the physiological ageing process.

Acht menschliche Gehirne mit den klinischen Zeichen der hepatocerebralen Degeneration (6 infolge alkohol-toxischer und 2 infolge posthepatitischer Cirrhosen) wurden in 4 verschiedenen Regionen auf ihren Gehalt an Cerebrosid, Sphingomyelin, Phosphatidylcholin, Phosphatidyläthanolamin und Cholesterin untersucht. Verglichen mit Normalgehirnen war der Lipidgehalt in den untersuchten Regionen reduziert. Die graue Substanz zeigte insbesondere eine Verminderung an Cerebrosid, während in den myelin-reichen Regionen ein signifikanter Verlust von Cerebrosid, Phosphatidylcholin, Phosphatidyläthanolamin und Cholesterin nachzuweisen war. — Von einem Ödem des Gehirnes abgesehen konnten auffallende morphologische Veränderungen nicht gefunden werden. Die Ergebnisse deuten darauf hin, daß es sich bei der hepatocerebralen Degeneration um eine prä-morphologische, allgemeine cerebrale Degeneration unter Mitbeteiligung der Strukturlipide handelt, welche durch den physiologischen Alterungsprozeß allein nicht erklärt werden kann.

The rising consumption of alcoholic drinks in the so called wealthy countries has resulted in alcoholic cirrhosis becoming an increasing social problem. The absolute amount of alcohol which leads to cirrhosis varies from person to person. As a general guide 100—180 grams of pure alcohol per day (1) can lead to cirrhosis within 5—20 years, although it is known that cirrhosis may occur within 1—2 years (2). Neurological and mental changes described as hepatocerebral degeneration are a frequent result of this liver disease.

"Hepatocerebral degeneration" is a syndrome in which liver and brain are simultaneously involved and show both morphological and functional changes (3). One widely recognised difficulty is that cerebral symptoms do not necessarily reflect the degree of liver damage (4). The clinical signs of hepatocerebral degeneration may appear days before hepatic coma or sometimes not at all. This lack of correlation is also illustrated by the Wernicke-Korsakoff syndrome in which by definition liver lesion is absent.

Liver insufficiency is accompanied by various metabolic changes. There is a clear rise in the blood level of certain metabolites, particularly those derived from protein breakdown. These include ammonia, free phenols, phenylcarbonic acids, indole and amines. One current view of the aetiology and pathogenesis of hepatocerebral degeneration is that the substances mentioned could bring about toxic damage (5) probably due to their lipid affinity (6).

Histological and electronmicroscopical changes are found in the brain in this type of degeneration. These have been described as nerve cell disintegration and parenchymal swelling (7), vacuolation of the astrocytes (8), enlargement of the astrocyte nucleus in the globus pallidus (9) or the appearance of carmine positive substances in the glial cell nuclei (10).

The metabolic abnormalities which preceed morphological damage are unknown and controversial (11). The problem of the metabolic and histochemical changes involved in hepatocerebral degeneration is not dealt with in one of the above accounts (3). As we have previously investigated the lipid and fatty acid composition of normal human brain we decided to make corresponding studies in hepatocerebral degeneration.

Materials and Methods

Normal brains

Six brains of patients aged 50 to 80 years who had died of non-cerebral disease and were free of morbid anatomical changes were examined. The data have been partly published (12, 13) but we carried out supplementary examinations for the purpose of this study.

Degenerated brains

Eight brains of patients who had died as a result of cirrhosis were examined. Six of these were from patients who had alcoholic cirrhosis, two from patients who had post hepatitic cirrhosis. The patients in the alcoholic group had a history of excessive alcohol consumption lasting for years (e. g. patient I and II total intake estimated at between 700 and 1500 l of pure ethanol in 2 years). All patients excepting number V had been on stationary treatment (tab. 1) several times for repeated bleeding from oesophageal varices or pronounced neurological or mental symptoms.

Anamnesis

The difficulties in obtaining exact data about the duration of a patient's alcohol history from himself or from his family are well known. In our patients statements fluctuated in the range 5 to 10 years with repeated inquiry. We are unable to give clear data about the history of the non alcoholic cirrhosis, but in both cases the acute hepatitis dates back to between 25 and 30 years before death. Thus with respect to the duration and severity of alcohol consumption and of the age of the non alcohol-related liver cirrhosis respectively we are dependent upon estimations.

Clinical examinations

During the patients' clinical treatment, the following neurological and mental symptoms were noticed: flapping tremor 6, dysphagia 2, apraxia 2, rigidity 3, drowsiness and periodic stupor 3, indifference 1, and changes in the sleep rhythm 1. Patient II had a coincident generalised psoriasis vulgaris. The serum content of ammonia, free phenols, *p*-hydroxyphenyl acetic acid (tab. 1) were determined in all 6 patients. These serum levels were not proportional to the severity of the clinical symptoms.

Brain regions

20 grams of tissue were taken from the following regions of the brains within 48 h of death:
grey matter (cortex) of cerebrum (frontal, parietal, occipital)
white matter of cerebrum
cerebellum (grey and white matter mixed)
medulla oblongata (and parts of pons).

Lipid extraction and separation

The detailed description of the lipid extraction appeared in previous publications (13, 14). The samples of the single brain regions were deep frozen and kept at — 15° to — 25°C until used. The isolation of the total lipid extract was done in identical ways with the same DEAE and silica gel columns and with the same organic solvents etc.

Using the method of FOLCH (15), 10 g fresh or frozen tissue of each region with 20 times the volume chloroform-methanol 2:1, was homogenized, washed with a salt solution (chloroform-methanol-water 3:48:47 v/v/v + 2.9 g NaCl + 267 mg $CaCl_2$ + 363 mg $MgCl_2$/l), dried, heat coagulated after being redisolved in small amounts of chloroform-methanol 2:1, filtered with extracted filter papers and dried. The substances found correspond to the total protein-free lipids (fig. 1). 400—1200 mg of total lipids were applied to a DEAE-Sephadex column. We eluted the non-acidic or so called neutral lipids (16) quantitatively with 300 ml chloroform-methanol 2:1. The subsequent elution procedure (acidic lipids, free fatty acids, etc., see fig. 1) is not of interest for this paper.

The neutral lipids were separated into 4 fractions with an ammonia silica gel column: cholesterol, cerebroside (incl. ceramide), phosphatidyl ethanolamine (incl. plasmalogens), and a mixture containing phosphatidyl choline and sphingomyelin. The last fraction

Tab. 1 Patients

Pat.	sex	age	Diagnosis	Cause of death	Brain		Clinical Symptoms		Serum levels	
					Autopsy	Histology	psychic	neurological	NH_3	*p*-HPAA*
I	♂	38	Decompensated, alcohol-related liver cirrhosis, Diabetes mellitus	Oesophageal piles haemorrhage	Oedema	(—)	++	+	↑ ↑	(↑)
II	♂	55	Decompensated, alcohol-related liver cirrhosis, Ascites, Psoriasis vulgaris	Hepatargy Hepatic coma Hypokalaemia	Oedema	(—)	+++	++	↑	↑ ↑
III	♂	56	Atrophic, alcohol-related liver cirrhosis	Oesophageal piles haemorrhage	cortic. atrophy	+	+	+++	↑	?
IV	♂	58	Decompensated, alcohol-related liver cirrhosis, Ascites	Cardiac failure Pulmonary oedema	Oedema	(+)	++	++	?	?
V	♀	58	Active, alcohol-related liver cirrhosis, Pyrazolon abuse	Gastric haemorrhage	?	?	+	+	?	?
VI	♂	50	Highly active, alcohol-related, liver cirrhosis	Hepatargy	Oedema	+	+++	++	↑ ↑	↑ ↑
VII	♂	59	Active, post hepatitic liver cirrhosis	Hepatargy, Gastric haemorrhage	(—)	—	—	(+)	—	—
VIII	♂	65	Atrophic, post hepatitic liver cirrhosis	Hepatargy	Oedema	—	++	+	—	—
Controls										
IX	♂	73	Bronchopneumonia	Circulatory insufficiency	normal		—	—	not studied	
X	♂	69	Myocardial infarction	Circulatory insufficiency	normal		—	—	not studied	
XI	♀	69	Pulmonary infarction	Cardiac insufficiency	normal		—	—	not studied	
XII	♀	70	Accident	Accident	normal		unknown		unknown	
XIII	♀	62	Carcinoma of the ovary	Cachexia	normal		—	—	not studied	
XIV	♂	79	Carcinoma of the prostate	Cachexia	normal		—	—	not studied	

* *p*-hydroxyphenyl acetic acid

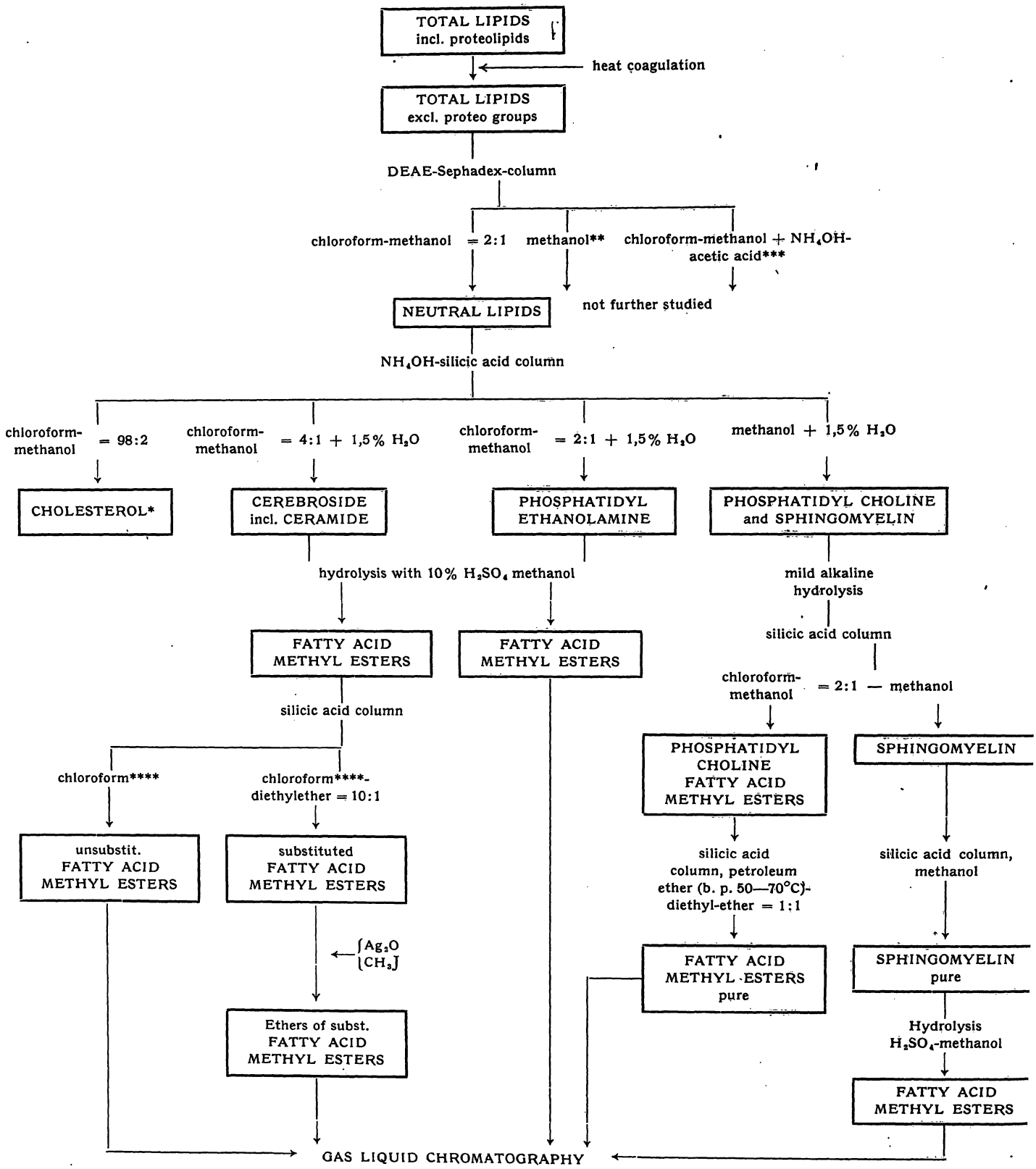

Fig. 1

Elution scheme for isolation of brain lipids and determination of fatty acid methylesters

*) This cholesterol fraction includes small amounts of cholesteryl esters and traces of glycerides

**) Methanol elutes very small amounts of water soluble nonlipid, lysophosphatidyl ethanolamine and some oxidation products of lysophosphatidyl ethanolamine and lysophosphatidyl choline

***) This fraction contains all acidic lipids (sulphatides, phosphatidic acids, phosphatidyl serine, phosphatidyl inositides, gangliosides, free fatty acids, and other minor components)

****) ethanol-free, amylene stabilized chloroform

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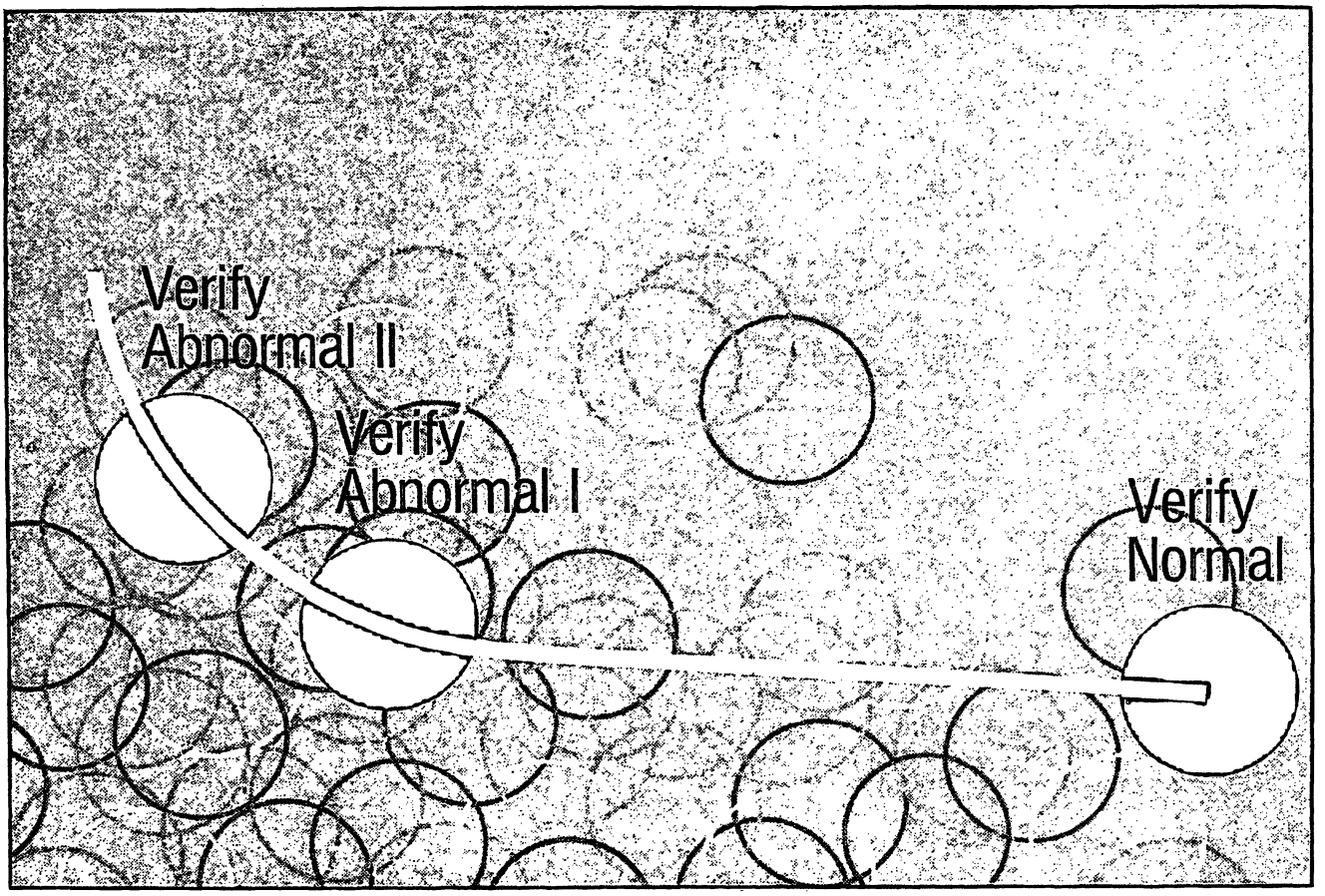

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Tab. 2

Dry weight and total lipids in % of fresh weight; neutral lipids, and lipid fractions in % of total lipids in brains of normals (n = 6) and of patients with alcohol-related liver cirrhosis (n = 6) and with posthepatitic cirrhosis (n = 2). Mean values and standard deviation

		Grey matter			White matter			Cerebellum			Medulla oblongata		
		normal brain	brains of patients with alcohol related	brains of patients with post-hepatitic liver cirrhosis	normal brain	brains of patients with alcohol related	brains of patients with post-hepatitic liver cirrhosis	normal brain	brains of patients with alcohol related	brains of patients with post-hepatitic liver cirrhosis	normal brain	brains of patients with alcohol related	brains of patients with post-hepatitic liver cirrhosis
Dry weight	$\bar{x}$	18.6	17.1	19.6	32.5	34.7	34.0	23.6	23.5	24.2	32.7	32.5	33.2
	s	1.27	1.47	5.16	3.13	2.00	0.78	2.69	1.94	2.47	2.41	2.82	3.11
Total lipids	$\bar{x}$	5.6	4.1	5.6	13.3	14.0	12.5	9.1	6.7	7.8	15.4	13.4	13.3
	s	0.64	0.72	0.64	1.28	2.87	3.04	1.50	1.36	0.49	2.48	2.76	2.55
Neutral lipids	$\bar{x}$	83.8	77.7*	81.7	84.3	67.0***	76.4**	83.0	76.3*	83.2	85.0	70.4***	77.2***
	s	2.50	4.04	0.71	0.85	4.95	1.27	2.32	4.93	1.06	2.09	4.89	0.49
Cholesterol	$\bar{x}$	22.1	21.2	23.5	25.1	21.3*	23.5	23.0	21.7	24.9	25.9	22.5*	23.9
	s	1.06	1.38	0.85	0.58	1.85	0.92	2.14	1.66	1.98	0.56	1.63	0.85
Cerebroside	$\bar{x}$	11.8	8.4*	8.2	21.0	16.3***	17.7	15.1	13.3	13.5	22.5	15.7***	16.1*
	s	2.55	1.87	1.48	2.37	1.19	1.13	1.94	1.69	1.41	1.55	2.21	1.63
Sphingomyelin	$\bar{x}$	5.3	6.3	6.9	8.1	7.1	8.0	7.5	7.4	8.5	8.4	7.2	9.0
	s	0.65	2.32	0.71	0.97	0.69	0.10	1.19	1.30	0.85	0.90	1.39	1.84
Phosphatidyl choline	$\bar{x}$	22.1	20.6	19.9	12.3	8.8**	10.2	15.7	15.2	14.2	11.3	9.4*	9.9
	s	3.24	3.27	1.70	1.61	0.52	0.99	2.31	1.69	1.20	1.20	1.02	1.56
Phosphatidyl ethanolamine	$\bar{x}$	21.3	19.6	21.4	16.3	12.0*	15.1	20.3	16.9	19.4	16.5	13.4*	15.7
	s	2.74	4.34	0.35	1.73	2.40	0.71	1.82	2.87	2.62	1.46	1.26	0.10
Phosphatidal ethanolamine	$\bar{x}$	2.0	1.5	1.9	1.6	1.6	2.1	2.0	1.9	2.8	1.6	1.7	2.4
	s	0.44	0.66	0.10	0.33	0.53	0.14	0.42	0.39	0.10	0.49	0.40	0.57

*) $p < 0.05$ **) $p < 0.01$ ***) $p < 0.001$

was then treated with alkali (0.5 mol/l sodium methoxide in chloroform-methanol (dry) 1:1) (17) for 15 h at room temperature. This hydrolyzed the phosphatidyl choline and the fatty acid methyl esters could be separated from the unchanged sphingomyelin on a silica gel column. The methyl esters and lipid could then be purified separately on further columns.

The phosphatidal ethanolamine (plasmalogen) was determined as the fatty aldehydes by gas chromatography analysis of the fatty acid methyl ester mixture of the phosphatidyl ethanolamine. The amount of the fatty aldehydes was subtracted from the fatty acid mixture to give a value for the calculation of the phosphatidal ethanolamine. In table 2 second row, are shown the amounts of phosphatidyl ethanolamine after the above correction of the determined phosphatidal ethanolamine.

Control of purity

After the gravimetric determination of the total pure lipid extracted, the degree of purity was examined microanalytically (phosphate and hexose determination) and by thin layer chromatography. The cerebrosides of white matter contained nearly theoretical amounts of hexose (92—98%) but these of grey matter seemed to be more contaminated (hexose 87—92% of theoretical amount).

Results

Total and neutral lipids

The total lipids which had been extracted and separated from the protein-containing lipids showed marked differences between the different regions. In the alcohol-related hepatogen degenerated brains the highest lipid content was found in the white matter of cerebrum (14% of the fresh weight), the lowest in the grey matter of cerebrum (4.1%). But the lipid concentration in all parts of the pathological brains examined was lower than in the normal brains. However, the differences are not significant (STUDENT's t-test).

The neutral lipids are obtained by separation on a DEAE-Sephadex column. In pathological brains the neutral lipids constitute 77.7% of the total lipids in grey matter of cerebrum and 67.0% in white matter of cerebrum. The neutral lipids of the pathological brains are therefore significantly diminished because the total lipids of the healthy control brains show more than 80% neutral lipids (tab. 2) in the 4 studied regions. This decrease can also be shown to a lesser degree in both the brains with post hepatitic cirrhosis.

Cholesterol

In the parts of the brain abundant in myelin sheaths (the white matter of cerebrum and medulla oblongata), a significant decrease of cholesterol was found. This decrease was 3—4% in relation to the total lipids or 13—15% in comparison to the cholesterol content in the normal brains (tab. 2). In the regions of the brain with a small content of myelin sheaths (the grey matter of cerebrum and cerebellum), no clear difference between normal and pathological brains was found.

Neutral Sphingolipids

Of all the lipid fractions examined the cerebroside fraction showed the most marked decrease. In 3 regions of pathological brains there was a decrease of 3—7% in relation to the total lipids as opposed to the normal brains: the content was about 8% in the grey matter of cerebrum and 16% in the white matter of cerebrum. However, the sphingomyelin of the pathological brains showed no change compared to normal brains with a concentration of between 6.0 and 7.5%.

Glycerophosphatides (tab. 2)

Of the glycerophosphatides, the phosphatidyl choline and phosphatidyl ethanolamine were examined. In the grey matter of cerebrum roughly 20% lecithin was found and in the cerebellum 15%, with approximately 9% in the white matter of cerebrum and medulla oblongata. The differences in these regions are significant in the pathological brains as well as in the normal brains. The pure phosphatidyl ethanolamine showed roughly the same concentration as the phosphatidyl choline in the scanty myelin regions of the grey matter of cerebrum and cerebellum; its content was about 40% more than the phosphatidyl choline in the regions rich in myelin with a level of between 12% and 13% in comparison to the total lipids. Compared with normal brains, a significant decrease of phosphatidyl choline and phosphatidyl ethanolamine was found in the white matter of cerebrum and medulla oblongata of the pathological brains.

The phosphatidal ethanolamine was equally well represented in the pathological and normal brains. The main components of the separated fatty aldehydes were identified as those with 16 and 18 C-atoms.

Discussion

The lipids of the human brain are essential constituents of the cell membranes or myelin sheaths (18), and specifically participate in the oxidative phosphorylation of nerve cells and the conduction of action potentials in the nerve fibres (19).

From birth until the end of the myelination phase, these structural lipids undergo quantitative changes, but with the exception of slight structural differences in the sphingosine bases (20) qualitative changes could not be demonstrated. The total lipid content increases with ontogenetic development. At birth it amounts to 2—3% of the fresh weight (21, 22), in adults to 5—6% in the grey matter and to 12—16% in the white matter. The proportion of particular lipids also changes with development: phospatidyl choline and cholesterol decrease as myelination proceeds whilst cerebroside and sphingomyelin increase relatively and absolutely (23, 24, 25, 26, 27). In old age a slight and not statistically significant (13) loss of lipid concentration occurs, especially in the cerebroside, which is apparently the result of the physiological ageing process; but this opinion is not uncontested (24).

Similar but more marked quantitative changes are pertinent to hepatocerebral degeneration. The proved significant decrease in neutral lipids (tab. 2) can now be discussed. The proportion of neutral lipids in the main fraction of total lipids amounts is only 67% (normal brains = 84%) in the white matter of cerebrum, about 70% (85%) in the medulla oblongata and 78% (84%) in the grey matter of cerebrum. The decrease of lipids in the non-alcoholic hepatogenic degenerated brains is small and insignificant. The loss of neutral lipids is partly due to lowered cerebroside especially in the grey matter of cerebrum where no other lipid loss could be shown. The decrease of cerebroside in the hepatogen degenerated brains of alcoholic type exceeds that associated with the physiological ageing process and can therefore be considered pathological.

Apart from this a further factor must be taken into consideration regarding the lower content of neutral lipids in the total lipids of the pathological brains: corresponding to the definition, the total lipids no longer contain any proteo groups. According to the methods of FOLCH et al. (15) the separation of the protein components from the protein-containing total lipids (fig. 1) results entirely from the heat coagulation in the extract of normal brains, but not in the pathological brains: According to our studies, the total lipids of these brains still include small amounts of proteo groups, probably with a different composition from those in normal brains. Only these remaining proteins are bound to the DEAE-Sephadex column. The loss of eluted neutral lipids therefore appears too high because the total lipids are not free of protein and for this reason are found in too large amounts.

Apart from the cerebroside losses, the white matter of cerebrum and medulla oblongata show a slight decrease ($p < 0.05$) of cholesterol, phosphatidyl choline and phosphatidyl ethanolamine (tab. 2). Thus there is a regular decrease of all lipids except sphingomyelin in the regions of brain rich in white matter. The general reaction of the brain to disease is the diminution of the lipids (11), particularly of the "myelin lipids", e. g. in the descending WALLERIAN degeneration of peripheral nerves after section (28, 29) in which the content of all lipids regularly falls. The novum of lipid loss in the cerebral degeneration with hepatogenic or/and alcoholic toxic aetiology is the non-uniform diminution of the neutral sphingolipids.

The brains I and VI which we examined showed only discret morphological changes such as oedema and traces of cortical atrophy. The histological characteristics of hepatocerebral degeneration could only be shown in two questionable cases. According to our examination, therefore, there is no exact correlation between morphology and altered lipid composition in hepatocerebral degeneration.

However, the decrease of the neutral lipids i. e. cerebroside, phosphatidyl choline, phosphatidyl ethanolamine and cholesterol is striking. We think, therefore, that the present findings must be classified as a pre-morphological, biochemically manifest degenerative lesion of hepatic and/or alcoholic toxic aetiology.

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