While the sub-units obtained in alkaline and urea solvents may have the same origin, the sedimenting entities are not identical. A loss of lipid occurs in urea solutions and this may result from the formation of urea adducts of the fatty acids. Actually, the reported chemical analyses are compatible with the loss of one fatty acid molecule from each molecule of triglyceride or phospholipid. The sedimentation coefficient ($s^\circ_{20,w}$) of the sub-units is 7.4 S in alkaline solution[2] and only approx. 6.0 S in urea, a difference that can be explained by the changes in the protein moiety caused by urea denaturation.

The molecular weights obtained in urea range from 1.8 to $2.2 \cdot 10^5$, which is half that of lipovitellin ($4.0 \cdot 10^5$) in *M* NaCl. Although the quantitative measurements cannot be made with the same accuracy in 4 *M* urea as in dilute buffer solution, lipovitellin evidently dissociates into two sub-units that sediment as a single boundary, but may not be identical in size or composition.

ACKNOWLEDGEMENT

The first author is a post doctorate fellow of the National Research Council of Canada (1957–1959).

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STUDIES ON THE PLASMALOGENS OF NERVOUS TISSUE

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(Received March 9th, 1960)

SUMMARY

The concentration and distribution of plasmalogens in various regions of the human nervous system and in nervous and other tissues of some animal species have been investigated.

Only traces of choline plasmalogen were detected in any region of the human or animal nervous systems examined. About 50 % of the ethanolamine phosphatide of grey matter and probably all or nearly all that of white matter and spinal cord is present as plasmalogen.

INTRODUCTION

The total plasmalogen content of nervous tissue has been studied by a number of authors in recent years. STAMMLER, STAMMLER AND DEBUCH[1] and RAPPORT AND

LERNER[2] have investigated some regions of the human nervous system, while studies on brain in various animal species have been reported by CHRISTL[3], MINDER AND ABELIN[4] and WITTENBERG, KOREY AND SWENSON[5]. FOLCH-PI[6] and more recently KOREY AND ORCHEN[7], DAVISON AND WAJDA[8] and ERICKSON AND LANDS[9] have studied the variations in plasmalogen content of whole brain as a function of age in animals.

A lipid mixture extracted from whole ox brain was found to contain only very little choline plasmalogen[10]; it was further found that the plasmalogen in the cephalin fraction of whole brain is present mainly as the ethanolamine type, only small amounts being present in the serine phosphatide fraction[11,12].

Although it is now well recognised from these previous studies that white matter contains considerably more plasmalogen than grey matter, little information seems to be available concerning possible variations in the amounts of the different types of plasmalogen lipid, or in the proportion of ethanolamine phosphatide present in the plasmalogen form, in the various regions of the central and peripheral nervous system. In view of increasing interest in these lipids and lack of knowledge concerning their biological significance, it seemed desirable to investigate these points. During the development of procedures suitable for the study of the biochemistry and metabolism of the aldehydogenic moiety of the plasmalogens some information on these questions was obtained and is presented below. Similar data on a few non-nervous tissues are also included for comparison.

EXPERIMENTAL

Materials

Human nervous tissues were obtained post-mortem as soon as possible after death.

Ox brain was obtained from the slaughter house from freshly killed animals.

Monkey spinal cord was removed from the animal immediately after death and deep-frozen for several days before extracting the lipids.

Rats and hens were killed by decapitation and the tissues to be examined removed immediately.

Separation of lipids

The lipids were extracted from the tissues by homogenization in a Waring blendor in 19 volumes chloroform–methanol (2:1, v/v) and the extracts, after filtration, washed with $^1/_5$ their volume of water as described by FOLCH, LEES AND SLOANE-STANLEY[13]. Aliquots of the washed extracts containing 0.3–2.0 mg lipid phosphorus were evaporated to dryness in vacuo at 60° and the lipids separated on columns of alumina[14]. 5 g of alumina (Hopkin and Williams, "Activated, for chromatography") were used for each column; the columns used were 7 cm long and 1 cm in diameter. $CHCl_3$-insoluble protein, which separated from proteolipid during evaporation of the solvent from the washed total lipid extract, was filtered off on the column. Lipids were eluted with a succession of solvents according to the technique of LONG described by DAVISON AND WAJDA[8].

Four fractions were obtained by elution with (a) 20 ml $CHCl_3$, (b) 30 ml chloroform–methanol (1:1, v/v), (c) 15 ml chloroform–methanol–water (7:7:1, v/v/v) and (d) 30 ml chloroform-ethanol–water (2:5:2, v/v/v). These four fractions contained

respectively (a) cholesterol, (b) choline phosphatide (lecithin plus sphingomyelin), (c) cerebroside and (d) cephalin.

In preliminary experiments with rat brain lipids it was found that the fraction eluted with $CHCl_3$ was free of phosphorus and fuchsin-positive material. In the above procedure slight tailing of the choline phosphatide occurred in the final stages of the elution with chloroform–methanol (1:1, v/v) and traces of phosphorus (3–4 % of the initial column loading) were present in the cerebroside fraction; a finding in agreement with that of DAVISON AND WAJDA[8]. Very slight traces of plasmalogen, but no ninhydrin-positive material were detected in the cerebroside fraction, which was therefore combined with the second fraction from the columns for the determination of choline plasmalogen and phosphorus.

Analytical methods

Phosphorus was determined by the method of ERNSTER, ZETTERSTRÖM AND LINDBERG[15] as modified by STRICKLAND, THOMPSON AND WEBSTER[16]. Plasmalogen was determined by the modification of the method of LEUPOLD AND BUTTNER[17] described by GRAY AND MACFARLANE[18]; pure dimethyl acetal of palmitaldehyde (kindly provided by Dr. G. M. GRAY) was used as a standard. Ethanolamine and serine were estimated by the fluorodinitrobenzene method of AXELROD, REICHENTHAL AND BRODIE[19], following hydrolysis of the lipid samples in 6 *N* HCl in sealed tubes for 3 h at 100°. Since the high water content of the cephalin fraction caused considerable foaming if the solvents were evaporated directly, the water and methanol contents were reduced by washing aliquots of this fraction with 2/3 their volume of 0.1 *N* NaCl, two phases then formed and were separated by centrifuging. The phospholipids were recovered quantitatively from the lower $CHCl_3$-rich phase.

Solvents: Chloroform and methanol (Hopkin and Williams, AR) and ethanol (99.4 % Burrough, RR Grade) were used as supplied.

RESULTS

Recoveries of lipids from alumina columns

The total recoveries of lipid phosphorus and plasmalogen in the second and fourth fractions eluted from the columns during chromatography of lipid extracts of various nervous tissues are shown in Table I.

It can be seen that except in the case of human sciatic nerve 75–87 % of the lipid phosphorus applied to the columns was recovered in these fractions. The recovery of lipid phosphorus of human sciatic nerve was rather less (70 %).

The total amount of plasmalogen recovered in these two fractions from human and rat central nervous tissues was 91–103 % of the initial column loading. The recovery of plasmalogen from human sciatic nerve and all the hen nervous tissues studied was, however, less complete (79–87 %).

In a more detailed study of the cephalin fractions from some human and rat nervous tissues it was found that only traces of serine were present in this fraction, which, however, contained 81–91 % (mean 88 %) of the ethanolamine present in the total lipid extracts applied to the columns. It seems likely, therefore, that the incomplete recovery of lipid phosphorus from the column may be due largely to failure to elute the serine phosphatide component of the cephalins.

TABLE I

RECOVERY OF LIPIDS FROM ALUMINA COLUMNS

Sum of amounts of lipid phosphorus and plasmalogen in choline phosphatide (sphingomyelin plus lecithins) and cephalin fractions, expressed as percentage of amounts applied to the column.

Species	*Tissue*	*% recovery*	
		P	*Plasmalogen*
Human	Cortex	78	101
	Putamen	78	101
	Internal capsule	78	95
	Corpus callosum	75	95
	Spinal cord	78	103
	Sciatic nerve	70	79
Hen	Cerebrum	87	87
	Spinal cord	82	80
	Sciatic nerve	81	82
Rat	Cerebrum	79	96
	Spinal cord	81	91

Nervous tissue

The values found for total lipid phosphorus and plasmalogen contents in various regions of the human nervous system are set out in Table II. The choline plasmalogen content, expressed as a percentage of the total, is also shown.

The total amount of plasmalogen in central white matter and in the spinal cord is 3–4 times that found in the cortex or putamen, and the percentage of phospholipid present as plasmalogen in the white matter (30–36 %) was between one and a half and two times that found in grey matter. In peripheral nerve the plasmalogen content of the total phospholipids resembled that of white matter and spinal cord.

TABLE II

PLASMALOGEN OF HUMAN NERVOUS TISSUES

Tissue	*μmoles/g fresh tissue*		*B as % of A*	*Choline plasmalogen as % of B*
	Total lipid P (A)	*Total plasmalogen (B)*		
Cortex	47.0	9.2	20	1.5
	44.5	7.2	16	1.7
Putamen	64.3	13.4	21	2.1
	57.4	9.4	17	3.2
Internal capsule	108.3	32.8	30	3.0
	123.8	40.6	33	3.4
	84.8	30.8	36	3.4
Corpus callosum	108.3	32.9	30	2.9
	99.6	34.5	35	3.5
Spinal cord	88.3	29.6	34	2.6
	104.9	35.9	34	3.7
Sciatic nerve	37.6	13.9	37	3.6
	54.2	16.8	31	3.3

The results found for nervous tissues from various animal species are shown in Table III. Spinal cord in both the hen and the rat contained about twice the amount of plasmalogen present in human cord (Table II). On a molar basis approx. 50% of the phospholipid in the spinal cords of these species was in the form of plasmalogen.

TABLE III

PLASMALOGEN OF ANIMAL NERVOUS TISSUES

Species	*Tissue*	*μmoles/g fresh tissue*		*B as % of A*	*Choline plasmalogen as % of B*
		Total lipid P (A)	*Total plasmalogen (B)*		
Hen	Cerebrum	58.0	15.0	26	2.0
		67.2	16.7	25	1.2
		60.6	14.4	24	1.2
	Spinal cord	111.2	63.9	57	2.4
		149.0	67.6	45	2.5
		140.0	64.5	46	2.4
	Sciatic nerve	77.7	31.2	40	1.3
		71.6	24.9	35	1.0
		65.4	26.3	40	0.8
		79.0	34.3	40	1.7
Rat	Cerebrum	79.0	22.6	29	2.8
		80.0	25.9	32	2.4
		79.0	20.6	26	2.1
		70.0	18.7	27	2.2
		70.4	17.7	25	2.3
		71.7	18.0	25	1.9
	Spinal cord	127.8	64.4	50	3.7
		140.8	64.2	46	4.8
Ox	Cortex	59.0	10.3	17	2.4
	Internal capsule	125.0	37.0	30	3.5
	Medulla	169.0	52.6	31	2.5
Monkey	Spinal cord	120.0	43.4	36	2.4

TABLE IV

LIPID PHOSPHORUS AND PLASMALOGEN OF CEPHALIN FRACTIONS OF NERVOUS TISSUES RECOVERED FROM ALUMINA COLUMNS

Each figure is the mean of at least two experimental results.

Species	*Tissue*	*μmole/g fresh tissue*		*Molar ratios P: Plasmalogen*
		P	*Plasmalogen*	
Human	Cortex	17.1	8.2	1 : 0.48
	Putamen	22.0	11.6	1 : 0.52
	Internal capsule	39.0	33.0	1 : 0.87
	Corpus callosum	35.4	31.0	1 : 0.88
	Spinal cord	36.1	32.8	1 : 0.90
	Sciatic nerve	13.4	11.7	1 : 0.87
Hen	Cerebrum	26.5	13.1	1 : 0.50
	Spinal cord	56.7	50.8	1 : 0.90
	Sciatic nerve	28.8	23.1	1 : 0.80
Rat	Cerebrum	27.4	16.8	1 : 0.62
	Spinal cord	59.8	55.8	1 : 0.93

It is evident from Tables II and III that only traces of choline plasmalogens could be detected in any of the regions of human or animal nervous systems studied.

The results of analyses of the cephalin fractions obtained from human, rat and hen nervous tissues for lipid phosphorus and plasmalogen are shown in Table IV. It can be seen that in the cephalin fractions from various regions of human brain rich in white matter and also in those from spinal cord and peripheral nerve in all three species, 87–93 % of the phospholipid was present in the plasmalogen form. In human cerebral cortex and putamen, on the other hand, only 48 and 52 % respectively of the phospholipid was present as plasmalogen. The value for whole rat cerebrum (62 %) was intermediate between these values for human grey and white matter.

In view of these differences between grey and white matter experiments were carried out with some human and rat nervous tissue to obtain more detailed information concerning the composition of the cephalin fractions. The molar ratios of lipid phosphorus to ethanolamine in these fractions from the tissues examined

TABLE V

ANALYSIS OF CEPHALIN FRACTIONS OF NERVOUS TISSUES RECOVERED FROM ALUMINA COLUMNS

Species	*Tissue*	*μmole/g fresh tissue*				*Molar ratios*
		P	*Plasmalogen*	*Ethanolamine*	*Serine*	*P: Plasmalogen: Ethanolamine: Serine*
Human	Cortex	17.2	7.5	14.7	0.8	1 : 0.44 : 0.85 : 0.05
	Internal capsule	32.3	29.3	29.1	0.5	1 : 0.91 : 0.90 : 0.01
	Corpus callosum	36.8	31.7	35.5	0.5	1 : 0.86 : 0.96 : 0.01
Rat	Cerebrum	27.6	16.8	24.8	1.8	1 : 0.61 : 0.90 : 0.07
		27.1	16.9	25.7	2.3	1 : 0.62 : 0.95 : 0.08
	Spinal cord	59.8	55.4	55.7	4.2	1 : 0.93 : 0.93 : 0.07
		59.8	56.2	57.7	3.1	1 : 0.94 : 0.96 : 0.06

TABLE VI

PLASMALOGEN OF NON-NERVOUS TISSUES

Species	*Tissue*	*μmoles/g fresh tissue*		*B as % of A*	*Choline plasmalogen as % of B*
		Total lipid P (A)	*Total plasmalogen (B)*		
Rat	Heart	33.0	4.4	13	21
		34.0	5.3	14	19
		40.0	3.8	10	12
	Skeletal muscle	19.0	2.9	15	13
		18.5	2.0	11	16
	Kidney	58.5	4.1	7	10
		61.1	4.8	8	11
	Liver	45.0	1.0	2	19
		55.0	0.7	1	25
Hen	Heart	43.8	7.5	17	40
		32.1	7.9	25	46
	Skeletal muscle	9.1	2.8	31	34
		11.6	3.5	30	40
	Liver	36.7	1.1	3	14
		32.2	0.9	3	7

were between 1:0.85 and 1:0.96, while only traces of serine were present. These results are set out in Table V.

Non-nervous tissues

The results of analyses of a small number of non-nervous tissues from the rat and the hen, shown in Table VI, are in general agreement with the findings reported by other workers for the tissues of various animal species. With the exception of hen heart the total amount of plasmalogen in these tissues is considerably less than that of nervous tissues. Liver contains only traces in both species.

Hen heart contains 1.5–2.0 times as much as rat heart, but the values for skeletal muscle are similar in the two species. The percentage of plasmalogen in the total phosphatide was also found to be rather higher in heart and approximately twice as high in skeletal muscle of the hen as compared with the rat. The choline plasmalogen content of heart and skeletal muscle is also considerably greater in the hen than in the rat.

DISCUSSION

In general, in earlier studies on plasmalogen content of tissues the values reported have referred to the wet or dry weight of the tissue. In the present study lipid phosphorus has been used in addition to wet weight as a standard of reference and when expressed in this way the results show good agreement with those of RAPPORT AND LERNER[2].

The results for peripheral nerve, which even after careful dissection still contains an appreciable proportion of connective tissue, show that the proportion of plasmalogen in the total phospholipid is closely similar to that of central white matter and spinal cord in both the hen and man.

The total plasmalogen content of hen heart is comparable to that of the rabbit[2] and the ox[18]. The relatively smaller amount in rat heart would seem to be due at least partly to its lower choline plasmalogen content. The possible significance of this species difference and of the greater proportion of plasmalogen in rat and hen cord as compared with human cord, is obscure because of our ignorance of the biological role of plasmalogens in tissues.

With regard to the composition of the cephalin fractions obtained from nervous tissues the data given in Tables IV and V show that this probably consists of about 85–95 % ethanolamine phosphatide accompanied by traces only of serine phosphatide. Nearly all the plasmalogen and 81–91 % of the ethanolamine phosphatide of the tissues examined was recovered in this fraction. DAVISON AND WAJDA (personal communication) have shown, in similar studies with rabbit brain, that the inositol phosphatide is quantitatively recovered from alumina columns in the cephalin fraction. The amount of inositol phosphatide in brain is, however, less than $^1/_{10}$ that of ethanolamine phosphatide[20]. The molar ratios given in Tables IV and V show further that in the cephalin fractions obtained from human grey matter and from rat and hen cerebrum about half the total phospholipid is present as plasmalogen, while in those from white matter, spinal cord and sciatic nerve 80–90 % of the phospholipid is in this form.

It was tentatively suggested some years ago by BRANTE[2], from calculations

based partly on his own results and partly on earlier chemical and histological data on the distribution of lipids in the nervous system, that "most or all of the ethanolamine phospholipids contained in the myelin sheaths are present in the form of plasmalogens". The findings reported in this paper provide direct evidence in support of this suggestion.

ACKNOWLEDGEMENTS

My thanks are due to Professor R. H. S. THOMPSON for his constant encouragement, to Dr. G. M. GRAY for a gift of pure dimethyl acetal of palmitaldehyde and to Dr. A. N. DAVISON and Miss M. WAJDA for permission to refer to some unpublished results of their work. Miss J. FIDLER gave valuable technical assistance.

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