The Magnesium-Ion-Dependent Cleavage of the Vinyl Ether Linkage of Brain Ethanolamine Plasmalogen

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1. There was a significant decrease in the amount of endogenous ethanolamine phospholipids when preparations of whole brain were incubated in bicarbonate-Ringer solutions, leading in particular to the hydrolysis of vinyl ether groups. 2. The hydrolysis of ethanolamine phospholipids in such preparations was abolished in the absence of bivalent cations. 3. An enzyme present in extracts of acetone-dried brain powders that cleaved the vinyl ether linkage in ethanolamine plasmalogen maximally at pH 7.4 required Mg^{2+} for activity. 4. The cleavage of the vinyl ether linkage of an ethanolamine lysoplasmalogen was enhanced in the presence of Mg^{2+} but the requirement was not absolute.

The enzymic processes leading to the catabolism of phospholipids in brain tissue have received far less attention than the anabolic reactions, though it is very possible that the distribution of the relevant enzymes and the control of catabolism are factors in the demyelinating diseases. It is also likely that phospholipids are degraded to provide energy-yielding substrates in the absence of exogenous oxidizable substrates (see below).

Sperry (1947) showed that, when whole-brain homogenates were incubated in saline-bicarbonate, there was a loss of lipid P of about 2 μ moles/g./hr. A much smaller loss was observed by Tyrrell (1950), who found that 16% of the 'kephalins' and 12% of the lecithin were hydrolysed in 24 hr. Johnson, McNabb & Rossiter (1949) also showed a loss of the 'kephalin' fraction in cerebral slices but could not demonstrate a loss of lecithin P.

Extracts of brain cortex possess phospholipase-A (phosphatide-acyl-hydrolase) activity towards added phosphatidyl-ethanolamine, -choline and -serine (Gallai-Hatchard, Magee, Thompson & Webster, 1962). Phospholipase-B (lysolecithin-acyl-hydrolase) activity towards added lysolecithin is also exhibited by brain homogenates (Marples & Thompson, 1960). There may be some balance *in vivo* between phospholipase-A activity and the mechanism whereby lysolecithin is acylated to the diacylphospholipid (Webster, 1962). Roitman & Gatt (1963) reported the presence in brain of a phospholipase C (phosphatidylcholine phosphatidohydrolase), active towards phosphatidylcholine and sphingomyelin.

The most active phospholipases in brain appear to be those degrading phosphoinositides (Sloane-

Stanley, 1953; Rodnight, 1956; Dawson & Thompson, 1964; Thompson & Dawson, 1964*a,b*) and phosphatidic acid (Smith, Weiss & Kennedy, 1957; Coleman & Hübscher, 1962). None of the reports so far has dealt extensively with the breakdown of the ethanolamine phospholipids. Evidence for their hydrolysis has come, however, from two studies on hypoglycaemia. Knauff, Marx & Mayer (1961) and DeRopp & Snedeker (1961) observed that there was a release of free ethanolamine and other lipid bases in the brains of animals subjected to insulin hypoglycaemia, i.e. when glucose concentrations in the brain are minimal. These studies showed that the fatty acids of some phospholipids of the brain may be used in the absence of the oxidizable substrates. The experiments described below stem from an investigation of the breakdown of phospholipids *in vitro* in the absence of added oxidizable substrate, of which a brief report has already been given (Ansell & Spanner, 1963*b*).

MATERIALS AND METHODS

Materials

Aluminium oxide ('for chromatographic adsorption analysis') was a British Drug Houses Ltd. product. Solvents were of A.R. quality.

The ethanolamine plasmalogen (glycerol 2-acyl-1-alkenyl-3-phosphorylethanolamine) used throughout the experiments was prepared by the method of Ansell & Spanner (1963*c*) from ox-brain corpus callosum, a tissue particularly rich in this lipid (Webster, 1960). A typical preparation had P:ethanolamine:acyl ester:vinyl ether molar proportions 1.0:1.0:1.0:0.99. Occasionally preparations had a lower unsaturated ether content, owing to the presence of the saturated ether analogue.

A lysoplasmalogen (glycerol 1-alkenyl-3-phosphoryl-ethanolamine) was prepared as follows. The ethanolamine lipid fraction ($\approx 160 \mu\text{moles of P}$), free from cerebrosides but containing some phosphatidylethanolamine (Ansell & Spanner, 1963c), was dissolved in a mixture of 8 ml. of carbon tetrachloride, 75 ml. of ethanol and 6.5 ml. of water. After the addition of 2.5 ml. of N-NaOH the mixture was incubated at 37° for 1 hr. This hydrolysis deacylated the phosphatidylethanolamine, the ethanolamine plasmalogen and any saturated ether lipid present (Ansell & Spanner, 1963a); the mixture was then neutralized by the addition of 4 ml. of ethyl formate and evaporated to dryness under reduced pressure at $< 50^\circ$. The residue was dissolved in 10 ml. of 'upper phase' and 20 ml. of 'lower phase' of the phases obtained by mixing chloroform, 2-methylpropan-1-ol and water (containing 0.25% of cetyltrimethylammonium bromide) in the proportions 4:2:1 (by vol.) (Dawson, Hemington & Davenport, 1962). After being warmed and shaken, the mixture was centrifuged, the lower phase evaporated to dryness under reduced pressure and the residue dissolved in 15 ml. of chloroform-methanol (2:1, v/v). This solution was applied to a column of 25 g. of aluminium oxide, already equilibrated with the same solvent. Ethyl esters of fatty acids were not adsorbed and, after these had been washed through, elution with chloroform-ethanol-water (2.5:2, by vol.) (Davison & Wajda, 1959) brought off a lipid containing no ester groups and with P:vinyl ether:N molar proportions 1.0:1.0:1.3 (a second sample had P:vinyl ether:ethanolamine molar proportions 1.0:1.0:1.2).

Tissue preparations

Dispersions. Adult rats were killed, and the brains were weighed and dispersed in Krebs bicarbonate-Ringer solution (Krebs & Henseleit, 1932; Krebs, 1933) in an all-glass homogenizer of the Potter-Elvehjem type (A. W. Dixon and Co., London, S.E. 20). After dilution to 125 mg. fresh wt. of brain/ml., 3 ml. samples were pipetted into Warburg vessels and incubated at 38° in an atmosphere of $\text{O}_2 + \text{CO}_2$ (95:5).

'Prisms.' After removal of the brain it was cut transversely into slices about 3 mm. thick. These slices were chopped into prisms 0.2 mm.² in cross-section with the apparatus of McIlwain & Buddle (1953). These were dispersed in Krebs bicarbonate-Ringer solution to give a suspension of 150 mg. fresh wt./ml., which could be pipetted. Such a preparation contained a preponderance of whole cells, and 3 ml. was incubated at 38° in an atmosphere of $\text{O}_2 + \text{CO}_2$ (95:5).

After incubation, samples were mixed with 20 vol. of chloroform-methanol (2:1, v/v), heated briefly to the boiling point and redispersed. The mixture was filtered, the residue was washed with chloroform-methanol (2:1, v/v) and the combined filtrates were evaporated to dryness under reduced pressure; the residue was redissolved by the addition of chloroform-methanol (1 ml.) and evaporated to dryness twice more. This ruptured any proteolipid linkages (Folch, Ascoli, Lees, Meath & LeBaron, 1951). The lipid residue was dissolved in 10 ml. of chloroform-methanol (2:1, v/v) and was washed with 0.73% NaCl by the method of Folch, Lees & Sloane-Stanley (1957). Washed solutions of lipid were evaporated to dryness and dissolved in carbon tetrachloride.

Acetone-dried powders. All operations were carried out at 0° . A whole adult rat brain was dispersed in 4 vol. of ice-cold

acetone and the mixture filtered on a sintered-glass funnel. The residue was re-suspended in 4 vol. of acetone and re-filtered; the process was repeated with a further 6 vol. of acetone and 6 vol. of diethyl ether. The acetone-dried powder was kept in a vacuum desiccator at 4° for at least 1 hr. before use. A 1 g. sample of powder was equivalent to approx. 6.7 g. fresh wt. of tissue.

Extracts of powder were prepared in a 1:1 (v/v) mixture of 50% (v/v) glycerol and 1% (w/v) sodium bicarbonate, as described by Magee, Gallai-Hatchard, Sanders & Thompson (1962). Sometimes this was dialysed against the same concentration of glycerol-bicarbonate. Samples of the enzyme extract equivalent to 25 mg. of acetone-dried powder (0.5 ml.) were incubated with 0.5 ml. of 0.1 M-glycylglycine buffer, pH 7.4, containing 2 mg. of sodium deoxycholate and the appropriate lipid substrate. Lipids were extracted with 4 ml. of chloroform-methanol (2:1, v/v) (Magee *et al.* 1962). A water-methanol phase was obtained that contained any water-soluble breakdown products of phospholipids.

Phospholipid analyses

The so-called alkali-labile phospholipids, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidic acid, were determined on an amount of lipid equivalent to 350 mg. fresh wt. of brain by the method of Dawson (1960). Alkali-stable acid-labile phospholipids, the ethanolamine plasmalogen and traces of the plasmalogens of choline and serine, were determined by the method of Dawson *et al.* (1962) as modified by Ansell & Spanner (1963c). Lipid-bound ethanolamine was determined by the method of Axelrod, Reichenthal & Brodie (1953), after hydrolysis of the sample in methanolic 6 N-HCl for 3 hr. (Davison & Wajda, 1960). Phosphorus was determined by either the method of Fiske & Subbarow (1925) for amounts in the range 0.3–1.7 $\mu\text{moles of P}$, or that of Ernster, Zetterström & Lindberg (1950) for amounts in the range 0.03–0.3 $\mu\text{mole of P}$. Samples were wet-ashed with HClO_4 before determination of P. Vinyl ether groups in lipids were determined by the spectrophotometric method of Gottfried & Rapport (1962), and the correction suggested by Rapport & Norton (1962) was applied. Free and total fatty aldehydes were determined by the method of Gray & Macfarlane (1958), carboxylic esters by the method of Stern & Shapiro (1953) and total lipid N by the method of Weil-Malherbe & Green (1955).

RESULTS

Breakdown of phospholipids in preparations of whole rat brain

When brain-tissue preparations were incubated in oxygenated bicarbonate-Ringer for 3 hr. at 38° there was a considerable decrease in the total lipid-bound ethanolamine (Table 1). Analysis of the individual phospholipids indicated that there was a breakdown of both phosphatidylethanolamine and the ethanolamine plasmalogen. With prisms there was a slightly lower loss of ethanolamine lipids, but the most striking difference between dispersions and prisms was found in phosphatidylcholine; this lipid increased in amount in dispersions and de-

Table 1. *Changes in the amounts of phospholipids in brain preparations incubated in Krebs bicarbonate-Ringer solution*

Experimental details are given in the text. The results are expressed as $\mu\text{moles/g. fresh wt. of brain/3 hr.}$, and are given as means $\pm$ S.D. with the numbers of experiments in parentheses.

	Dispersions	Dispersions with no bivalent cations	Prisms
Decrease in total lipid ethanolamine	4.4 ± 0.23 (4)	—	—
Decrease in phosphatidylethanolamine	1.9 ± 0.37 (4)	0^* (4)	1.5 ± 0.33 (4)
Decrease in ethanolamine plasmalogen	1.76 ± 0.17 (4)	0^* (4)	1.2 ± 0.18 † (7)
Change in phosphatidylcholine	$+1.89 \pm 0.14$ (4)	$-0.48 \pm 0.3^*$ (4)	-1.35 ± 0.14 (4)
Increase in phosphatidic acid	0.57 ± 0.28 (4)	0^* (4)	2.85 ± 0.3 (4)

* Ca^{2+} and Mg^{2+} were omitted from the suspending medium and EDTA (final concn. 1 mM) was added.

† Measured by selective hydrolysis.

‡ Measured as vinyl ether groups on the alkali-stable fraction.

Table 2. *Breakdown of total brain phospholipids by extracts of acetone-dried brain powders*

Experimental details are given in the text. The results are expressed as $\mu\text{moles/g. of acetone-dried powder/hr.}$, and are given as means $\pm$ S.D. with the numbers of experiments in parentheses.

	Undialysed extracts	Dialysed extracts
Increase in water-soluble phosphate	8.4 ± 1.2 (4)	2.9 ± 0.3 (7)
Increase in water-soluble ethanolamine	3.7 ± 0.2 (6)	0 (4)
Decrease in total lipid P	10.7 ± 1.3 (3)	0.63 ± 0.02 (8)
Decrease in phosphatidylcholine	4.3 (2)	0 (2)
Decrease in phosphatidylethanolamine	2.7 (2)	0 (2)
Decrease in ethanolamine plasmalogen	2.7 (2)	0 (8)

creased when large numbers of whole cells were present. There was a significant increase of phosphatidic acid in the preparations of prisms; this increase was not seen in the dispersions. These changes in the amounts of phosphatidylcholine and phosphatidic acid have not been investigated further. It is conceivable that some methylation of phosphatidylethanolamine to phosphatidylcholine occurred.

When Ca^{2+} and Mg^{2+} were omitted from the Ringer solution and EDTA (1 mM) was added, the changes in the amounts of the phospholipids were almost completely inhibited (Table 1).

Breakdown of total brain phospholipids with extracts of acetone-dried brain powder

Gallai-Hatchard *et al.* (1962) have shown that an extract of acetone-dried powder made with glycerol-bicarbonate could convert added phospho-

tidylcholine and -ethanolamine into the corresponding lyso compounds (phospholipase-A activity). It was found that an extract prepared by this method was heavily contaminated with water-soluble phosphates that could be removed by dialysing the extract at 0° for 16 hr. against glycerol-bicarbonate.

Samples of rat-brain phospholipids ($\approx 5.4 \mu\text{moles of P}$), suspended in 0.5 ml. of 0.1 M-glycylglycine buffer containing 2 mg. of sodium deoxycholate, were incubated with 0.5 ml. of a glycerol bicarbonate extract (dialysed or undialysed) equivalent to 25 mg. of acetone-dried powder at 38° . Table 2 shows that, in the presence of the dialysed extract, there was a release of $2.9 \mu\text{moles of P/g. of powder/hr.}$ and $0.7 \mu\text{mole of ethanolamine/g./hr.}$ (this free ethanolamine was determined in a sample of the aqueous phase obtained on extracting the incubated mixture with chloroform-methanol). There was a breakdown of phosphatidylcholine, phosphatidylethanolamine and the ethanolamine plasmalogen. No breakdown was observed when boiled extracts were used. With dialysed extracts the release of water-soluble phosphates was decreased and there was only a slight breakdown of phospholipid. Since the ethanolamine plasmalogen is the predominant ethanolamine lipid in brain the action of acetone-dried brain powder on this phospholipid was studied further.

Breakdown of ethanolamine plasmalogen by extracts of acetone-dried brain powder

For these experiments $3.0\text{--}5.4 \mu\text{moles of ethanolamine plasmalogen}$, solubilized with deoxycholate, were incubated with an amount of extract equivalent to 25 mg. of powder and glycylglycine buffer, as described above, for 3 hr. In the presence of undialysed extracts there was a loss of vinyl ether groups, fatty acyl groups and lipid P. The loss in

Table 3. *Hydrolysis of ethanolamine plasmalogen by extracts of acetone-dried brain powders incubated at 38° for 3 hr.*

Experimental details are given in the text. The results are expressed as μ moles/g. of acetone-dried powder/hr., and are given as means $\pm$ S.D. (where appropriate) with numbers of experiments in parentheses.

	Increase in water-soluble phosphate	Decrease in lipid P	Decrease in acyl ester groups	Decrease in vinyl ether groups
Undialysed extract	8.9 ± 4.0 (4)	7.1 (2)	17.6 ± 6.7 (4)	12.0 ± 1.3 (4)
Dialysed extract	0 (4)	1.7 (2)	0 (4)	0 (4)
Dialysed extract + Mg^{2+} (10 mM)	0 (4)	0 (6)	0 (6)	14.5 ± 2.5 (9)
Dialysed extract + Ca^{2+} (10 mM)	0 (2)	0 (2)	3.9 (2)	$14.4 \pm 2.98^*$ (4)
Dialysed extract + Ca^{2+} (10 mM) + Mg^{2+} (10 mM)	0 (2)	0 (2)	0 (2)	0 (2)
Dialysed extract + Mn^{2+} (10 mM)	—	0 (3)	9.3 ± 0.7 (5)	8.5 (2)
				2.5 ± 2.1 (7)

* Measured by the release of free aldehyde (Gray & Macfarlane, 1958).

Fig. 1. pH-activity curve for the cleavage of the vinyl ether linkage of ethanolamine plasmalogen by an extract of acetone-dried brain powder. The incubations were performed as described in the text with glycylglycine buffer (final concn. 0.05 M) at the appropriate pH.

lipid P was paralleled by an increase in water-soluble phosphates, but dialysis abolished all these effects (Table 3). This effect of dialysis confirms the results obtained with whole brain dispersions (Table 1) and with acetone-dried powder and total brain lipids (Table 2).

Effect of bivalent cations. The addition of Mg^{2+} (10 mM) completely restored the ability of the dialysed extract to cleave the vinyl ether linkage of the plasmalogen (Table 3). The remainder of the molecule remained intact. In the system used, the

optimum concentrations of Mg^{2+} lay between 5 and 10 mM. However, Ca^{2+} (10 mM) did not activate the cleavage of the vinyl ether linkage but partially restored the capacity of the extract to hydrolyse the fatty acyl linkage; this effect was also seen with Mn^{2+} (10 mM), which caused, in addition, a slight breakdown of the vinyl ether linkage. A significant loss of lipid P was found only with undialysed extracts. None of the bivalent cations tested activated the cleavage of the glycerol-phosphate linkage.

Effect of pH. The optimum pH for enzyme activity lay between 7.25 and 7.4 (Fig. 1). All subsequent experiments were carried out at pH 7.4 in glycylglycine buffer. The breakdown in 0.05 M-borate buffer, pH 7.4, was identical with that obtained in glycylglycine buffer.

Effect of different methods of extraction. Extraction of the acetone-dried powder with glycerol-bicarbonate for longer periods had no effect on the amount of activity obtained on centrifuging the mixture.

Comparison between the loss of vinyl ether groups and release of free aldehyde. If the vinyl ether linkage is destroyed by hydrolytic cleavage, the long-chain fatty aldehydes, i.e. stearaldehyde and palmitaldehyde (Thannhauser, Boncoddo & Schmidt, 1951a,b), should be released at the same rate. That this was so is shown in Table 3.

Comparison between loss of vinyl ether groups and release of alkali-labile phosphorus. Removal of the fatty aldehydes from native plasmalogen should yield a lysophosphatidylethanolamine (glycerol 2-acyl-3-phosphorylethanolamine). This product, unlike the intact plasmalogen, yields a water-soluble phosphate ester (glycerol 3-phosphorylethanolamine) on mild hydrolysis with alkali in the method of Dawson (1960). Samples of ethanolamine plasmalogen were therefore incubated with acetone-

Fig. 2. Equivalence between the cleavage of the vinyl ether linkage of brain ethanolamine plasmalogen and the formation of an alkali-labile phospholipid. The incubations were carried out as described in the text. The upper curve does not pass through the origin because contaminating phosphatidylethanolamine was present. ●, Formation of alkali-labile phospholipid; ○, cleavage of vinyl ether linkages.

dried powder extracts under standard conditions for 1–4 hr. There was equivalence between the loss of vinyl ether groups and the release of alkali-labile phospholipid (Fig. 2).

Enzymic cleavage of lysoplasmalogen (glycerol 1-alkenyl-3-phosphorylethanolamine). Warner & Lands (1961) observed that the choline lysoplasmalogen was hydrolysed by liver microsomes, whereas the native choline plasmalogen was unaffected. It was important therefore to examine the activity of brain extracts towards the analogous ethanolamine lysoplasmalogen. Experiments performed with extracts of acetone-dried brain powder in the presence of Mg^{2+} showed that there was rather less cleavage of the vinyl ether linkage than with the native plasmalogen (Table 4). Moreover, dialysed extracts also showed some activity. The loss of vinyl ether linkage was paralleled by the loss of lipid P, since the first product of hydrolysis is glycerylphosphorylethanolamine. In the absence of Mg^{2+} all the water-soluble phosphate was accounted for as inorganic phosphate, indicating that the glycerylphosphorylethanolamine was hydrolysed to ethanolamine (by glycerolphosphorylethanolamine diesterase) and glycerophosphate, which was subsequently hydrolysed by an alkaline phosphatase. Alternatively, but less likely, the glycerylphosphorylethanolamine

Table 4. *Hydrolysis of an ethanolamine lysoplasmalogen with extracts of acetone-dried brain powder at 38° for 3 hr.*

Experimental details are given in the text. The results are expressed as $\mu\text{moles/g.}$ of acetone-dried powder/hr., and are given as means $\pm$ s.d. with the numbers of experiments in parentheses.

	Decrease in phospholipid P	Decrease in vinyl ether groups
Dialysed extract	4.5 ± 0.8 (7)	5.3 ± 1.4 (7)
Dialysed extract + Mg^{2+} (10 mM)	10.1 ± 2.2 (7)	10.4 ± 0.3 (7)

was hydrolysed by an enzyme similar to phospholipase C, and the product of the reaction, phosphorylethanolamine, hydrolysed by alkaline phosphatase. No inorganic phosphate was produced from lysoplasmalogen in the presence of Mg^{2+} .

DISCUSSION

The vinyl ether groups of the ethanolamine plasmalogen of rat brain are hydrolysed in tissue dispersions at a rate of $0.6 \mu\text{mole/g.}$ fresh wt. of tissue/hr. No loss of ether groups occurred in the absence of bivalent cations and in the presence of a sequestering agent. From experiments with acetone-dried powders, it is likely that the intact plasmalogen is attacked before other groups, e.g. acyl esters or phosphorylethanolamine, have been removed.

Extracts prepared from acetone-dried powders of rat brain hydrolysed the ether groups of added ethanolamine plasmalogen in glycylglycine buffer, in the presence of Mg^{2+} (optimum pH 7.4). However, Ca^{2+} inhibited the reaction to some extent and Mn^{2+} could not be substituted for Mg^{2+} . The products of the reaction in this system were shown to be a long-chain aldehyde and a glycerol 2-acyl-3-phosphorylethanolamine. Enzymes hydrolysing other parts of the plasmalogen molecule were either not present in the extract or they lacked the necessary cofactors.

This is, as far as we know, the first evidence for the presence in an animal tissue of an enzyme that attacks the vinyl ether linkage of a native plasmalogen. The enzyme in liver microsomes described by Warner & Lands (1961) attacked only the choline lysoplasmalogen and required no cofactors. It was not active toward the plasmalogen containing ethanolamine; such plasmalogens are in any event absent from liver (Klenk & Dohmen, 1955). In the experiments with ethanolamine lysoplasmalogen

described in the present paper there was some activity in the absence of Mg^{2+} . This finding suggests that the necessity for Mg^{2+} depends partly on the presence of fatty acyl groups in the plasmalogen molecule, though much greater activity was obtained when Mg^{2+} was added.

The activity of the phospholipases and related enzymes in mammalian tissues is low compared with those present in snake venoms and micro-organisms. It was calculated that the phospholipase A of brain studied by Gallai-Hatchard *et al.* (1962) cleaved phosphatidylcholine and phosphatidylethanolamine at a rate of approx. 0.5 μ mole/g. fresh wt. of tissue/hr., whereas the lysolecithinase of liver examined by Dawson (1956) had an activity of about 7 μ moles/g. of liver/hr. The phosphatidate phosphatase of brain hydrolysed added substrates at rates ranging from 17 μ moles/g. fresh wt. of tissue/hr. (Smith *et al.* 1957) to 6 μ moles/g. fresh wt. of tissue/hr. (Coleman & Hübscher, 1962). It is likely that the most active phospholipases in brain, however, are those degrading phosphoinositides. Thus Rodnight (1956) found that a 'diphosphoinositide' preparation was hydrolysed by a brain suspension at a rate of 300 μ moles/g. of tissue/hr. The breakdown of phosphoinositide is complex (Thompson & Dawson, 1964a,b; Dawson & Thompson, 1964) and it is not possible to calculate the activities of the triphosphoinositide phosphodiesterase and phosphomonoesterase in terms of the fresh wt. of the tissue from the results given by the authors. The enzyme obtained from acetone-dried brain powders and described in the present paper cleaved the vinyl ether linkage at a rate equivalent to 2.9 μ moles/g. fresh wt. of tissue/hr. This is five times the rate obtained with fresh dispersions in bicarbonate-Ringer. Though it is difficult to compare this activity with that of the enzyme of Warner & Lands (1961), it appears that the enzyme from liver microsomes had a slightly greater activity.

The significance of an enzyme cleaving the vinyl ether linkage of a plasmalogen is at present only speculative. The enzyme is active at physiological pH and the product of the reaction is a lysophospholipid that might have some detrimental action on brain cells *in vivo*. According to the work of Cuzner, Davison & Gregson (1964) the ethanolamine plasmalogen is a relatively stable constituent of the brain white matter and is not significantly 'turned over' in adult life. A knowledge of the distribution in the brain of any enzyme capable of degrading plasmalogens would therefore be of some interest.

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