complete, and doubling the time of extraction with saline did not significantly increase the yield. The average yield from rat cerebrum corresponded to 1.6 (s.d. 0.09) μmol of lecithin hydrolysed/h per g of tissue; the specific activity was 0.24 (s.d. 0.04) $\mu\text{mol/h}$ per mg of protein.

The phospholipase A_1 activities in dialysed and taurocholate-treated preparations of human cerebral cortex and white matter gave broader pH peaks than rat brain, with maximal activities at pH 4.6, averaging 0.85 and 0.62 $\mu\text{mol/h}$ per g of tissue respectively. The substrate specificity of purified A_1 prepared in this way from rat cerebrum or from human cerebral cortex was in general agreement with that reported for rat brain by Gatt (1968). In the presence of taurocholate, (1-[^3H]stearoyl) lecithin and (1-[^3H]palmitoyl) lecithin were hydrolysed most rapidly, at similar rates, and (1-[^3H]stearoyl) phosphatidylserine about 20–30% as fast. Only slight activity against (1-[^3H]stearoyl) phosphatidylethanolamine was detected in this system but, as Gatt had found, there was appreciable hydrolysis in a mixture of taurocholate and a non-ionic detergent. Only relatively slight breakdown of (1-[^3H]stearoyl)lysolecithin, glycerol tri[^{14}C]oleate or cholesteryl [^{14}C]oleate was found.

Preparations showing phospholipase A_2 activity free of phospholipase A_1 have not as yet been isolated. Some preliminary information about this enzyme was obtained, however, with unpurified saline extracts. The pH optimum for phospholipase A_2 activity in human grey and white matter was 5.0, somewhat less than in rat brain, and no activity was detectable above pH 6.5.

Comparative studies on the regional distribution of phospholipase A in the human nervous system were carried out with (1-[^3H]stearoyl)lecithin as substrate for both types of activity. Phospholipase A_1 activity was calculated from the amounts of [^3H]stearic acid released by dialysed and taurocholate-treated extracts at pH 4.6, and phospholipase A_2 from the [^3H]lysolecithin formed by incubation with crude saline extracts at pH 5.0. In the central nervous system phospholipase A_1 activity showed a wide range of values between various regions, in contrast with phospholipase A_2 , which was more uniformly distributed. Phospholipase A_1 /phospholipase A_2 activity ratios therefore differed considerably (cerebellar cortex, 5.0:1; cerebral cortex, 3.1:1; centrum semiovale, 2.0:1; cervical cord, 1.4:1); a small phospholipase A_1 activity but no phospholipase A_2 was found in sciatic nerve.

The finding that certain lysophosphatides can be actively reacylated by brain (Webster, 1965), in the manner described originally in liver by Lands (1960), suggested that in normal nervous tissue phospholipase A plays a part in fatty acid exchange reactions. Whether also some abnormality of intrinsic phospho-

lipase A action is involved in loss of lipid in degenerative neurological disease is still unexplored.

This work was supported by a grant from the Multiple Sclerosis Society.

- Gallai-Hatchard, J., Magee, W. L., Thompson, R. H. S. & Webster, G. R. (1962). *J. Neurochem.* **9**, 945.
 Gatt, S. (1968). *Biochim. biophys. Acta*, **159**, 304.
 Gatt, S., Barenholz, Y. & Roitman, A. (1966). *Biochem. biophys. Res. Commun.* **24**, 169.
 Lands, W. E. M. (1960). *J. biol. Chem.* **235**, 2233.
 van Deenen, L. L. M. & de Haas, H. (1966). *A. Rev. Biochem.* **35**, 157.
 Webster, G. R. (1965). *Biochim. biophys. Acta*, **98**, 512.
 Webster, G. R. & Cooper, M. F. (1968). *J. Neurochem.* **15**, 795.

The Catabolism of Ethanolamine Phospholipids in Brain Tissue

By G. B. ANSELL and SHEILA SPANNER. (*Department of Experimental Neuropharmacology, The Medical School, University of Birmingham, Birmingham 15, U.K.*)

Some 9% of the dry weight of whole brain consists of ethanolamine phospholipids and 85% of this is in membranes. Undoubtedly a considerable proportion, particularly the ethanolamine plasmalogen, serves a structural function and is metabolically stable in adult life. In pathological conditions where demyelination occurs and loss of ethanolamine lipid is evident, this loss can be extremely serious. The measurement of the rates of breakdown of the ethanolamine lipids of the brain *in vivo* has never been achieved, though it is apparent from the measurements of the uptake of radioactive phosphate and ethanolamine in adult brain that the phosphate base moiety of both the major ethanolamine phospholipids undergoes some turnover (Ansell & Spanner, 1967).

Although early studies indicated the presence of enzymes hydrolysing ethanolamine phospholipids in brain (see Ansell & Hawthorne, 1964), the first demonstration of a specific enzyme, phospholipase A, was by Gallai-Hatchard, Magee, Thompson & Webster (1962). Some corroborative evidence of their breakdown *in vivo* was demonstrated by Knauff, Marx & Mayer (1961), who showed a rise in the free ethanolamine concentration in brain in insulin hypoglycaemia, which indicated a breakdown of ethanolamine lipid.

When a brain homogenate was incubated in oxygenated Krebs-Ringer bicarbonate medium for short periods, there was a breakdown of the endogenous phospholipids including those containing ethanolamine. The low rate of loss of ethanolamine lipids of about 1.5 $\mu\text{mol/h}$ per g fresh wt. was accompanied by a rise in the concentration of water-soluble ethanolamine compounds, particularly ethanolamine

(1.3 $\mu\text{mol/h}$ per g fresh wt.) and phosphorylethanolamine (0.07 $\mu\text{mol/h}$ per g fresh wt.). Under these conditions there was no apparent accumulation of glycerophosphorylethanolamine, but there was a small yield of radioactive glycerophosphorylethanolamine when whole brain homogenates were incubated with added phosphatidylethanolamine labelled with [1,2- ^{14}C]ethanolamine (G. B. Ansell, S. Spanner & D. J. Williams, unpublished work). This particular finding is of interest in view of Dawson's (1955) finding that in liver *in vivo* glycerophosphorylethanolamine is a major catabolic product of phosphatidylethanolamine.

There are four possible sites for enzyme attack of the ethanolamine phospholipid molecule, yielding as breakdown products (a) lysophosphatidylethanolamine (1- or 2-acylglycerophosphorylethanolamine) plus fatty acids, (b) phosphorylethanolamine plus 1,2-diacylglycerol or (c) 1,2-diacylglycerophosphate plus ethanolamine. Catabolism of the ethanolamine plasmalogen would yield analogous groups of compounds with free aldehydes or alkenyl-substituted compounds of glycerol. Evidence for some of these reactions in brain tissue has been found.

Gallai-Hatchard *et al.* (1962) showed that when phosphatidylethanolamine was incubated with an extract of an acetone-dried powder of brain there was a hydrolysis of the lipid to yield lysophosphatidylethanolamine at a rate of 1.2 $\mu\text{mol/h}$ per g fresh wt. By varying the cation present, Ansell & Spanner (1966) observed that there were a number of different enzymes present in brain capable of degrading both phosphatidylethanolamine and ethanolamine plasmalogen to simple compounds.

When ethanolamine plasmalogen was incubated with an undialysed extract of an acetone-dried powder of brain there was a loss of acyl ester groups, vinyl ether groups and lipid-bound phosphorus. If the extract was dialysed no hydrolysis occurred. The activity of the phospholipase A that removed the acyl group from the 2-position could be restored by Mn^{2+} and to a smaller extent by Ca^{2+} . Evidence was also obtained for a phospholipase C that requires Zn^{2+} . It has been established that the enzyme cleaving the vinyl ether linkage from the 1-position requires Mg^{2+} (Ansell & Spanner, 1965).

A similar pattern has been found for phosphatidylethanolamine and phospholipase C activity was greater with this substrate than with the plasmalogen (1.8 $\mu\text{mol/h}$ per g fresh wt. compared with 0.96 $\mu\text{mol/h}$ per g). The possibility that there are two phospholipases active towards phosphatidylethanolamine in brain, i.e. A_1 and A_2 , has not yet been established. The loss of fatty acids from phosphatidylethanolamine at the observed rate of 3.8 $\mu\text{mol/h}$ per g fresh wt. could involve the A_1 and A_2 phospholipases or the lysophospholipase described in brain by Leibovitz & Gatt (1968). These workers found an enzyme in a

sonicated crude mitochondrial fraction in brain that would remove the fatty acid from 1-acylglycerophosphorylethanolamine. This enzyme appeared to require no cofactors. Gatt (1968) has also shown the presence of phospholipase A_1 in brain. This enzyme, which is probably lysosomal, has a pH optimum of 4.0, unlike most of the other phospholipid degrading enzymes, which have pH optima at about 7.0. The action of this enzyme produces a 2-acylglycerophosphorylethanolamine.

An interesting feature of the studies on the catabolism of endogenous phospholipid *in vitro* was the production of free ethanolamine. At neutral pH the activity of a phosphatase active towards phosphorylethanolamine would be minimal (Strickland, Thompson & Webster, 1956), and in any event the production of free ethanolamine was greatly in excess of the phosphorylethanolamine present. Conceivably the ethanolamine was being produced by the action of a phospholipase D on the intact lipid, but this enzyme, though present in plant tissue, has never been found in animal tissues. On the other hand, a Ca^{2+} -activated ethanolamine exchange has been demonstrated with a brain microsomal preparation and this could be due to an enzyme like phospholipase D (G. B. Ansell & S. Spanner, unpublished work).

Further study of the plasmalogenase showed it to be present in the 'mitochondrial fraction' of white matter of brain. It is probably present in the glial cells, though its exact location has yet to be established.

In various demyelinating diseases of the central nervous system it has been shown that the concentration of the ethanolamine phospholipids falls. Of the ethanolamine phospholipids, the plasmalogen is the most affected. Studies have been carried out on the plasmalogenase activity of the white matter of the spinal cord of monkeys with vitamin B_{12} deficiency, a condition causing demyelination in the dorsal horns of the cord in the thoracic region. It was found that in the spinal cord of the monkeys showing marked demyelination there was a considerable elevation of enzyme activity from 1.2 $\mu\text{mol/h}$ per g fresh wt. in the normal animals to 3.76 $\mu\text{mol/h}$ per g in the deficient animals (Ansell & Spanner, 1968). A limited number of studies have also been made on human brain biopsy material to test whether there is any correlation between enzyme activity and the concentration of ethanolamine plasmalogen present. The white matter around a plaque showed a rise in enzymic activity, which was twice the value of normal tissue. An interesting finding has emerged from studies of the brain biopsies from patients with Alzheimer's disease. The ethanolamine plasmalogen concentrations of white matter are low and there is a high plasmalogenase activity.

Work is in progress on the subcellular distribution

of the enzymes involved in the breakdown of the ethanolamine phospholipids and this will be discussed.

This work has been supported in part by a grant from the Multiple Sclerosis Society.

- Ansell, G. B. & Hawthorne, J. N. (1964). *Phospholipids*, p. 318. Amsterdam: Elsevier Publishing Co.
 Ansell, G. B. & Spanner, S. (1965). *Biochem. J.* **94**, 252.
 Ansell, G. B. & Spanner, S. (1966). *Biochem. J.* **98**, 23P.

- Ansell, G. B. & Spanner, S. (1967). *J. Neurochem.* **14**, 873.
 Ansell, G. B. & Spanner, S. (1968). *Biochem. J.* **108**, 207.
 Dawson, R. M. C. (1955). *Biochem. J.* **59**, 5.
 Gallai-Hatchard, J., Magee, W. L., Thompson, R. H. S. & Webster, G. R. (1962). *J. Neurochem.* **9**, 545.
 Gatt, S. (1968). *Biochim. biophys. Acta*, **159**, 304.
 Knauff, H. G., Marx, D. & Mayer, G. (1961). *Hoppe-Seyler's Z. physiol. Chem.* **299**, 248.
 Leibovitz, Z. & Gatt, S. (1968). *Biochim. biophys. Acta*, **164**, 439.
 Strickland, K. P., Thompson, R. H. S. & Webster, G. R. (1956). *J. Neurol. Neurosurg. Psychiat.* **19**, 12.

MOLECULAR ENZYMOLOGY GROUP COLLOQUIUM ON 'MECHANISMS OF OXYGEN ABSORPTION AND UTILIZATION'

Free-Radical Intermediates in the Reduction of Oxygen

By R. C. BRAY. (*Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London S.W.3, U.K.*)

Free-radical species derived from oxygen have frequently been proposed as intermediates in biochemical reactions (e.g. Norman & Radda, 1962; Yamazaki & Piette, 1963; Staudinger & Ullrich, 1964; see also Guroff *et al.* 1967). It may therefore be useful to review some of the known properties of oxygen radicals in aqueous solution. We shall not be concerned with radicals co-ordinated to metal ions, though these may be of considerable biochemical importance.

Oxygen reduction can in principle occur in four single-electron steps:

The redox potentials at pH 7 for these reactions have been calculated as about -0.6, +1.1, +0.4 and +2.3V respectively (George, 1965; Mason, 1965). Thus quite strongly reducing conditions are required to add the first electron to oxygen, and the two free radicals, HO₂ and OH, are oxidizing agents, the latter a powerful one. There is also the possibility of oxygen atoms being formed in the reduction, by the O-O bond breaking in the second of the above reactions, to give water and O, instead of H₂O₂ (George, 1965).

Quite a number of oxygen free radicals have been known to physical chemists for some years past, and their formation and reactions have been quite extensively studied. Radiation techniques particularly with high-energy electrons have played a major role in this work. Both HO₂ and OH can undergo acid dissociations, giving O₂⁻ and O⁻

respectively, with pK values of 4.5 and 11.9 (Schested, Rasmussen & Fricke, 1968; Rabani & Matheson, 1964). Thus at pH values of biochemical interest O₂⁻ and OH might occur, HO₂ also being a possible species in the low pH range. There appears to be no conclusive evidence for the existence of the species OH⁺ (Samuel, 1968). Radiation work has given data on the lifetimes of the oxygen radicals. Though unstable, they do have measurable lifetimes in aqueous solution at room temperature. In the absence of anything else for them to react with, two free radicals react together. The least stable of the three radicals of interest is OH, which at moderate concentrations has a half-life in the region of a few tens of microseconds only (Schested *et al.* 1968). However, in the neutral pH region both O₂⁻ and HO₂ are distinctly more stable than this, with half-lives coming up to tens of milliseconds (Czapski & Dorfman, 1964). Further, it has recently come to light that in the alkaline pH range the stability of O₂⁻ is markedly increased, e.g. it has a half-life of about 200ms at pH 10 (R. Nilsson, F. M. Pick, R. C. Bray & E. M. Fielden, unpublished work; see also Knowles, Gibson, Pick & Bray, 1969).

In general, electron paramagnetic resonance is now the method of choice for detecting and identifying free-radical species, and the electron-paramagnetic-resonance parameters in frozen aqueous solution of HO₂, O₂⁻ and OH are all known (Wyard, Smith & Adrian, 1968; Knowles *et al.* 1969; Bravati, Symons, Tinling, Wardale & Williams, 1967). However, the usefulness of electron paramagnetic resonance in this field is severely limited both by instrument sensitivity, the minimum useful radical concentration being in the region of 10 μM, and by lack of time-resolution, since it usually takes several minutes to obtain an electron-paramagnetic-resonance spectrum.

It may not be surprising that so far, apparently, only the most stable oxygen radical, O₂⁻ on the alkaline side of neutrality, has conclusively been identified by electron paramagnetic resonance in a biochemical system. O₂⁻ was observed by Knowles