population, and was the result of an increase in bound enzyme content, in accordance with the conclusions of Gregson & Williams (1969). A second population, with a mean buoyant density of 1.166 was characterized by a low and relatively constant cytochrome oxidase/monoamine oxidase activity ratio with a mean value of 0.19.

It has been claimed that in mixed mitochondria obtained from whole mammalian brain homogenates, submitted to sucrose-density-gradient centrifugation, the resolution of cytochrome oxidase, monoamine oxidase and protein concentration into two peaks showed the potential feasibility of using such a procedure to separate glial and neuronal mitochondria (Hamberger et al., 1970). We have not obtained such clear-cut resolutions of these enzymic and biochemical mitochondrial parameters along the sucrose gradient from the crude mitochondrial fraction of the chick brain at any stage of development studied. In some experiments a 6h centrifugation time was used, which did not modify the relative position of the isopycnic bands.

From the results obtained in this study, we confirm the existence of more than two heterogeneous populations of mitochondria in the chick brain, the evolution of which is different during embryogenesis and postnatal growth and leads to various ratios of inner to outer membrane mass.

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Ascorbic Acid in Developing Brain: Possible Function as an Inhibitor of Lipid Peroxidation

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The immature brain of both the rat (Allison & Stewart, 1973; Adlard et al., 1973) and man (Adlard et al., 1974) contains high concentrations of ascorbic acid. Amounts in the rat brain during the first week of postnatal life are approx. 4μmol/g, twice those in adult brain. The presence of such high concentrations suggests that ascorbate may act as an anti-oxidant preventing membrane lipid peroxidation in the developing nervous system.

Ascorbate at low concentrations (<1.0μmol/ml) is recognized as a cofactor of lipid peroxidation in vitro (Bernheim et al., 1948). Peroxidation is usually measured as formation of malonaldehyde, a minor, but constant product of membrane polyunsaturated fatty acid destruction (May & McCay, 1968). Microsomal fractions, or other...
Ascorbate (pmol/ml)

Fig. 1. Lipid peroxidation at various ascorbate concentrations by a microsomal fraction from foetal human forebrain (●) and by homogenates of newborn rat brains (○)

The brain was obtained fresh from an 18 week human foetus and deep frozen at -20°C for 3 weeks. Microsomal fractions were prepared by the method of Cuzner & Davison (1968). Newborn rat brains were homogenized in 9 vol. of iso-osmotic KCl. Malonaldehyde formation was determined by the method of Kitabchi & Williams (1968) with tissue from 25 mg of brain in 1 ml of incubation mixture. The curve for newborn rats represents the mean of four determinations.

membrane preparations, from rat liver (Wills, 1969) or brain (Bishayee & Balasubramanian, 1971) form malonaldehyde when incubated with NADPH or ascorbate. Rates of peroxidation in the presence of ascorbate show an optimum at cofactor concentrations of 0.1–1.0 µmol/ml. The results of Fig. 1 suggest that human foetal brain microsomal fractions are similar. Above 1.0 µmol of ascorbate/ml peroxidation rate sharply declined suggesting that at high concentrations the anti-oxidant property of ascorbate predominates.

Newborn rat brain homogenates peroxidized rapidly without added cofactors (Fig. 1). Endogenous brain ascorbate would have given a concentration of 0.1 µmol/ml in the incubation system. Further addition of this cofactor increased the rate slightly, but peroxidation declined rapidly when ascorbate exceeded 1.0 µmol/ml. This critical concentration above which ascorbate begins to behave as an anti-oxidant has been found to be the same (approx. 1 µmol/ml) in a variety of species, tissues and membrane preparations: adult rat liver microsomal fractions (Wills, 1969), adult guinea pig brain and liver homogenates, adult rat brain microsomal fractions and synaptosomes and newborn rat brain microsomal fractions (B. P. F. Adlard, unpublished results).

Thus in tissues where ascorbate could act as a cofactor of peroxidation, a high amount might prevent such a destructive process occurring. However, there may be other cofactors determining peroxidation rates, and the importance of ascorbate in relation to these would therefore have to be assessed. An approach to this problem has been made by examining malonaldehyde formation in vitro in tissues from ascorbic acid-deficient guinea pigs.

Guinea pigs (4–6 weeks old) received an ascorbic acid-free diet ad libitum (Hughes &
Table 1. Lipid peroxidation by tissues from normal and ascorbic acid-deficient guinea pigs

Results (mean±S.D.) are based on five control and six deficient animals. Malonaldehyde formation was determined by using 30mg of tissue/ml of incubation mixture. When added, ascorbate was at a concentration of 2.0μmol/ml in liver assays and at 1.5μmol/ml (for controls) or 0.3 μmol/ml (for deficient animals) in cerebellar assays. Differences were compared by using Student’s t test.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ascorbic acid-deficient</th>
<th>P</th>
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<tbody>
<tr>
<td>Ascorbic acid (μmol/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.96±0.16</td>
<td>0.05±0.05</td>
<td>&lt;0.001</td>
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<tr>
<td>Cerebellum</td>
<td>1.47±0.08</td>
<td>0.33±0.05</td>
<td>&lt;0.001</td>
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<tr>
<td>Malonaldehyde formation without added</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.06±0.41</td>
<td>2.17±0.48</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>9.0±0.5</td>
<td>2.7±0.3</td>
<td>&lt;0.001</td>
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<tr>
<td>Malonaldehyde formation with added</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Liver</td>
<td>0.38±0.16</td>
<td>3.17±1.56</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>12.2±0.6</td>
<td>13.6±1.4</td>
<td>0.05–0.1</td>
</tr>
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</table>
Hurley, 1969) for 25 days. Control animals received ascorbic acid daily in the drinking water (1 g/100 ml) but experimental animals did not receive this supplement. Assays of lipid peroxidation in vitro (malonaldehyde formation) were performed by the method of Kitabchi & Williams (1968). Some peroxidation studies were also carried out with forebrain which gave similar results to those of cerebellum. Tissue ascorbic acid was determined by the method of Roe & Kuether (1943).

Liver homogenates of ascorbic acid-deficient guinea pigs showed higher rates of peroxidation than controls whether or not ascorbate was added to the medium (Table 1). Membrane damage or other pathological change in ascorbate-deficient animals may have enhanced the intrinsic tendency of membrane lipids to peroxidize in vitro. This group showed very low amounts of liver ascorbate (undetectable in three animals) despite high rates of peroxidation. This suggests that cofactors other than ascorbate may be of relatively greater importance in promoting peroxidation in liver.

In contrast, peroxidation by homogenates of cerebellum was probably almost completely dependent on ascorbate as cofactor (Table 1). It can be estimated that endogenous ascorbic acid contributed 0.04 μmol/ml to the ascorbate concentration of control incubation media and 0.01 μmol/ml to those of deficient cerebella. These concentrations were in the range where increasing amounts of ascorbate stimulate malonaldehyde formation in cerebellar homogenates. Deficient animals, having a cerebellar ascorbate 22% of the control value showed a peroxidation rate 30% of that in control animals. The addition of a physiological concentration of NADPH (0.2 μmol/ml) increased malonaldehyde production by only 6% in control animals but by 42% in ascorbate-deficient animals. The difference between the two groups in peroxidation rate in the absence of ascorbate was abolished when this cofactor was added to give the concentration found in vivo (Table 1).

In the period 11–19 weeks gestation a group of 16 human forebrains showed ascorbic acid concentrations in the range 2.5–7.1 μmol/g (Adlard et al., 1974). At these concentrations ascorbate was inhibitory to peroxidation as assessed in vitro (Fig. 1). It is not established to what extent malonaldehyde formation in vitro may reflect processes which occur in vivo causing tissue damage (Demopoulos et al., 1972; Green, 1972; Tappel, 1972; Wills, 1971). It nevertheless seems worth considering whether brain lipid peroxidation might be enhanced in the human foetus by maternal ascorbic acid deficiency, particularly when the foetal brain has suffered some initial trauma (Demopoulos et al., 1972). Such a possibility might be investigated by using the foetal guinea pig as a model.

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