J. Physiol. (1985), **359**, *pp.* 417–429
With 5 text-figures
Printed in Great Britain

CHANGES IN BRAIN PHOSPHORUS METABOLITES DURING THE POST-NATAL DEVELOPMENT OF THE RAT

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(*Received 3 July 1984*)

SUMMARY

1. Changes in brain phosphorus metabolites during the post-natal development of the rat and in neonatal and adult guinea-pigs have been studied *in vivo* using ^{31}P nuclear magnetic resonance spectroscopy (n.m.r.s.).

2. The brain spectra showed clear differences with age, particularly during the first 3 weeks post-partum.

3. The spectra from 4-day-old rats resembled those of new-born human infants. We suggest that the differences between human and animal brains seen in previously published spectra arise because of an age difference rather than a species difference.

4. The phosphocreatine (PCr) to nucleoside triphosphate (NTP) ratio increased from around 1·0 in 3-day-old rats to 1·8 in adult animals. The adult ratio is larger than that previously reported from *in vitro* chemical analyses.

5. An unknown compound in the phosphomonoester (PME) region of the spectra predominated in young animals, but decreased in concentration relative to NTP with age and reached adult values by around 2 weeks post-partum.

6. Neonatal guinea-pigs, which are much more developed at birth than the rat, had a significantly greater PCr/NTP ratio than the neonatal rat, but their brain spectra also contained the large PME peak.

7. The intracellular pH of cerebral tissue was estimated to be $7{\cdot}21 \pm 0{\cdot}02$ and did not show any change with age.

8. The changes we find in the phosphorus compounds in the brain may be of importance in post-natal development, and the possible functional significance of these results is discussed.

INTRODUCTION

Using ^{31}P nuclear magnetic resonance spectroscopy (n.m.r.s.) and surface coils, brain phosphorus metabolites can be examined *in vivo*, as first shown by Ackerman, Grove, Wong, Gadian & Radda (1980). In particular, it is possible to determine the relative concentrations of nucleoside phosphates, phosphocreatine (PCr) and inorganic phosphate (P_i) and intracellular pH simultaneously from living (anaesthetized) animals.

Recently, the brains of new-born infants have been examined (Cady, Costello, Dawson, Delpy, Hope, Reynolds, Tofts & Wilkie, 1983). In a study of birth-asphyxiated

babies, low ratios of PCr/P_i (<0·8), were found to be associated with a very bad prognosis for survival and early neurodevelopmental outcome (Hope, Costello, Cady, Delpy, Tofts, Chu, Hamilton, Reynolds & Wilkie, 1984). The spectra from human infant brains differed from those found previously for animal brains (e.g. Ackerman *et al.* 1980; Thulborn, Boulay, Duchen & Radda, 1982) in that the PCr peak was smaller and the most prominent peak in infant brain spectra, which was in the phosphomonoester (PME) region, was rather small and insignificant in the animal brain spectra. These differences could be related to species or to age. Whereas the human study was on infants, the animal studies were all performed on adult animals. We therefore decided to study the development of the rat brain by ^{31}P n.m.r.s. In this way it should be possible to determine: (i) if there are changes in the rat brain phosphorus metabolites with age and (ii) whether brain spectra of young rat resembles that of infant brain. Another reason for undertaking such a study was to determine whether the concentrations of energetically important phosphorus compounds, particularly PCr, change in early life. Previous analyses of excised brain tissue had given inconsistent results (e.g. Lolley, Balfour & Samson, 1961; Miller & Shamban, 1977).

As different species of animals vary in the amount of development which they undergo before birth, we decided to compare a well-developed (precocious) neonate, the guinea-pig, with the underdeveloped (altricious) neonatal rat. We carried out a ^{31}P n.m.r.s. study on rats and guinea-pigs aged between 3 days and adult. The results show that there are significant changes in the levels of phosphorus metabolites of the brain with age in both the rat and guinea-pig. Furthermore, as the spectra from young rat brain are very similar to the spectra from infant brain, we suggest that the differences in previously published spectra of animal and human brain are due to differences in age.

A preliminary account of some of these results has been presented to the Physiological Society (Tofts & Wray, 1984*a*, *b*).

METHODS

Animals

Sprague–Dawley rats of either sex from pups 3 days old to adult (up to 300 g body weight) were used. Pups were weaned after 3 weeks. Food (Dixon's animal diet pellets, GR3-EK) and tap water were supplied *ad libitum*. Litters of five to twelve pups were used and the age of the pups was known to within 24 h. Dunkin–Hartley guinea-pigs, 3–5 days old and adult (≃525 g body weight) were also examined.

The animals were anaesthetized with 36% (w/v) urethane (ethyl carbamate, 1 ml/200 g body weight) given I.P. A n.m.r. surface coil (see below) was placed on top of the shaved head in young animals or, in animals older than 24 days, on the skull with skin reflected and the muscle at the side of the head removed so as to prevent it from contributing to the n.m.r. signal (see Results). The coil was always placed in the same position with reference to the ears and held there with adhesive tape. Two of the young animals were allowed to recover from the anaesthesia and were re-examined on four subsequent occasions. Rectal temperature was measured and a heated enclosure and cotton-wool were used to maintain the animal's temperature at 37 °C while it was inside the bore of the magnet. Blood samples were taken at the end of an experiment and blood gases measured and found to be within the normal range.

N.m.r.s.

A 20 cm diameter bore superconducting magnet (Oxford Research Systems) was used at a field strength of 1·89 T. It operated at a frequency of 32·5 MHz for ^{31}P. A two-turn, circular planar 15 mm diameter surface coil made from copper wire was used to transmit radiofrequency pulses to, and receive signals from, the brain. The shape of the tissue from which signal was received was approximately a hemisphere with its base on the coil (Tofts, 1984). This penetrated approximately 7 mm into the head and had a volume of about 0·9 cm^3. A pulse duration of 7 μs was used with a pulse interval of 12 s. The flip angle at the centre of the coil (i.e. the angle by which the sample magnetization is rotated away from the direction of the static magnetic field, by the radiofrequency pulse) was about 90 deg. The number of pulses varied from 100 to 1000 but the usual number was 300.

Spectral information

Processing of data. Spectral peaks arise only from metabolites which are mobile, i.e. in solution. Less mobile phosphorus nuclei in skull bones, and membrane phospholipids, give rise to a very broad component upon which the spectral peaks are superimposed (see Fig. 1*A*). Data were collected as signal-averaged free induction decays (f.i.d.). To obtain a spectrum such as shown in Fig. 1*B* the f.i.d. was processed and Fourier transformed to remove the broad components and obtain values of peak areas and positions (Gordon, Hanley & Shaw, 1982).

The broad component in the spectrum (see Fig. 1*A*) was removed by multiplying the f.i.d. by the following function:

$$M = \frac{1}{1 + F \exp(-W\pi t)},$$

where t is the time from the start of the f.i.d. and F and W are chosen semi-empirically to be 4 and 200–400 Hz respectively. W is approximately the width of the broad component to be removed.

We also removed the broad component using a different technique to see how good the method described above was at removing signal from less mobile nuclei and not metabolites. The broad component may be removed directly from the n.m.r. signal by irradiating at a single frequency between peaks 2 and 3, as shown by the arrow in Fig. 1*C*. It can be seen that the resulting spectrum, Fig. 1*C*, is similar to that obtained with computer removal of the broad component (Fig. 1*B*). In our experiments, the irradiation slightly reduced the peaks close to where it was applied and so could not be used routinely. We used a delay of 120 μs which with our band width of 8 kHz eliminated the need for frequency-dependent phase correction (Hoult, Chen, Eden & Eden, 1983). A frequency-independent phase correction was adjusted to give a symmetrical nucleoside triphosphate (NTP) peak. A linear base-line correction was applied to the spectrum, adjusted to pass through the part of the spectrum immediately to the left of the PME peak and to the right of the NTP peak.

To measure peak areas, a set of gates were used, one around each peak. The position of each gate was determined by visual inspection of spectra from a range of rats, and kept constant for all subsequent analysis. No shifts in peak position were observed. The gated area under the spectrum was used as the estimate for the peak area. In the case of overlapping peaks this leads to some error (as do most methods of analysis); however, we believe this procedure to be preferable to the more operator-dependent ones which involve estimating where the peak (approximately Lorentzian in shape) meets the base line.

Spectral peaks and metabolite concentrations. The positions of most peaks in ^{31}P n.m.r. spectra are well established. The position of the peaks is indicative of chemical environment and is given in parts per million (p.p.m., 1 Hz = 0·031 p.p.m. at 32·5 MHz). PCr is assigned as resonating at 0 p.p.m. and thus other resonance positions are given relative to it. In Fig. 1 the peaks are: nucleoside phosphates (NP); three peaks 1, 2 and 3, arise from the different phosphorus atoms in the nucleotides. The resonance positions of the individual nucleosides are indistinguishable (Shoubridge, Briggs & Radda, 1982). Peak 1 is contributed to only by NTP. Peak 2 includes a contribution from nicotinamide adenine dinucleotide (NAD and NADH) which can sometimes be seen as a hump on the right-hand side of this peak at about −8·0 p.p.m. The phosphodiester peak (PDE) is a composite peak and will include contributions from glycerol phosphoryl choline, glycerol phosphoryl ethanolamine and membrane phospholipids (Cerdan, Egan & Hilberman, 1984). A small

peak from P_i can be seen. The peak at around 6·6–6·7 p.p.m. (PME), is in the sugar phosphate or phosphomonoester region of the spectrum. Its identity is still not known with certainty. The intracellular pH was calculated from the PCr–P_i shift, using the formula:

$$\mathrm{pH} = \mathrm{p}K + \log_{10} \frac{\delta - 3{\cdot}27}{5{\cdot}69 - \delta},$$

where δ is the chemical shift difference between P_i and PCr and the pK value was 6·72 (Dawson & Wilkie, 1984).

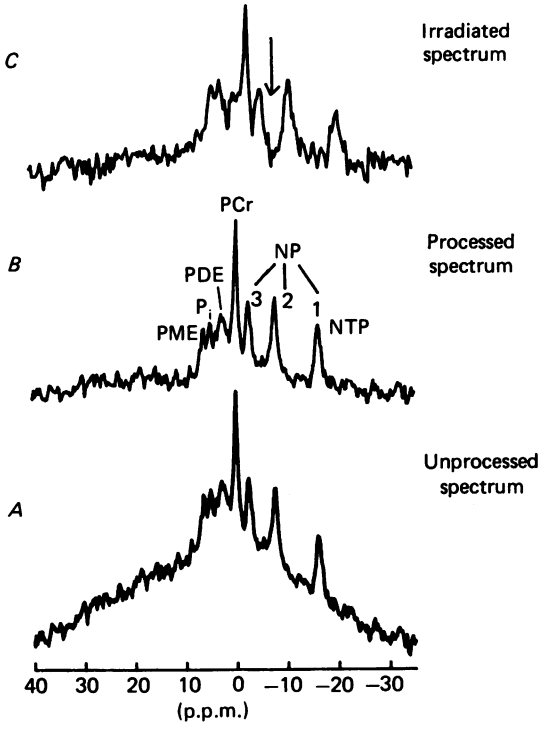

Fig. 1. Spectra from adult rat brain, showing: *A*, unprocessed spectrum, *B*, spectrum after computer processing to remove the broad component and *C*, spectrum after irradiating at the point indicated by the arrow, to remove the broad component. The number of radiofrequency pulses was 400 in each case and the pulse interval in this experiment was 2 s. The identity of the metabolites in the spectra are as follows: NP, nucleoside phosphates; three peaks arise from NPs (1, 2 and 3). Peak 1 contains contributions only from nucleoside triphosphates (NTP). PCr, phosphocreatine; PME, phosphomonoester; PDE, phosphodiester; P_i, inorganic phosphate. Chemical shift is given as parts per million (p.p.m.) from PCr.

Provided that the phosphorus nuclei have time to return to their equilibrium state between the radiofrequency pulses, then the peak areas are directly proportional to the concentration of metabolite present. In order to investigate the effect of pulse interval, spectra were collected at intervals of 2, 5, 10, 12 and 20 s in a single experiment. System stability during the accumulation of these data was monitored by repetition of the two second spectra at intervals throughout the experiment. The results showed that signals obtained with pulse intervals of 12 and 20 s were the same. Therefore, an interval of 12 s was sufficient to allow relaxation of the nuclei, and we used this in subsequent experiments. The peak areas were obtained by integration as described above.

The reproducibility of our methods was tested by studying the same adult animal three times. The concentrations, relative to NTP, of PCr, P_i and PME were calculated. The standard deviations as a percentage of the mean value were 6, 10 and 11 % respectively.

Figures given throughout are mean values of n observations with the S.E. of the mean. Significance of differences was tested using unpaired t tests.

RESULTS

The effects of skull muscle

Before investigating the origin of the differences between human and rat brain spectra, we thought it essential to exclude one possible artifactual explanation. In rats, the skull is surrounded by musculature from the ears, jaws and neck. It is therefore possible that muscle tissue and skin may contribute to the spectra obtained

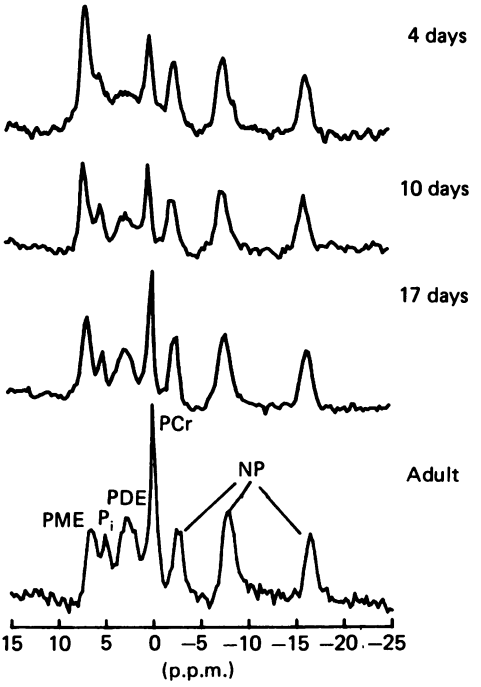

Fig. 2. Spectra of rat brain obtained from animals aged 4, 10, 17 days and adult (top to bottom). The spectra were scaled to contain the same NTP peak area. The peak identities are as described in Fig. 1. The number of radiofrequency pulses was 200–400. The pulses were at 12 s intervals.

with surface coils. This effect would be expected to be greater in the rat than the human since the jaw and ocular muscles are more prominent in the former. Since muscle contains a high concentration of PCr (around 28 mM, Edwards, Dawson, Wilkie, Gordon & Shaw, 1982) this might account for the larger PCr and relatively smaller PME peak seen in the rat brain. We have therefore examined spectra obtained before and after removing muscle and skin from around the sides of the head and the neck in adult rat. It was found on some occasions (three out of six, presumably

depending upon anatomical differences and slight variations in the coils position), that the spectrum had higher PCr/NTP before removing muscle and skin. The removal of muscle did not cause any deterioration in the cerebral tissue; unchanged, normal spectra were obtained for many hours after removing the muscle. We therefore decided to examine adult rat brains with muscle removed.

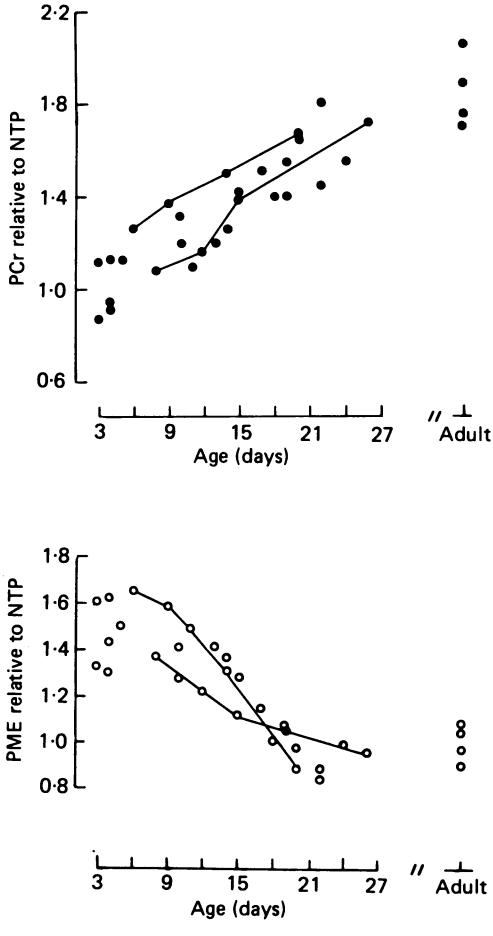

Fig. 3. Graphs showing the changes in the relative concentration of PCr (top) and phosphomonoester (bottom) with post-natal development (in days) in the rat. Points joined by a continuous line were obtained from the same animal examined at different ages. All other points are from individual animals. The points were all obtained from spectra containing at least 100 radiofrequency pulses, the usual number being 300, and the pulse interval was 12 s.

The effects of skull bones

Adult skull bones are known to give rise only to broad components in the brain spectra (Ackerman *et al.* 1980). Since the neonatal rat skull is softer, i.e. much less calcified than the adult, it was not clear that this was necessarily the case in the younger animals. We therefore obtained spectra from 4-day-old rats with and without

the skull and from skull alone, to investigate the contribution made by the neonatal skull. It was found that without the skull there still remained a large broad component, from membrane phospholipids. The skull made only a small contribution to the broad component. Spectra from brain slices also contain a large broad component (Cox, Morris, Feeney & Bachelard, 1983).

Changes with age

Fig. 2 shows typical spectra from 4, 10, 17-day-old and adult rats. There are large differences between young and adult brain spectra. In particular the PME peak decreases relative to the other peaks with age while PCr increases.

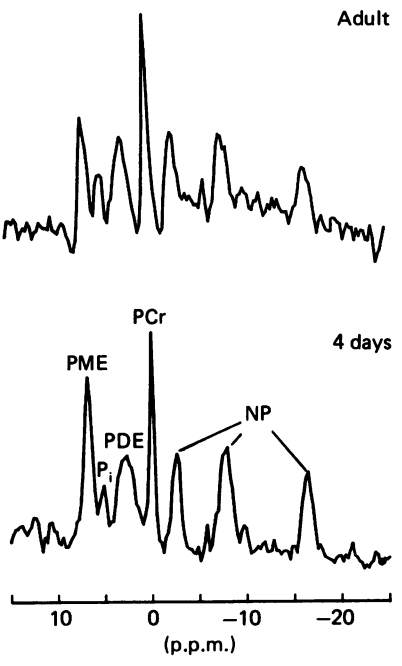

Fig. 4. Brain spectra from neonatal (bottom) and adult (top) guinea-pigs. The peaks are as described in Fig. 1. The number of radiofrequency pulses was 300 in both spectra, with 12 s between pulses.

The differences in the spectra were quantified by measuring the peak areas. It is not yet possible to obtain absolute millimolar concentrations directly in these *in vivo* studies. We have therefore expressed most of the results relative to the magnitude of the NTP peak which is composed largely of ATP (Lolley *et al.* 1961). This peak was chosen for two reasons. First, other studies have indicated that ATP and NTP do not change greatly with age (Samson, Balfour & Dahl, 1960; Mandel & Edel-Harth, 1966; Miller & Shamban, 1977). Secondly, ATP concentration is maintained constant during the course of an experiment by PCr acting as a buffer (see e.g. Carlson & Wilkie, 1974). This makes ATP a more appropriate metabolite to choose as a standard rather than, for example, P_i or PCr.

The relative concentrations of PCr and PME are plotted against the age of the animal in Fig. 3 ($n = 32$). Each point in Fig. 3 is from a different animal, except for the points joined together by the lines. Each continuous line shows successive measurements made on the same animal. The relative concentration of PCr increased linearly with the age of the rat from 3 to 27 days. Thus, by the time of weaning (3 weeks post-partum) around 90 % of the adult concentration of PCr had been reached compared with 57 % at 3–5 days.

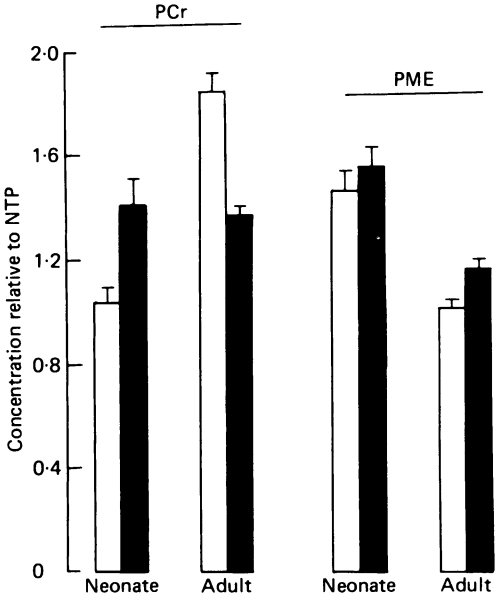

Fig. 5. The mean concentration (relative to NTP) of PCr and PME in neonatal rats ($n = 5$), adult rats ($n = 4$), neonatal guinea-pigs ($n = 3$) and adult guinea-pigs ($n = 4$). Rat, □; guinea-pig, ■.

The PME/NTP ratio showed changes in the opposite direction, decreasing with age. By weaning it had decreased to adult levels, being 60 % of the level at 3–5 days. In the two animals which were examined at five different ages the same pattern of changes was seen as that from the data pooled from all the rats.

The resonance position of ATP depends upon the amount of magnesium bound to ATP (see, e.g. Gadian, 1982). The mean resonance position of the ATP peak (peak 1), was $-16{\cdot}20 \pm 0{\cdot}02$ p.p.m. and did not change with age. This position indicates as expected, that the ATP is almost all (95 %) magnesium bound.

Comparison of guinea-pig and rat brain

The results from neonatal and adult guinea-pigs are shown in Fig. 4. The brain spectra from 3–5-day-old guinea-pig, like neonatal rat brain, contained a large contribution from the PME peak. However, the PCr peak in the neonatal guinea-pig was much larger than that found in the neonatal rat brain spectra, see Fig. 2. The concentrations of PCr and PME relative to NTP were calculated from the guinea-pig

brain spectra and the values obtained are shown in Fig. 5 along with the corresponding values from rats. It can be seen that the changes in the PME peak with age are in the same direction in both species. There was no significant difference in its concentration in neonatal rats and guinea-pigs. In the guinea-pig, unlike the rat, PCr relative to NTP does not seem to change with age. The ratio of PCr/NTP was $1{\cdot}41 \pm 0{\cdot}12$ in neonatal guinea-pigs and $1{\cdot}04 \pm 0{\cdot}06$ in the neonatal rat. The mean ratio of PCr/NTP in the adult rat ($1{\cdot}85 \pm 0{\cdot}08$, $n = 5$) was greater than in the adult guinea-pigs examined ($1{\cdot}37 \pm 0{\cdot}03$, $n = 5$).

Intracellular pH

The mean intracellular pH of cerebral tissue was calculated (see Methods section) to be $7{\cdot}21 \pm 0{\cdot}02$ ($n = 40$). There was no significant variation in the pH of cerebral tissue with age.

Metabolite concentrations

As mentioned above, direct calibration of results from n.m.r.s. using surface coils is difficult as external reference capillaries or internal standards cannot be used. We have therefore obtained absolute concentration values by assigning a concentration value to NTP of 3·7 mM, obtained from *in vitro* studies (Chapman, Westerberg & Siesjo, 1981). The rest of the spectral peaks can then be calibrated with respect to NTP. Using this method, the [PCr] and [PME] in 3–5-day-old rats was 3·8 and 5·4 mM respectively, and 6·8 and 3·7 mM respectively, in adult rats.

DISCUSSION

The spectra obtained from the neonatal rats resembled very closely those of human neonatal brain (Cady *et al.* 1983). In particular, the PME peak at 6·7 p.p.m. was prominent in both species and was also found in new-born guinea-pigs. Comparison of the metabolic ratios is not possible because of the different processing methods used. Bottomley, Hart, Edelstein, Schenck, Smith, Leve, Mueller & Redington (1983) have published spectra from adult human brain, using a 1 m bore spectrometer which has been designed to perform n.m.r.s. and n.m.r. imaging. These spectra were qualitatively similar to the adult rat spectra. Furthermore, brain spectra from adult rabbits (Delpy, Gordon, Hope, Parker, Reynolds, Shaw & Whitehead, 1982), and Monogolian gerbils (Thulborn *et al.* 1982) are also similar to those of the rat. It appears, therefore, that the differences between previously published animal and human spectra are due to the age difference in the studies.

There were marked changes in phosphorus metabolites during post-natal development. We expressed the changes quantitatively by calculating them relative to NTP concentration. With this method, any changes in triphosphate concentration with age will influence the result. However, it changes only slightly or not at all with age (Samson *et al.* 1960; Miller & Shamban, 1977; Mandel & Edel-Harth, 1966). The scatter seen in our results can largely be accounted for by the reproducibility of our method. Measurements made on the same animal on different occasions give a standard deviation of about 10 % of the mean value. The estimate of the intracellular

pH of the brain did not vary with age. The mean value found, $7{\cdot}21 \pm 0{\cdot}02$, is similar to the value found in human neonates, $7{\cdot}14 \pm 0{\cdot}10$ (Hope *et al.* 1984). It should be noted, however, that pH values depend upon the calibration curve used and that the apparent resonance position of a peak can be influenced by large, overlapping neighbouring peaks. Since the P_i peak borders the PME peak, which is especially large in young animals, it is possible that our estimate of pH may be shifted slightly in the alkaline direction, compared to the actual value. A shift of up to 0·03 p.p.m., which may occur due to this effect, would alter the pH value by around 0·03, i.e. the actual value would be reduced to 7·18. Using indirect methods to calculate pH gives values of around 7·05 (Siesjo, 1978).

Comparison with in vitro measurements

The results are from rats which were anaesthetized with urethane. Anaesthesia, other than with barbiturates, is not thought to alter cerebral phosphorus levels and energetic state (Siesjo, 1978). The increases in [PCr] with age during post-natal development were seen clearly in the ^{31}P n.m.r. spectra (Fig. 2). Earlier studies of cerebral [PCr] had given conflicting results: a decrease with age (Lolley *et al.* 1961); no change with age (Ozand, Stevenson, Tilden & Cornblath, 1975) or an increase (Miller & Shamban, 1977).

There are no studies using modern analytical techniques which have measured both [NTP] and [PCr] during development in the rat, but values for ATP and PCr exist. Thus, to compare our metabolite levels to those found in *in vitro* studies it is necessary to relate [NTP] to [ATP]. This can be done by taking ATP to be 75% of the total NTP pool in cerebral tissue (Chapman *et al.* 1981). When this is done our PCr/ATP ratio is 1·3 in 3–5-day-old rats and 2·4 in adults. The value for neonatal rats is comparable to those in the literature (e.g. 1·3, Miller & Shamban, 1977; Ozand *et al.* 1975). However, the ratio of PCr/ATP calculated from the n.m.r. spectra of adult rat brains is higher than those previously reported, from *in vitro* studies (e.g. 1·5–1·7, Miller & Shamban, 1977; Lust, Passoneau & Veech, 1973). Using values of 3·7 mM for [NTP] (Chapman *et al.* 1981) and 4 mM for [PCr] (Veech, Harris, Veloso & Veech, 1973) then the PCr/NTP ratio can be calculated to be 1·1. This value is also lower than the value determined by n.m.r. which was 1·8. N.m.r. values of [PCr] are often higher than those from chemical analysis, because in the latter PCr hydrolysis may occur (see, e.g. Dawson & Wilkie, 1984).

Possible functional significance of the developmental changes

The signals detected by n.m.r.s. are not from a single discrete region of the brain. There has been discussion as to the exact region from which signal is detected with surface coils (see, e.g. Pettegrew, Minshew, Diehl, Smith, Kopp & Glonek, 1983 and Tofts, 1984). The 15 mm surface coil used will sample roughly 0·9 cm^3 of cerebral tissue and it is from this region that the present results have been obtained. The identity of the PME peak is not yet known with absolute certainty. Earlier suggestions that it was ribose-5-phosphate (Glonek, Kopp, Kot, Pettegrew, Harrison & Cohen, 1982) are now not thought to be correct (see Hope *et al.* 1984). Examination of rat brain extracts by gas chromatography and n.m.r. have suggested that it is ethanolamine phosphate (S. Krywawych & P. G. Morris, personal communication; Hope *et al.* 1984), present at a concentration of 5 mM in 3-day-old rat brains

(S. Krywawych, personal communication). This value is close to our estimate of 5·4 mM in 3–5-day-old rats and to the value found in 1-day-old rats by Agrawal, Davis & Himwich (1966). It has been shown to decrease markedly in concentration during post-natal development in the guinea-pig, mouse, cat and dog (Himwich & Agrawal, 1969), as well as in the rat, where it is reported to fall by 74% in the first 14 days of life (Agrawal *et al.* 1966). The concentration of ethanolamine phosphate in adult rats was found to be 1·4 mM ($n = 3$) by Agrawal and colleagues (1966), which is a lower value than our concentration estimate of 3·7 mM. A possible explanation of this is that another metabolite may be present in the PME region of adult rat brain spectra. Ethanolamine phosphate is an intermediate in the biosynthesis of phosphatidyl ethanolamine (cephalin), a major component of cell membranes. The large quantities of phosphoethanolamine in the young animals could be a store to be used as cell division and myelination proceeds throughout the C.N.S. It is also possible that it represents increased membrane turnover. This PME peak was the most prominent peak in spectra of infant brains (Cady *et al.* 1983). It has also been observed in the spectra from a human cancer (Griffiths, Cady, Edwards, McCready, Wilkie & Wiltshaw, 1983) and rat uterus (Dawson & Wray, 1983). Earlier reports in the literature on the occurrence of phosphoethanolamine indicate that high concentrations of this substance seem to be associated with growing tissue (see Ferrari & Harkness, 1954). It has long been known that there are biochemical differences between neonatal and adult animals, such as greater usage of the hexose monophosphate shunt, increased anaerobic glycolytic capacity and an increased resistance to anoxia in neonates (McIllwain, 1966). The activities of all glycolytic enzymes in brain increase by 50% or more between birth and 20 days (see, e.g. Lowry & Passonneau, 1964). Glucose and oxygen utilization also increase during post-natal development (Himwich, Sykowski & Fazekas, 1941; Duffy, Kohle & Vannucci, 1975). The changes in the energetic P compounds in the brain, detected by n.m.r.s., occur over the same period and may well be a reflexion of these biochemical changes. The increase in [PCr] with age may reflect a greater neuronal organization and activity in the older animal. More energy will be expended in pumping ions out of cells to maintain the greater fraction of functioning neurones in the older animals. The effects of a decreased amount of ATP may well be more deleterious in the adult than in the neonate which is physically and electrophysiologically less active. On the other hand, it is equally plausible that as development nears completion less energy is required for synthetic reactions and thus a greater energetic reserve of PCr can be held.

The results from the neonatal guinea-pigs showed that these animals also had a large PME peak in their brain spectra just as was found in the rats. However, the relative concentration of PCr to NTP in neonatal guinea-pigs was much larger than that found in spectra from neonatal rats. The large amount of PCr in the neonatal guinea-pig brain may well be there as an energy reserve because of its greater level of cerebral activity compared to the neonatal rat. The guinea-pig, unlike the rat, is a precocious animal; at birth its eyes and ears are open, it has fur and resembles the adult animal.

In many animals, including man, the brain undergoes considerable development after birth. These developmental changes have been associated with both morphological and electrophysiological changes in cerebral tissue. In this paper we have

shown large changes in energetically important phosphorus metabolites in the developing rat brain. It may be that changes such as the increase in [PCr] have to occur during development before the electrophysiological changes can take place. Further work is required to examine the role of these metabolites in normal and abnormal development.

S.W. is grateful to Action Research – The National Fund For Research into Crippling Diseases – for financial support. We would like to thank colleagues at University College London for many helpful discussions, in particular E. Cady, M. J. Dawson, D. Delpy, E. O. R. Reynolds and D. R. Wilkie. We would also like to thank D. A. Eisner, P. H. Ellaway, A. C. Elliott and A. R. Gardner-Medwin, for reading an earlier version of this manuscript. We are grateful to M. Gardner for help with the analysis of blood gases.

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