Pediat. Res. *5:* 173–180 (1971)

amino acids hypoxia
anoxia newborn
brain protein
growth temperature

# Postnatal Changes in Protein Metabolism of Brain. II. Effects of Alteration of Ambient Temperature and Gaseous Composition of Inspired Air

R. J. SCHAIN[34] AND K. WATANABE

Division of Pediatric Neurology and the Mental Retardation Program, Neuropsychiatric Institute, University of California at Los Angeles Center for the Health Sciences, University of California, Los Angeles, California, USA

*Extract*

A number of environmental factors affecting the incorporation of L-leucine-$^{14}$C into brain proteins of newborn rats and guinea pigs under *in vivo* conditions have been investigated. Reduction of ambient temperature from 35 to 22° results in a 35% decrease in the incorporation of leucine into brain proteins in the newborn rat. Similar alterations do not affect incorporation rates in the newborn guinea pig.

Graded reductions of the oxygen concentration of inspired air do not affect incorporation rates into brain proteins of the newborn rat until total anoxia is achieved. In contrast, reduction of incorporation of leucine into liver proteins occurs at $O_2$ concentrations below 10% in inspired air.

Anoxia does not affect the entry of an inert amino acid, $^{14}$C-$\alpha$-aminoisobutyric acid, into brain substance under *in vivo* conditions. It is concluded that alteration of levels of radioactivity in free amino acid pools under similar circumstances is not attributable to structural damage of the vascular bed.

*Speculation*

These data indicate that reduction of ambient temperature alters protein biosynthetic processes in the brain of the newborn animal. The effect appears to be produced by direct alteration of body temperature rather than by energy depletion accompanying thermogenesis. This finding leads to the consideration that prolonged neonatal hypothermia may decelerate brain development with resulting cerebral deficits at a later time. By contrast, the lack of effect of severe hypoxia upon incorporation of amino acids into brain proteins suggests that hypoxic episodes in the neonate may have relatively little effect upon growth of the brain during the newborn period.

## *Introduction*

Previous studies in this laboratory have revealed an abrupt decrease in the incorporation of amino acids into several brain areas of the newborn miniature pig [14, 15]. This change in pattern of brain protein biosynthesis under *in vivo* conditions was shown to be related to the event of birth rather than to the conceptional age of the animals. The immediate postnatal period represents a time of special vulnerability of the developing brain; it occurs at a time of rapid growth and development [8] and is associated with the transition to extrauterine life, an event which places great demands upon the adaptive capacities of the newborn.

The purpose of this study was to determine the effects of alteration of the ambient temperature and of oxygen and carbon dioxide in the inspired air upon protein metabolism in newborn rats and guinea pigs. The use of radioactive amino acids as tracers provided an opportunity to view "acute" changes in cellular protein metabolism in the intact animal as influenced by environmental alterations.

## *Materials and Methods*

### *Animals*

Infant rats of the Wistar strain [21] and guinea pigs [22] were utilized in these experiments because they differ markedly in their levels of neurological and behavioral development at birth. The newborn rat is highly immature (furless, sightless, and deaf), has few motor abilities, and is not able to maintain body temperature in even moderately cool environments. The ability to suckle is virtually its only well-established neurological function. In contrast, the newborn guinea pig has locomotor abilities established shortly after birth, possesses well-developed homeothermic mechanisms, and manifests a number of infantile reflexes. Its physical appearance is quite mature since it is completely furred and possesses fully developed sight and hearing.

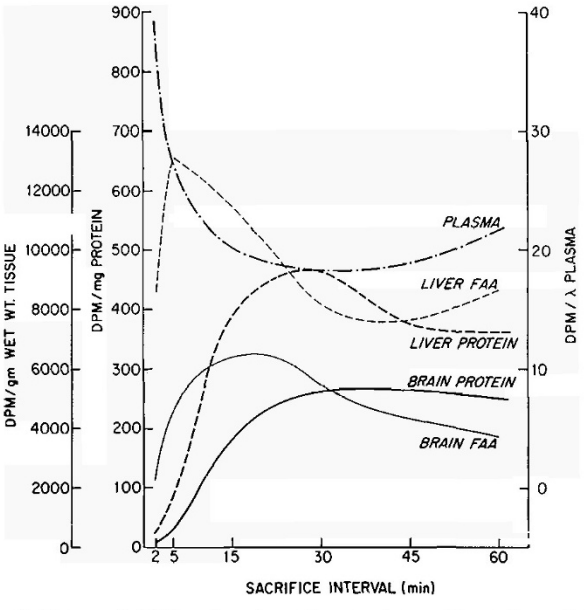


*Fig. 1.* Entry of $^{14}$C-leucine into free amino acid pools and incorporation into tissue proteins. Plasma radioactivity refers to activity in raw plasma. Sacrifice interval refers to minutes between pulse labeling (0.2 $\mu$Ci/10 g body weight) and death of animal. Infant rats were 1 day old and were obtained from one litter. Ambient temperature: 30°. DPM: disintegrations per minute. FAA: free amino acids.

Infant rats or guinea pigs less than 24 hr old were designated as newborn (although most of the animals so classified were less than 16 hr old). The maternal rats were maintained on rat ration [23]. The nursing mothers and offspring were kept at 24° prior to the experiments. All experiments were carried out on unweaned animals (newborn to 21 days in the case of the rats, newborn to 7 days for the guinea pigs).

### *Experimental Procedures*

Tracer dosages (0.2$\mu$Ci/10 g body weight) of radioisotopes were administered subcutaneously into a highly vascularized area over the back of the neck. This site of injection was found to be more reliable in infant animals than the usual intraperitoneal route; time graphs of radioactivity in plasma revealed extraordinarily rapid absorption with peak plasma levels reached less than 2 min after administration (Fig. 1). Peak activity in the chloroform-methanol-soluble fraction occurs within 5–15 min after administration of the pulse; peak activity in the protein fraction characteristically occurs approximately 30 min after injection. Infant guinea pigs were injected at a similar site.

The animals were decapitated 45 min after administration of the radioisotope in all experiments. Brain, liver, and blood were removed within 3 min. Brain and liver were frozen over Dry Ice and stored at −16°. Plasma was separated from blood and similarly stored.

The radioisotopes used were leucine-$^{14}$C (uniformly labeled) (specific activity 160–278 mCi/mmole) [24, 25], and $\alpha$-aminoisobutyric-1-$^{14}$C acid (specific activity 204 mCi/mmole) [24].

### *Biochemical Analysis*

Tissues were homogenized in 20 volumes of chloroform-methanol (2:1, v/v) and centrifuged at 2000 rpm for 40 min, and the supernatants were saved. The pellets were rewashed with 10 volumes of chloroform-methanol. The combined supernatants were extracted with 0.1 M NaCl according to the method of Folch *et al.* [10]. An aliquot of the aqueous (upper) phase of this extraction, containing free amino acids, was counted in Bray's solution [3]. This fraction is designated as the free amino acid fraction (FAA) and contained labeled, unmetabolized leucine as well as other labeled products of leucine metabolism, in particular dicarboxylic amino acids and their derivatives [13].

The residue from the second chloroform-methanol extraction was washed once with 20 volumes 95%

ethanol and three times with 5% trichloroacetic acid (TCA). The second TCA wash was carried out at 90° for 30 min in order to remove nucleic acids. After a final wash with 100% methanol, the pellets were scraped out onto watch glasses, taken to visual dryness *in vacuo*, and dried overnight at 110°. These residues were regarded as purified protein. Ten-milligram samples were digested in 0.5 ml solubilizer [26] at 50° for 1–2 hr, combined with 10 ml PROPOP scintillation fluid, and counted in a liquid scintillation spectrometer [27]. The counts obtained were corrected for quenching and expressed as disintegrations per minute per milligram protein or, for the chloroform-methanol-soluble fraction, as disintegrations per minute per gram wet weight tissue. Radioactivity in plasma was counted directly in PROPOP scintillator, after digestion with solubilizer [26], and expressed as disintegrations per minute per microliter.

*Environmental Alterations*

Temperature studies were conducted in an incubator [28] in which the air temperature is thermostatically regulated. The animals were exposed to the stated temperatures for 30 min prior to administration of the tracer. In some experiments subcutaneous temperature was monitored with a telethermometer [29] during the 45-min interval. A flexible, thin probe [30] was inserted into the loose tissue over the dorsum with the tip at the midthoracic areas. Experiments requiring alteration of ambient gas tensions were conducted in a 10-liter desiccator jar placed in an incubator [31] at 30–32° (nest temperature). Gas mixtures of known concentration were passed through the jar at the rate of approximately 4 liters/min. Oxygen tensions in the inspired air were monitored with an oxygen analyzer [32]. The animals were maintained at the stated gaseous conditions for the full 45-min interval between pulsed injection of the tracer and death. In most experiments, half of the litter served as controls.

## *Results*

*Alteration of Ambient Temperature*

Figure 2 illustrates the incorporation of labeled leucine into brain and liver proteins as a function of age at two different ambient temperatures. It is evident that a significantly greater degree of incorporation (35%) in brain proteins occurred in newborn rats which were maintained at 35° than in those at the ambient temperature of 22°. This difference steadily diminished during the first few days of life and was no longer present by 4 days of age. In contrast, differences of incorporation into liver protein were not significantly different until 4 days of age, when increased incorporation occurred in animals maintained at room temperature.

The radioactivity in the chloroform-methanol-soluble fraction of these experiments is presented in graphic form in Figure 3. The variance present in these data is too great to make statistically significant comparisons at any single time point (except for the newborn brain and liver data). However, the trend is clear: radioactivity in the chloroform-methanol-soluble fraction of brain tissue was higher in the experiments conducted at an ambient temperature of 22° than at 35°. The data from the brain chloroform-meth-

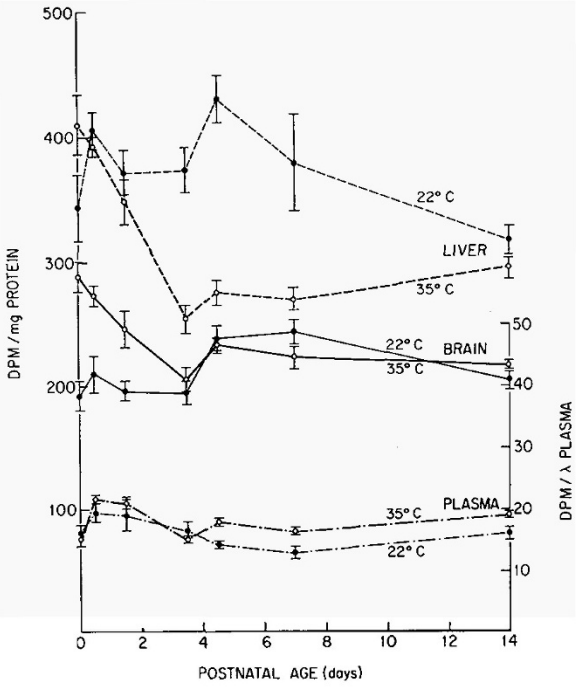


*Fig. 2.* Incorporation of $^{14}$C-leucine into protein fraction of rat brain and liver as a function of postnatal age. Ambient temperatures 22 and 35°. Forty-five-minute interval between pulse labeling (0.2 μCi/10 g body weight) and death of animal. Vertical bars are standard errors about the mean. DPM: disintegrations per minute.

*P* values for difference of means at two temperatures for first 7 days (Student's *t* test)[1]

| | 0 | 1 day | 2 days | 4 days | 7 days |
|---|---|---|---|---|---|
| Brain | <0.01 | <0.01 | <0.02 | NS | NS |
| Liver | NS | NS | NS | <0.01 | <0.01 |

[1] Means derived from 6–10 animals.

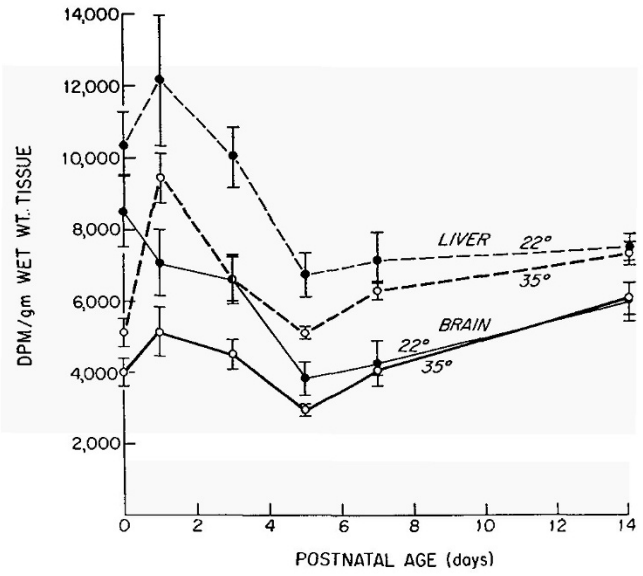


*Fig. 3.* Radioactivity in the aqueous extract of the chloroform-methanol-soluble fraction of rat brain and liver as a function of postnatal age. Individual points represent means from a minimum of animals. Experimental conditions as in Figure 2.

anol-soluble fractions resemble a mirror image of the brain protein data in Figure 2. This suggests that, at least in brain, decreased incorporation in these experiments was associated with increased radioactivity in the chloroform-methanol-soluble fraction.

Similar experiments were carried out on newborn guinea pigs. Figure 4 illustrates the incorporation of leucine into protein and the radioactivity of the chloroform-methanol-soluble fraction in newborn guinea pigs at two different ambient temperatures. Similar data from the rat experiments are also presented for purposes of comparison. It is evident that ambient temperature alterations do not affect incorporation rates in newborn guinea pigs as in newborn rats. The same is true for radioactivity in the chloroform-methanol-soluble fraction.

The 30-min preexperimental exposure to the stated ambient temperature was found to be adequate for the maintenance of relatively stable body temperature during the 45-min experimental period. The subcutaneous temperatures of newborn rats are approximately 10° apart at ambient temperatures of 24° and 35°. This difference is still quite evident at 4 and 7 days of age; by 14 days of age the rat is much more effective in defending body temperature against cool external conditions. The subcutaneous temperature of the guinea pig varied by no more than 1–3° regardless of age, again confirming the thermal stability of the newborn guinea pig compared with species such as the rat, which are born in a more immature state. Values of subcutaneous temperatures determined on a separate series of rats and guinea pigs are given in Figure 5.

Changes in the subcutaneous temperature of small infant mammals may be assumed to give a fair indication of similar changes in colonic temperatures, even though temperature gradients are present in different somatic sites. Hull [12] found relatively small gradients to exist between colon, brain, and subcutaneous tissue in newborn rabbits at ambient temperatures of 25–35°. The mean difference between brain and subcutaneous sites was 0.8°.

### *Alteration of Inspired Gases*

Experiments relating graded degrees of hypoxia to the incorporation of leucine into brain and liver proteins of the intact newborn rat are outlined in Figures 6 and 7. The rats maintained in atmospheres of 2 and

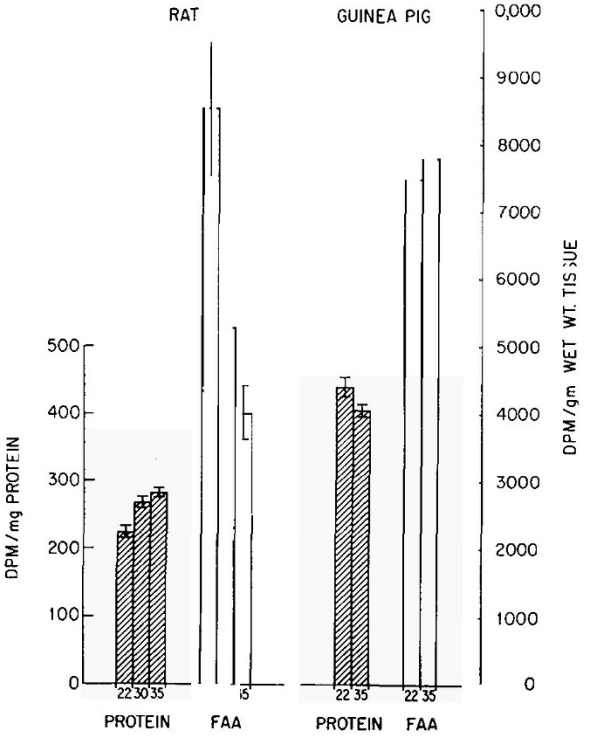


*Fig. 4.* Histograms of means representing entry of $^{14}$C-leucine into newborn guinea pig and rat brain and incorporation into proteins at graded ambient temperatures. Forty-five-minute interval between pulse labeling and death. Individual bars represent means from 3–4 guinea pigs and 8–28 rats. Numerals at column bases refer to temperature in degrees centigrade. FAA: free amino acids. The differences between the means of guinea pig protein disintegrations per minute were not significant. The means of the rat protein disintegrations per minute were significantly different (22 and 35°) at a $P$ level of 0.01 (Student's $t$ test).

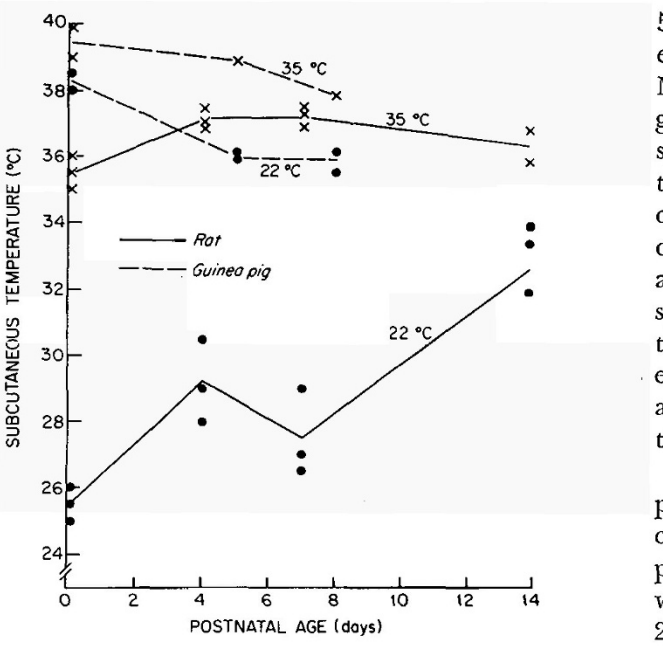


*Fig. 5.* Subcutaneous temperatures in dorsal thoracic region after exposure to stated ambient temperatures for 45 min. Each point represents a temperature recording from one animal.

5% oxygen (20 and 50 mm Hg) manifested intense exploratory activity initially and then became torpid. Marked cyanosis was noted in the animals in 2% oxygen. Animals maintained in pure nitrogen ceased spontaneous activity and often ceased active respiration, but exhibited a continuous heart beat at the time of decapitation. Figure 6 shows that the incorporation of leucine into brain proteins of newborn rats is resistant to severe reductions in the $O_2$ content of the inspired air. The animals required exposure to pure nitrogen before a reduction of incorporation rate was evident. Radioactivity in plasma and chloroform-methanol-soluble fractions revealed the usual inverse relation to incorporation levels.

In comparison to the brain protein data, the incorporation of leucine into liver proteins was more susceptible to hypoxia (Figure 7). The major fall of incorporation occurs when the ambient $O_2$ concentration was reduced from 5 to 2%. It should be noted that at 20% $O_2$ concentration, the incorporation of leucine into liver proteins was approximately twice that of incorporation into brain proteins. At 2% oxygen, this ratio was more than reversed, with brain displaying three times the activity obtained in the liver.

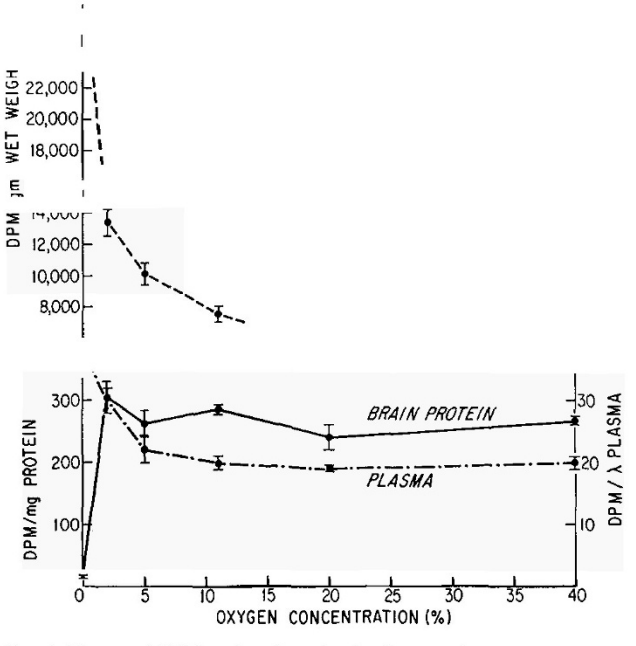


*Fig. 6.* Entry of $^{14}$C-leucine into brain free amino acid pool and incorporation into brain proteins of newborn rats at graded $O_2$ concentrations in inspired air. Forty-five-minute interval between pulse labeling and death. Ambient temperature: 30°. Individual points represent means from 5–13 animals. Other details as in Figure 1.

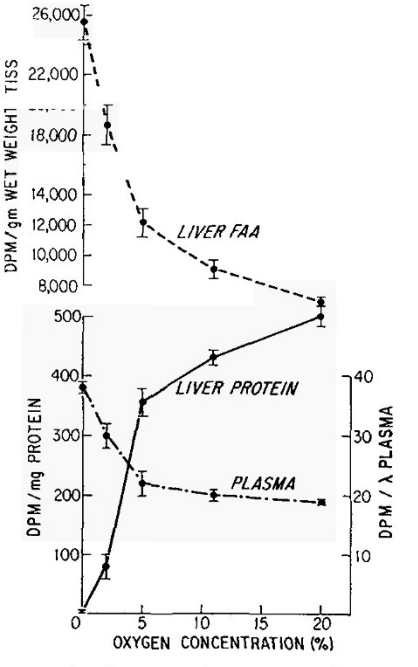


*Fig. 7.* Entry of $^{14}$C-leucine into liver free amino acid pool and incorporation into liver proteins of newborn rat at graded $O_2$ concentrations in inspired air. Forty-five-minute interval between pulse labeling and death. Ambient temperature: 30°. Individual points represent means from 5–13 animals. Other details as in Figure 1.

*Table I.* Effect of anoxia upon entry of $^{14}$C-AIB into brain and liver[1]

| Inspired gas | Brain | Liver | Plasma |
|---|---|---|---|
| | dpm/g wet wt tissue | | dpm/μl |
| Room air | 10,228 ± 192[1] (8)[2] | 31,843 ± 2,778 (9) | 34 ± 2 (9) |
| 100% $N_2$ | 10,193 ± 743 (6) | 24,860 ± 1,077 (6) | 48 ± 3 (6) |

[1] SE.
[2] Figures in parentheses represent number of animals.

In order to determine whether the increased radioactivity in the chloroform-methanol-soluble fraction obtained in the severe hypoxia experiments was due to a breakdown of physical barriers between the vascular system and brain substance, experiments were conducted utilizing radioactively labeled α-aminoisobutyric acid ($^{14}$C-AIB). This inert, synthetic amino acid has been used to study transport phenomena in the brain since it is not metabolized in animal tissues [16]. As indicated in Table I, severe hypoxia does not influence the entry of $^{14}$C-AIB into the brain. It can be concluded that the increased levels of $^{14}$C-leucine under the same circumstances are not attributable to structural damage of the vascular wall, secondary to hypoxia.

The effects of hypercapnia upon the incorporation of leucine into protein was studied by carrying out a group of experiments in 5% $CO_2$, 20% $O_2$, balance $N_2$ (Table II). Little difference was noted in radioactivity in the control and experimental brain protein fractions. However, the increased radioactivity of the chloroform-methanol fraction of brain in the hypercapnic animals was statistically significant and may be attributable to the cerebral vasodilatation produced by elevated plasma $CO_2$ tensions.

## *Discussion*

Our data indicate that the incorporation of leucine into brain proteins of infant rats is a function of ambient temperature during the first few days of life. The body temperature of the newborn rat closely approximates that of its environment because of an ineffective thermogenic response to cold [1, 18]. Unlike newborn rats, newborn guinea pigs are born in a mature state with relatively efficient homeothermic mechanisms. Newborn guinea pigs incorporate leucine into brain proteins at a rate unaffected by ambient temperature within the temperature range of these experiments. Therefore, we believe that environmental temperature alterations affect incorporation primarily by altering body temperature rather than by diverting energy supplies into thermogenesis. This opinion is reinforced by the hypoxia data, which indicate that marked reduction of available energy does not appreciably effect the synthesis of brain proteins. Amino acid incorporation into brain proteins is similar to protein-synthesizing systems in other tissues, requiring activating enzymes, an adenosine triphosphate-generating source, ribonucleic acids, and appropriate amino acid substrates [20]. Such systems are temperature-sensitive, as are all chemical reactions, enzymatic or nonenzymatic. These experiments demonstrate this sensitivity under *in vivo* conditions within physiological temperature ranges.

The effect of ambient temperature alterations upon incorporation of leucine into brain proteins as a function of age does not exactly parallel the effect upon subcutaneous temperatures. In 4- to 7-day-old rats, subcutaneous temperatures are 8° lower at ambient temperatures of 22° than at 35°, while incorporation into brain proteins at these ages is similar at the two ambient temperatures. It may be that brain temperature is better maintained in a cool environment than is subcutaneous temperature at these ages, even though previously described gradients between these sites in newborn rabbits are small [12]. A second possibility is that the high level of brain protein incorporation which occurs during the first few days of life is more sensitive to hypothermic conditions. Direct recording of brain temperature is required to settle this issue.

*Table II.* Effects of hypercapnia upon incorporation of $^{14}$C-leucine into brain and liver proteins

| Inspired gas | Protein | | Chloroform-methanol-soluble fraction | | Plasma |
|---|---|---|---|---|---|
| | Brain | Liver | Brain | Liver | |
| | dpm/mg protein | | dpm/g wet wt tissue | | dpm/μl |
| Room air | 305 ± 11[1] (7)[2] | 577 ± 20 (7)[3] | 4,010 ± 164 (7)[4] | 6,025 ± 1,620 (7) | 21 ± 0.3 |
| 5% $CO_2$ 20% $O_2$ 75% $N_2$ | 310 ± 4 (7) | 642 ± 13 (7)[3] | 5,586 ± 362 (7)[4] | 6,315 ± 197 (7) | 19 ± 0.3 |

[1] SE.
[2] Figures in parentheses refer to number of animals.
[3] Significant difference, $P < 0.05$.
[4] Significant difference, $P < 0.01$.

The significance of the dependence of amino acid incorporation into brain proteins upon thermal stability of the newborn is of some interest in regard to the human neonate. It has been well documented that the survival rate of premature infants can be raised by maintaining them in environments which place the least possible stress on their themoregulatory capacities [4, 7, 17], in spite of the evidence in experimental animals that hypothermia prolongs survival time under asphyctic conditions [13]. It is reasonable to speculate that thermoregulatory stress may have an effect upon the developing central nervous system short of producing actual demise. This issue is important in view of the recent report that human prematures manifest increased cold resistance when raised at thermal conditions below thermal neutrality [11].

The experiments conducted in graded degrees of hypoxia reveal a remarkable ability of the newborn rat to maintain the biosynthesis of brain proteins under conditions of severe hypoxia. At an oxygen concentration of 2% in inspired air, blood oxygen tension was not higher than 20 mm Hg, representing a profound degree of hypoxia. The relative resistance of this newborn animal to prolonged anoxia is well known [9]. Avery [2] has reported a "striking resistance" of the newborn rat to prolonged hypoxia (3–4% $O_2$ in inspired air). It appears from our data that total anoxia is required before suppression of leucine incorporation into brain proteins occurs. By contrast, the incorporation of leucine into liver proteins is depressed severalfold at ambient $O_2$ tensions unaffecting brain incorporation rates. The liver data are more in accord with evidence that the $O_2$ consumption of a number of newborn animal species falls when the inspired air is reduced to below 10% [6]. It may be presumed that the brain possesses a specific metabolic property allowing it to maintain protein biosynthesis under hypoxic conditions. This property is probably a low metabolic rate occurring during the newborn period since, contrary to common belief, there is no evidence that glycolysis provides a special source of energy to the brain of the newborn [19].

The postnatal fall of incorporation of amino acids into brain proteins as previously reported has been observed in these experiments in both rats and guinea pigs, although thermostable conditions are required for demonstration in the newborn rat (Fig. 2). None of the environmental alterations reported in this study account for this phenomenon. The explanation for postnatal reduction of brain protein synthesis is not yet available but may be assumed to be associated with some metabolic or hormonal change associated with the transition to extrauterine life.

We have suggested that incorporation of tracers into the heterogeneous proteins of organ homogenates is best regarded as being analogous to the metabolic rate of whole organisms since it is a result of incorporation into numerous protein fractions existing in regional and subcellular locations [15]. A previous study of the labeling of subcellular fractions of brain proteins *in vivo* has revealed the highest rate of incorporation during 0.5–3 hr after injection to occur in the microsomal protein fraction [5]. The 45-min interval between pulse labeling and death in the experiments we report here makes it probable that the bulk of the labeling has occurred in proteins deriving from the microsomal, or more specifically, ribosomal, subcellular fraction.

*References and Notes*


1. Adolph, E. F.: Ontogeny of physiological regulations in rat. Quart. Rev. Biol., *32:* 89 (1957).
2. Avery, G. B.: The effect of hypoxia on newborn animals, with reference to hyaline membrane disease. Pediatrics, *32:* 801 (1963).
3. Bray, G. A.: A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem., *1:* 279 (1960).
4. Buetow, K. C., and Klein, S. W.: Effect of maintenance of "normal" skin temperature on survival of infants of low body weights. Pediatrics, *34:* 163 (1964).
5. Clouet, D. H., and Richter, D.: Incorporation of ($^{35}S$) labelled methionine into the proteins of the rat brain. J. Neurochem., *3:* 219 (1959).
6. Dawes, G. S.: Foetal and Neonatal Physiology, p. 203 (Year Book Medical Publishers, Chicago, 1968).
7. Day, R., Caliguiri, L., Kamenska, C., and Ehrlich, F.: Body temperature and survival of premature infants. Pediatrics, *34:* 171 (1964).
8. Dobbing, G. J.: Vulnerable periods in the developing brain. In: A. N. Davison and J. Dobbing: Applied Neurochemistry, p. 287 (F. A. Davis, Philadelphia, 1968).
9. Fazekas, J. F., Alexander, F. A. D., and Himwich, H. E.: Tolerance of the newborn to anoxia. Amer. J. Physiol., *134:* 281 (1941).
10. Folch, J., Lees, M., and Sloane-Stanley, G. H.: A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem., *226:* 497 (1957).
11. Glass, L., Silverman, W. A., and Sinclair, J. C.: Effect of thermal environment on cold resistance and growth of small infants after the first week of life. Pediatrics, *41:* 1033 (1968).
12. Hull, D.: Oxygen consumption and body temperature of newborn rabbits and kittens exposed to cold. J. Physiol., *177:* 192 (1965).
13. Miller, J. A., and Miller, F. S.: Interactions between hypothermia and hypoxia-hypercapnia in neonates. Fed. Proc., *25:* 1338 (1966).
14. Schain, R. J., Carver, M. J., and Copenhaver, J. H.: Protein metabolism in the developing brain: Influence of birth and gestational age. Science, *156:* 984 (1967).

15. SCHAIN, R. J., CARVER, M. J., AND COPENHAVER, J. H.: Postnatal changes in protein metabolism of brain. I. Studies in newborn miniature pigs at varying conceptual ages. Pediat. Res., *3:* 135 (1969).
16. SCHAIN, R. J., COPENHAVER, J. H., AND CARVER, M. J.: Entry of L-($^{14}$C)phenylalanine and alpha-aminoisobutyric acid into the cerebrospinal fluid and brain of dogs. J. Neurochem., *14:* 195 (1967).
17. SILVERMAN, W. A., FERTIG, J. W., AND BERGER, A. P.: The influence of the thermal environment upon the survival of newly born premature infants. Pediatrics, *22:* 876 (1958).
18. TAYLOR, P. M.: Oxygen consumption in newborn rats. J. Physiol., *154:* 153 (1960).
19. THURSTON, J. H., AND MCDOUGAL, D. B., JR.: Effect of ischemia on metabolism of the brain of the newborn mouse. Amer. J. Physiol., *216:* 348 (1969).
20. ZOMZELY, C. E., ROBERTS, S., AND RAPOPORT, D.: Regulation of cerebral metabolism of amino acids. III. Characteristics of amino acid incorporation into protein of microsomal and ribosomal preparations of rat cerebral cortex. J. Neurochem., *11:* 567 (1964).
21. Simonsen Laboratories, Gilroy, Calif.
22. Laboratory Associates, Kirkland, Wash.
23. Purina Rat Chow, Ralston-Purina Company, St. Louis, Mo.
24. New England Nuclear Corporation, Boston, Mass.
25. Schwarz BioResearch, Orangeburg, N. Y.
26. NCS, Nuclear-Chicago Corp., Des Plaines, Ill.
27. Packard TriCarb, Packard Instrument Company, Downers Grove, Ill.
28. Armstrong Care-ette, Ohio Chemical and Surgical Equipment Company, Madison, Wis.
29. Yellow Springs Instrument Company, Yellow Springs, Oh.
30. YSI 402, Yellow Springs Instrument Company.
31. Beckman Instrument Company, Fullerton, Calif.
32. Presented in part at the 39th meeting of the Society for Pediatric Research, Atlantic City, N. J., 1969.
33. This investigation was supported by Public Health Service Grants nos. NB-5231 and HD-4612.
34. Requests for reprints should be addressed to: R. J. Schain, Department of Pediatrics, UCLA School of Medicine, Los Angeles, Calif. 90024 (USA).
35. Accepted for publication June 12, 1970.

Copyright © 1971 International Pediatric Research Foundation, Inc. *Printed in U.S.A.*