Growth and Development of Brain of Artificially Reared Hypoketonemic Rat Pups (43152)

NANCY AUESTAD,[1] ROBIN FISHER, FRANCESCO CHIAPPELLI, ROSE A. KORSAK, AND JOHN EDMOND[2]

Division of Nutritional Sciences, University of California at Los Angeles School of Public Health and the Department of Biological Chemistry, Department of Anatomy and Cell Biology, and the Mental Retardation Research Center, and Psychoneuroimmunology Program, Departments of Psychiatry and Biobehavioral Sciences, and Microbiology and Immunology, The Brain Research Institute, University of California at Los Angeles School of Medicine, Los Angeles, California 90024-1737

Abstract. **Three groups of rats were reared: mother-reared controls; artificially reared controls (AR-c), which were fed a milk substitute with the same composition of macronutrients as natural rat's milk; and an artificially reared test group (AR-h), which was fed a milk substitute identical to that fed AR-c pups except that the component of fat containing medium chain length fatty acids was omitted (medium chain triglyceride deficient) and replaced on an isocaloric basis with carbohydrate. The AR rats were fed the milk substitute from postnatal Day 5 until Day 17 by fitting them with gastric cannulas through which the milk could be infused automatically. The nutritional impact of the milk substitutes on growth and the integrity of the brain was assessed by a comparison of morphologic and biochemical markers. Pups in the AR-h group were hypoketonemic. Animals in all groups attained the same body weight by Day 17 and there was no difference in the morphologic markers among the groups with one exception: the vibrissal "barrel fields" of the somatosensory cortex of rat pups in both AR groups were reduced in size but not in number or distribution from those of the mother-reared groups. Furthermore, the brains of the rat pups in the AR groups were not different in weight, but they weighed less than brains of mother-reared controls. Our data show that although there are many similarities in the status of AR rat pups when compared with mother-reared controls, distinctive differences associated with artificial rearing are evident. We conclude that medium chain fatty acids in milk fat and the circulating ketone bodies are not mandatory substrates for growth and the development of the brain. Mechanisms must exist whereby alternative substrates are used to compensate when these metabolites are diminished in supply.** [P.S.E.B.M. 1990, Vol 195]

Considerable evidence indicates the detrimental impact of under- and malnutrition on mammalian brain development (1, 2, and references therein). In an animal model such as the rat, brain is most vulnerable to nutritional factors during the postnatal growth spurt. Malnutrition in this critical period yields a number of brain deficits including: (i) reductions in cell size and frequency (3, 4); (ii) impaired synaptogenesis (5); (iii) abnormal electrophysiologic activity (6); (iv) reduced concentrations of brain lipids (2), DNA, and RNA (4, 7); (v) delayed neuronal, glial, and microglial differentiation and proliferation (1); and (vi) retarded myelination (8, 9). Experimental determinations of the micronutrients associated with particular brain defects are largely uncertain due to (i) the complex composition of milk and (ii) the difficulty of manipulating nutrient in *ad libitum* suckling animals. In light of these problems, the purpose of our study was 2-fold. First, a specific substitution to the composition of the milk formula, where one caloric component was omitted (medium chain triglycerides of fat) and replaced by an alternative energy source (carbohydrate) was introduced to test the hypothesis that the medium chain

[1] Present address: Department of Paediatrics, The Research Centre, University of British Columbia, Vancouver, British Columbia, Canada V5Z 4H4.

[2] To whom reprint requests should be addressed at Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024-1737.

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length fatty acid components of milk fat and ketone bodies in blood are mandatory substrates in early development. Second, the morphology and chemical composition of brains of rat pups reared without maternal contact and on a milk substitute closely resembling rat's milk in composition are compared with that of brains of rat pups reared by their mother. The experiments were designed not to create a state of undernourishment for the neonatal rat, but to impose a state of isonutrition that would introduce a distinct metabolic environment from normal during the period of milk dependence. Isocaloric substitution of the medium chain triglycerides with a glucose polymer resulted in a reduction in circulating ketone bodies in an artificially reared (AR) test group (AR-h) to levels 20–25% of those in both the AR control groups (AR-c) and mother-reared control group (MR).

Our focus on ketone bodies was due to recent reports indicating that ketone bodies, which circulate at concentrations about 10-fold higher in the suckling rat than in the well-fed adult rat (10, 11), are important energy substrates (11, 12) and carbon sources for the formation of lipids (13–17) during the brain growth spurt. Hawkins *et al.* (11) estimate that circulating ketone bodies could supply between 48 and 76% of the energy required for brain development during this period. In addition, Bhat and Volpe (18) suggest that most of the cholesterol in brain derives from synthesis *in situ*. Finally, ketone bodies may supply a substantial proportion of the carbon for the synthesis of cholesterol and fatty acids in the brain (14, 19).

A broad spectrum of biochemical and morphologic measurements was performed to determine if normal growth and brain development occur in rat pups fed milk substitutes on the system to artificially rear them. The biochemical measurements are designed to assess if the reduction in the ketone body supply in the AR-h group influences the lipid content of brain or results in changes in the activity level of enzymes responsible for their utilization as key substrates in energy production and lipid synthesis. Morphologic investigations are directed at regions of brain actively developing during the period of milk feeding to assess if conditions of artificial rearing and/or the impact of an isonutritional condition influence the integrity of developing brain.

Materials and Methods

Animals. To secure a pool of about 48 rat pups, from which 24 could be selected for a particular rearing experiment, we routinely obtained six- to eight-time pregnant Sprague-Dawley rats at 14-day gestation from Bantin and Kingman, Fremont, CA. These animals were housed in an isolated area as described previously (20). Postnatal Day 0 was the calendar day on which the rat pups were born. Litters remained undisturbed until Day 5. Routinely, five litters containing 10 to 12 pups were born within a 24-hr period. Litters, which were included in the pool for selection, were chosen if they and their mother were deemed healthy and of appropriate weight for pups 4 to 5 days of age (11–13 g body wt). Litter size was reduced to eight or nine by discarding the light or heavy pups, or both. Litters, which were undersized because of the birth of a large number of pups, or were exceptionally heavy because the litter size was small, were excluded from the selection.

Surgery and Rearing Conditions. On postnatal Day 5, pups were assigned randomly to a MR, AR-c, or AR-h group. Pups within individual litters were weight-matched and divided among the three groups. The AR groups consisted of eight rats per group, and the MR group consisted of 10 pups. The MR group was allowed to suckle *ad libitum*, the AR-c group was artificially reared from 4 to 17 days of age on a rat milk substitute (RMS) resembling rat milk in composition as described previously (20), and the AR-h group was artificially reared on the same formula modified only in its fat and carbohydrate content. The composition of the milks is shown in Table I. The procedure of Hall (21) was used to implant the gastric feed cannulas. Each pup was housed individually in a plastic deli cup, measuring 8-cm deep and 10.5 cm in diameter as described by Hall (21). The cups contained a layer of dust-free "Bed o Cob" (Anderson's Cob Division, Delphi, IN), which was replaced daily and served as bedding. Each cup was fitted with a lid that contained five air holes of 3/8 inches in diameter. The artificially reared animals were maintained daily when the syringes containing fresh milk were replaced and the cannulas were flushed out with 0.2 ml of water. Each animal was stimulated to urinate by stroking the perianal region with a Q-tip; the animals were weighed and examined to check that the cannulas were properly placed and

Table I. Composition of Milks[a]

	Rat milk, MR group	Rat milk substitutes	
		AR-c group	AR-h group
	kcal/100 ml milk		
Energy content	124–223	171[b] 166[c]	172[b] 172[c]
	g/100 g milk		
Protein	6.9–11.8	9.7	9.8
Carbohydrate (total)	2.8–3.7	4.7	10.2
Lactose	2.6	2.5	2.5
Fat	9.3–17.5	12.6	10.2

[a] The values for the composition of rat's milk were taken from Auestad *et al.* (20). The compositions of the rat milk substitutes were determined as described in Materials and Methods. The values shown are the averages from duplicate or triplicate determinations.
[b] Calculated based on average values for protein, carbohydrate, and fat using: protein, 4 kcal/g; carbohydrate, 4 kcal/g; fat, 9 kcal/g.
[c] Determined by calorimetry.

the sites of entry to the stomach were without infection (20, 21). Rat pups that served as controls were reared by their mother in cages that were housed in the same room as the rearing system. Control animals were weighed every 3 days.

The basic procedure for the preparation of the RMS for the AR groups was described previously as RMS-2B (20) with cholesterol supplementation (22). Cholesterol as 0.5 g of cholesteryl oleate and 0.2 g of free cholesterol per liter milk substitute, respectively, was dissolved in the oil mixture at 60°C for 3 hr, then cooled to room temperature before blending into the milk. The AR-c group was fed the cholesterol-supplemented RMS-2B by an automated intragastric feeding regime (20). The AR-h group was fed a modified preparation of RMS-2B in which the medium chain triglyceride (MCT) components (MCT Oil and Captex-MCT) were omitted and replaced on an isocaloric basis with 8.36 g of a glucose polymer, Polycose, and 1.76 of corn oil/100 ml of milk. The compositions of the milks fed AR-c and AR-h rat pups were determined by Bioserv Inc., Frenchtown, NJ (20) (Table I).

Biochemical Measurements. Samples of blood were collected as a mixture of arterial and venous blood from the neck stump following decapitation at 15 to 30 min after the end of a feeding period for the AR groups and from well-fed rat pups in the MR group. Deproteinated plasma filtrate was prepared by centrifuging plasma at 1500*g* through the Amicon Centrifree System on a Sorval centrifuge at 4°C for 90 min. The concentrations of the ketone bodies, acetoacetate and D(−)-3-hydroxybutyrate, in plasma filtrates were determined as described previously (20). Glucose was then measured using a Beckman Glucose analyzer.

Brains were dissected to exclude olfactory bulbs and spinal cords, weighed, and hemispheres were separated. One-half of the brain was used for measuring the concentration of cholesterol, desmosterol, and cerebroside sulfate. The other half was used for measuring the activities of the enzymes pyruvate dehydrogenase (EC 1.2.4.1), 3-oxo-acid-CoA transferase (EC 2.8.3.5), and acetoacetyl-CoA ligase (EC 6.2.1.16). For lipid analyses, half brains were saponified and lipids extracted into petroleum ether/methanol as described previously (16). Sterols were measured by gas chromatography (23), and cerebroside sulfate was measured according to the method of Kean (24). The activities of the enzymes pyruvate dehydrogenase, 3-oxoacid-CoA transferase, and acetoacetyl-CoA ligase were measured in brain homogenates as described (25–27) with modifications (20). In the optimization of the assay for pyruvate dehydrogenase, we observed that activity levels greater than reported previously were obtained when the coupling enzyme for the assay, arylamine acetyl transferase, was prepared at the highest possible concentration and used from this stock in the standard assay of Ksiezak-Reding *et al.* (25). To obtain the developmental profile for pyruvate dehydrogenase in brain from fetal Day 17 to postnatal Day 40, brains from fetal animals and rats were prepared for the assay of pyruvate dehydrogenase as described by Ksiezak-Reding *et al.* (25). The values were used to make comparisons with the values obtained for pyruvate dehydrogenase in brain of artificially reared rats. Protein concentration was determined by the Bradford method (28).

Morphologic Studies. Twelve rats from two different litters (four animals per group) provided tissue for anatomical studies. The animals were sacrificed by barbiturate overdose (phenobarbital, 100 mg/kg body wt). Transcardial perfusion of saline washout and aldehyde fixative solutions were accomplished after the inferior vena cava was ligated.

The brains (excluding spinal cords) were divided into two tissue blocks by midsagittal section. The left hemispheres were encased immediately in gelatin and serially sectioned in the sagittal plane (Vibratome; 100-μm section thickness). Alternating sections in this series were stained with cresyl violet or were processed histochemically for cytochrome oxidase activity by methods adapted from Wong-Riley (29). These specimens were used to assess the gross regional and histologic arrangement of the brains of rat pups from each treatment group. The cytochrome oxidase materials provided an anatomical evaluation of the aerobic metabolic capacities of different brain regions with particular reference to the vertical and horizontal organizations of the vibrissal "barrel field" representation in the sensorimotor neocortex (30). Although composed of pre- and postsynaptic components, these morphologic patterns are known to be quite sensitive to postnatal shifts in the functional activity and the spatial arrangement of axonal connections in the brain (31).

The right hemispheres were fixed for an additional 24 hr. They were then encased in gelatin and serially sectioned in the coronal plane (Vibratome; 100-μm section thickness). Alternating sections were processed for silver chromate to show fine cellular morphology impregnation (single-section Golgi method of Gabbott and Somogyi (32) or the immunohistochemical detection of selected cell line molecular markers to assess molecular differentiation (glial fibrillary acidic protein), proteolipid protein, neuron-specific enolase, neurofilaments and synapsin); unlabeled antibody peroxidase-antiperoxidase methods were adapted from Sternberger (33). The tissue was examined by light microscopy for comparisons between treatment groups.

Statistical Analyses. Analysis of variance using the Bonferroni *t* test was used to evaluate the reliability of differences between groups.

Results

General. The AR-c and AR-h groups grew as well as the MR groups and all groups had similar body weights at Day 17 when biochemical and morphologic measurements were taken. With the exception of a slight deficit in brain weight for the AR group versus the MR group and a distinctive difference between the AR group and the MR group in the morphology of the vibrissal barrel fields of the somatosensory cortex, no obvious differences could be discerned among the three groups for the many other morphologic and biochemical markers that were investigated.

Milk Composition. The concentrations of protein, carbohydrate, and fat, and the energy content of the rat milk substitute fed to the AR-c group were within the range of reported concentrations of these nutrients in rat milk (Table I). The carbohydrate content of the RMS fed to the AR-h group was 300% higher and the fat content 20% lower than in the control RMS and rat milk. As occurs in rat milk, the medium chain length fatty acids (caprylic, caproic, and lauric acids) accounted for approximately 30% of the total fat by weight in the control RMS, whereas these fatty acids accounted for less than 2% of the total fat by weight in the milk substitute fed the AR-h group (Table II). The concentrations of the minerals and vitamins in the two milk substitutes (data not shown) were not different from those reported previously (20).

Body Growth and Plasma Metabolites. Body growth, as determined by body weight measurements between 5 and 17 days of age, was similar in the three groups (Tables III and IV). The concentration of glucose in the plasma (filtrate) was not different among the AR-h group and the two control groups. However, the circulating concentrations of the ketone bodies in the AR-h group were reduced to levels of 20–25% of values obtained from MR and AR-c groups (Table IV). Earlier on Day 12, ketone bodies were barely detectable in the AR-h group in comparison to their concentration in the MR and AR-c groups, (<0.08, 1.9 ± 0.14, and 1.57 ± 0.09 m*M*, respectively, $n = 5$ or 6 in each group, data not shown).

Biochemical Measurements in Brain. The concentrations of two lipids (sterols and cerebroside sulfate) associated closely with myelin were not different among the three groups (Table V). The concentrations of total sterols in brain were 8.98 ± 0.34, 9.25 ± 0.39, and 9.21 ± 0.19 mg/g brain in the MR, AR-c, and AR-h groups, respectively. Although the mean concentration of cholesterol in brain was not different statistically, the mean concentration of desmosterol was lower ($P < 0.05$) in both AR groups compared with the MR rats (0.34 ± 0.04 and 0.32 ± 0.04 vs 0.46 ± 0.05, respectively). Cerebroside sulfate, a biochemical marker of myelin, was 1.19 ± 0.25, 1.10 ± 0.11, and 1.10 ± 0.14 mmol/g brain in the MR, AR-c, and AR-h groups, respectively.

Since the isonutritional exchange of carbohydrate for MCT in the milk was reflected by a pronounced hypoketonemic state in the suckling rat, it was worthwhile to determine if the enzymatic potential for the utilization of acetoacetate and glucose by brain had adapted to the different metabolic condition. A precocious expression of pyruvate dehydrogenase, which is poorly developed normally in the brain in the mid-suckling period, could compensate for the excess availability of glucose in the experimental AR-h group.

The mitochondrial enzyme, 3-oxo-acid CoA transferase, activates acetoacetate to acetoacetyl-CoA for its subsequent oxidation to CO_2 in the citric acid cycle. Acetoacetyl-CoA ligase is the cytosolic enzyme that activates acetoacetate to acetoacetyl-CoA for synthesis of sterols and fatty acids. The activities of both of these ketone body-utilizing enzymes in the hypoketonemic AR-h group were not different from their respective activities in the MR and AR-c control groups (Table V).

Pyruvate dehydrogenase, essential for the complete oxidation of glucose to CO_2 and flux of carbon from glucose to sterols and fatty acids, is developed incompletely during the suckling period (34) (Fig. 1). It has been reported previously that pyruvate dehydrogenase in brain is barely detectable until Day 5 of age and by Day 15 attains an activity level 20% of the adult level (34). With the assay described by Ksiezak-Reding *et al.*

Table II. Individual Fatty Acids in the Milk Fat

Fatty acid[a]	Rat milk, MR[b] group	Rat milk formulas	
		AR-c[c] group	AR-h[c] group
	g/100 g milk		
8:0	0.30–0.78	0.47 ± 0.09	0.02 ± 0.01
10:0	1.04–2.09	1.61 ± 0.05	0.04 ± 0.02
12:0	1.14–1.51	1.60 ± 0.13	0.09 ± 0.01
14:0	1.10–1.72	0.34 ± 0.01	0.50 ± 0.17
16:0	2.21–3.91	1.58 ± 0.01	1.70 ± 0.06
16:1	0.22–0.28	0.09 ± 0.03	0.07 ± 0.02
18:0	0.29–0.50	0.53 ± 0.02	0.54 ± 0.03
18:1	1.82–2.45	2.29 ± 0.12	2.74 ± 0.12
18:2	0.72–1.37	3.66 ± 0.24	4.29 ± 0.34
18:3	0.07–0.16	0.23 ± 0.04	0.22 ± 0.03
	weight % of total fat		
MCFA[d]	20.7–36.5	29.2 ± 0.5	1.3 ± 0.4
LCSFA	30.0–51.1	19.5 ± 0.5	25.3 ± 4.5
MUFA	17.0–22.7	18.9 ± 0.3	25.7 ± 2.7
PUFA	6.6–18.7	30.9 ± 0.9	40.6 ± 2.8

[a] Number of carbons:number of double bonds.
[b] The range of values for individual fatty acids were calculated based on 12 g fat/100 ml milk and the values for weight percent of total fatty acids were given previously (20).
[c] The values shown are mean ± SE, $n = 3$ milk preparations.
[d] MCFA, medium chain length fatty acids; LSCFA, long chain saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table III. Body Weight with Age of Mother-Reared and Artificially Reared Rat Pups[a]

Age (days)	g Body weight			Volume of milk-fed AR groups (ml/24 hr)
	MR group ($n = 9$)	AR-c group ($n = 6$)	AR-h group ($n = 7$)	
5	11.7 ± 1.5[b]	12.2 ± 1.5	12.2 ± 1.5	3.2
7	16.9 ± 1.7	15.2 ± 1.4	15.0 ± 1.4	4.7
9	20.9 ± 2.0	18.8 ± 1.3	19.1 ± 1.1	6.1
12	26.4 ± 3.8	25.4 ± 1.8	26.4 ± 1.1	7.8
14	31.8 ± 3.3	30.6 ± 2.1	30.5 ± 2.5	9.3
17	37.9 ± 3.3	40.6 ± 1.8	39.7 ± 2.2	11.3

[a] Shown are the body weights of animals for one of the experiments and the volume of milk that was fed the AR rat pups. The details of the feeding protocol, selection of animals, and the rearing system are contained in Materials and Methods.
[b] Values for body weight are means with SD.

Table IV. Metabolites in Plasma of 17-Day-Old Rats[a]

	MR group	AR-c group	AR-h group
Body weight (g)	41.5 ± 3.3[b]	39.7 ± 2.9	40.1 ± 2.7
No. of rats	25	22	22
Ketone bodies (m*M*)			
Total	1.31 ± 0.21	1.23 ± 0.18	0.26 ± 0.09[c]
Acetoacetate	0.35 ± 0.09	0.38 ± 0.06	0.09 ± 0.02[c]
D(−)3-Hydroxybutyrate	0.98 ± 0.19	0.85 ± 0.15	0.17 ± 0.07[c]
No. of rats	10	13	12
Glucose (mg/dl)	150 ± 6	147 ± 6	146 ± 10
No. of rats	5	6	5

[a] Pups were reared as described in Materials and Methods. The concentrations of the ketone bodies and glucose were determined in deproteinated plasma filtrates.
[b] Values are means with SD.
[c] Significantly different from MR, $P < 0.005$, and AR-c, $P < 0.005$, by analysis of variance (Scheffe's multicomparison post-analysis of variance method).

(25), reasonable activity levels can be detected even in the fetal brain (Fig. 1). Pyruvate dehydrogenase activity in adult brain is 72 nmol acetyl CoA formed min^{-1} $mg/protein^{-1}$, a measurement in good agreement with the value determined at this age by Land *et al.* (34). The activity of pyruvate dehydrogenase was not different at 12 or 16 days of age as a result of either the AR condition or the high dietary carbohydrate (Table V).

Morphologic Measurements in Brain. In all groups, the anatomical indicators of brain development were strikingly similar with one notable exception. The sizes of the cytochrome oxidase-enriched vibrissal barrel fields were notably reduced in the AR-h and AR-c groups when compared with the MR group (Fig. 2). These neocortical barrel fields consisted of patches of histochemical labeling composed of cell bodies and finer elements in the neuropil as shown in previous reports (29, 30). Although these aspects of the cellular localization of the enzyme were maintained, the vertical and horizontal arrangements of the barrel fields were also equivalent in all groups. Thus, the diminished cytochrome patches probably reflected sensory deprivation common to the artificial rearing procedure of the AR-h and AR-c groups even though the development of aerobic metabolism and its structural correlates proceeded normally in the neocortex as well as the other inspected brain sites.

The regional and histologic arrangements of the brain were equivalent in the different groups as shown in cresyl violet specimens (Fig. 3). The observations focused particularly on late-forming structures including olfactory bulb, neocortex, dentate gyrus of hippocampus, and cerebellar cortex. All of these sites have neurons undergoing proliferative and migratory events during the postnatal period in rats (35). Their organization and cellular composition are known to be vulnerable to a variety of malnourishment and undernourishment conditions. Yet, no defects were apparent in these or any other brain sites in the AR-c or AR-h groups. The normal cellular and regional development of the brain was maintained despite the depressions of circulating ketone content and metabolism.

The Golgi material provided a structural check of the formation of cellular processes and specializations in the brain. As shown for neocortical pyramidal neurons in Figure 4, the dendritic arbor arrangements and dendritic spine specializations of complex neuronal cell lines were comparable in the different groups. These

Table V. Biochemical Indices of Brain Development[a]

	MR group	AR-c group	AR-h group
Brain weight (g)	1.37 ± 0.05[b]	1.26 ± 0.05[c]	1.25 ± 0.05[c]
No. of rats	25	22	22
Lipids			
Total sterols (mg/g brain)	8.98 ± 0.34	9.25 ± 0.39	9.21 ± 0.19
Cholesterol	8.52 ± 0.37	8.91 ± 0.41	8.89 ± 0.20
Desmosterol	0.46 ± 0.05	0.34 ± 0.04[d]	0.32 ± 0.04[d]
No. of rats	13	9	10
Cerebroside sulfate (μmol/g brain)	1.19 ± 0.25	1.10 ± 0.11	1.10 ± 0.14
No. of rats	10	11	12
Enzymes ($nmol \cdot min^{-1} \cdot mg\ protein^{-1}$)			
3-Oxoacid-CoA transferase	760 ± 60	760 ± 80	690 ± 60
No. of rats	5	5	7
Acetoacetyl-CoA ligase	7.7 ± 2.9	6.9 ± 2.1	5.9 ± 1.1
No. of rats	5	7	5
Pyruvate dehydrogenase			
12 Days old	31.3 ± 1.3	34.7 ± 1.0	34.0 ± 2.0
No. of rats	5	3	3
16 Days old	48.3 ± 0.6	45.9 ± 3.4	43.9 ± 1.0
No. of rats	10	5	5

[a] The data are from animals whose body weights are shown in Table IV. The concentrations of lipids and activities of enzymes were determined in their brains at 17 days of age. The methods to assay enzymes and lipids are presented in Materials and Methods.
[b] Values are means with SD.
[c] Significantly different from MR, $P < 0.0001$.
[d] Significantly different from MR, $P < 0.05$.

dendritic indicators represent late-formed morphologic features that are mainly established during early postnatal development in rats (35). They can be modified by nutritional status and, to a lesser extent, experience. Their normal development was maintained in the AR-c and AR-h groups. Similarly, the structural features revealed by the Golgi impregnation of both astrocytes and oligodendrocytes were established in an apparently normal fashion in the AR groups.

The patterns of expression and localization of the tested molecular markers for neurons and glia also matched these results and supported similar conclusions. None of the tested markers (glial fibrillary acidic protein for astroglia, proteolipid protein for oligodendroglia, and neuron specific enolase, neurofilament, and synapsin for neurons) demonstrated any clear differences between the groups. Of particular interest, normal expression and localization of synapsin in neocortex strongly suggested that typical synaptic contacts were formed in the AR groups. The expression of the neurofilament marker in the internal capsule indicates that corticifugal and corticipetal fibers were established.

Discussion

General. The milk of the rat has evolved through countless generations to provide a unique nutrient supply as the sole source of nutrients to meet adequate growth and development. Characteristic properties of rat's milk are its high content of medium chain length fatty acids in milk fat at 30% of total fatty acids by weight and a very low concentration of carbohydrate at about 3 g% lactose, which amounts to only 8% of the caloric content of the milk. Fat is the major caloric component of rat milk and provides 65–70% of total calories (36). It is the products of fat metabolism that dominate metabolic pools in the milk-feeding period (36). The artificially reared rat pup is admirably suitable to examine the significance of the characteristic properties of rat's milk, since milk substitutes can be formulated in different ways to omit or include particular components under controlled conditions. In the present study, our aim was to apply morphologic measurements to assess the adequacy of our rat milk substitute, which is similar in its composition to rat's milk (20), and to create an isonutritional condition of diet for the suckling rat in order to determine if the medium chain length fatty acids in fat in rat's milk are a mandatory component, necessary for growth and brain development.

The results of the morphologic studies suggest that the milk substitutes and the system to feed rat pups by intragastric infusion of these milks are adequate to produce a rat pup of a body weight comparable to that of mother-reared controls. The functional integrity of the brain is largely intact under these conditions. Two differences are observed between AR rats and MR rats. The reduction in brain weights (Table IV) and the reduced size of the vibrissal barrel fields in both AR groups (Fig. 2) are consistent with lack of sensory stimulation during postnatal brain development. A decrease in total brain weight in AR rats (Table IV) is also found in rats reared on a modified version of the

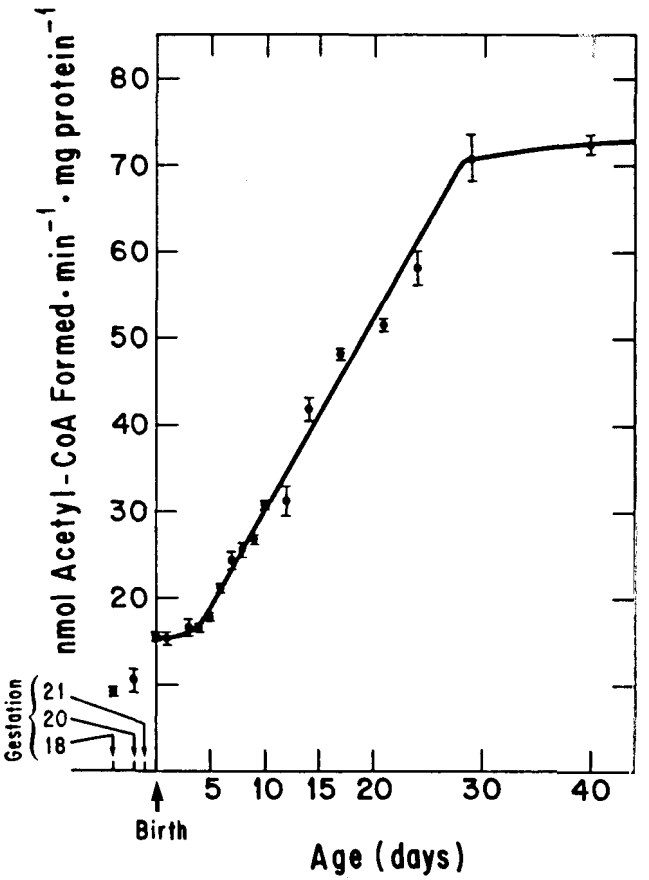

Figure 1. Brain from fetus, rat pups, and adult rats were prepared for the assay of pyruvate dehydrogenase as described in Materials and Methods. Each data point represents the mean of values for the assay of three to seven brains. Before 5 days of age, three to six brains were combined for assay. The assay, procurement of animals, and brain are described in Materials and Methods. The values are means with SD.

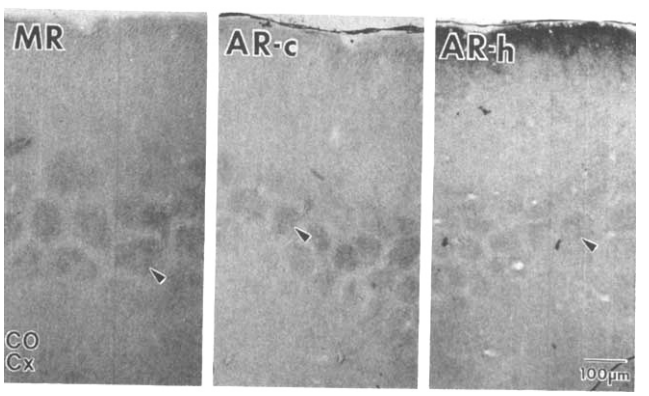

Figure 2. Photomicrographs comparing vibrissal barrel fields (arrows) defined by cytochrome oxidase histochemistry (CO) in the principal somatosensory cortex (Cx) of MR, AR-c, and AR-h rats of 16 days of age. Note the diminished areas of the stained patches in both samples of the artificially reared groups.

rat milk substitute (37) devised by Messer *et al.* (38). However, Smart *et al.* (39) observed no difference in the brain weights of 21-day-old rats reared on our RMS (20) supplemented with expressed rat's milk, suggesting

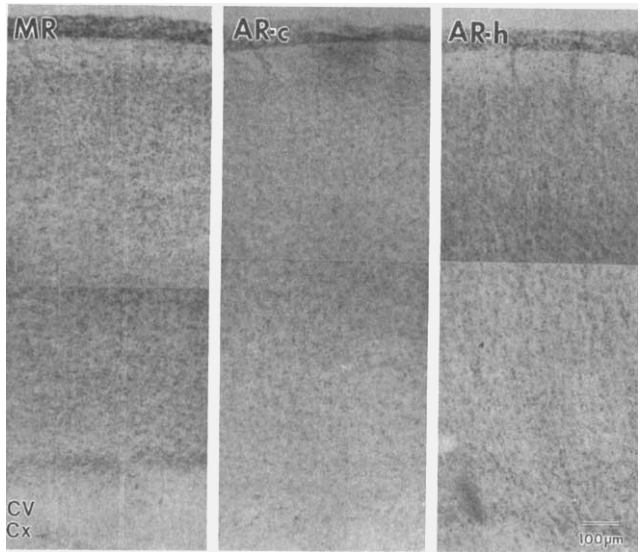

Figure 3. Photomicrographs comparing the laminar organization demonstrated by cresyl violet staining (CV) in the principal somatosensory cortex (Cx) of MR, AR-c, and AR-h rats of 16 days of age. The staining patterns were essentially identical in all of the groups.

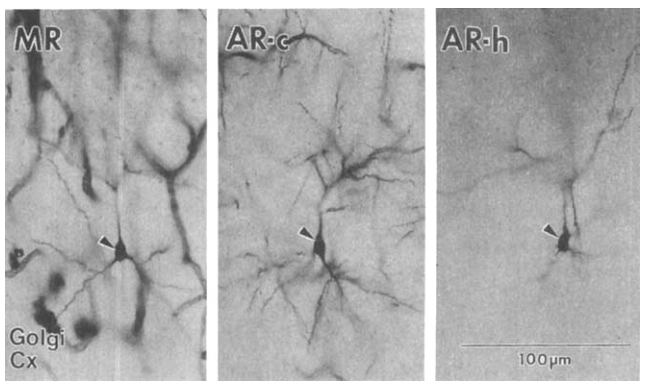

Figure 4. Photomicrographs comparing the somatodendritic features of neurons shown by silver chromate impregnation (Golgi) in the principal somatosensory cortex (Cx) of MR, AR-c, and AR-h rats of 16 days of age. These pyramidal cells were similar in all of the groups.

that rat's milk may contain a factor that is essential for promoting optimal rates of brain growth. Consequently one cannot exclude the possibility the RMS is deficient in a component essential for normal brain development. The lower values for desmosterol, proposed to be an inhibitor of hydroxymethylglutaryl-CoA reductase in developing brain (18), in both AR groups compared with the MR controls (Table IV) implies an altered cholesterogenic activity in brains of AR rats.

Isonutrition. Unlike malnutrition or undernutrition, a condition of isonutrition is introduced when all known essential nutrients are adequate in amount but a major exchange in components that could supply energy is introduced without altering the caloric content of the milk. The isocaloric replacement of fat containing medium chain length fatty acids by carbohydrate is a condition of isonutrition that can be tested in the

artificially reared rat. An immediate consequence of the dietary impact of this isonutritional condition is a pronounced hypoketonemia.

The concentration of ketone bodies in the plasma of neonatal rat pups is normally 1.5–2.0 m*M*, about 10-fold higher than in the well-fed adult (10, 11). The modification of the quantity and source of carbohydrate and fat in the rat milk substitute fed to the AR-h groups from 4 to 17 days of age resulted in substantially lower concentrations of plasma ketone bodies, >0.08 to 0.26 ± 0.09 (SE) m*M*. This hypoketonemia is probably due to both the absence of the dietary medium chain length fatty acids and increased concentration of dietary carbohydrate, which provides an ample supply of malonyl-CoA and inhibition of long chain fatty acid oxidation and ketogenesis (40). Previous studies have shown that when diets containing medium chain triglyceride, as a major portion of the dietary fat, were fed to suckling (41) and adult (42, 43) rats, the concentration of ketone bodies in plasma increased. Both rat milk and the control rat milk substitute contain approximately 30% by weight of the fat as medium chain length fatty acids, while the triglyceride of milk-fed AR-h animals contained negligible quantities of medium chain length fatty acids (Table II). The 3-fold increase in dietary glucose (Table I) and the elevated activity of fatty acid synthase in the liver of the AR-h rats (data not shown) are consistent with increased fatty acid synthesis, decreased fatty acid oxidation, and decreased production of ketone bodies.

Ketone bodies have been described as major carbon sources for energy production and lipogenesis in the rat brain during the suckling period (44). They are oxidized preferentially over glucose (17) and may supply potentially as much as 76% of the energy requirements of the brain of the rat (11) during the maximum growth period. Neurons and oligodendroglia derived from neonatal rat brain and grown in primary culture exhibit a marked preference for ketone bodies as oxidative substrates (45). The lipogenic role of these ketone bodies is well documented (44). The rate of 3β-hydroxy sterol formation and fatty acid synthesis in brain is enhanced over the course of the suckling period (14, 17, 19), with ketone bodies supplying a large proportion of the carbon for newly synthesized sterols and fatty acids by 18 days (14, 19). Based on these considerations, it is reasonable to predict that ketone bodies play a major role in supplying carbon for energy-requiring events and carbon for lipogenic events during the brain growth spurt in the rat. Neuronal events associated with brain growth include neuronal migration (46), dendritic growth (47), axonal collateralization (48), synapse formation (49, 50), and laminar differentiation (46, 51). Glial events include glial cell proliferation (52) and myelination (53–55). However, the biochemical (Tables IV and V) and morphologic data (Figs. 2–4) obtained in the present study indicate that within the limits of sensitivity of the parameters examined, plasma ketone bodies are not mandatory energy or lipogenic substrates for normal brain development in the rat.

We can conclude also, bearing in mind the limits of the sensitivity of our analysis, that the ketogenic medium chain length fatty acids of milk fat appear not to be essential for growth and development. Substrates other than ketone bodies must come into play to compensate for their reduced availability to organs such as the brain. The adapted metabolic environment neither decreases nor increases the activity levels of the two enzymes responsible for acetoacetate activation in brain, 3-oxo-acid CoA transferase, and acetoacetyl-CoA ligase (in mitochondria and cytoplasm, respectively). Furthermore, there is no evidence to suggest an enhanced utilization of glucose. Glucose is supplied in 3-fold excess in the diet as polycose, which is readily hydrolyzed to free glucose in the intestine (56). The key enzyme responsible for converting glucose-derived pyruvate to acetyl-CoA, pyruvate dehydrogenase, remained unchanged under these metabolic conditions. It has been demonstrated that enzymes of carbohydrate metabolism such as sucrase develop precociously in the intestine when the carbohydrate components of the diet are abnormal (57). Our findings illustrate that the three enzymes measured are controlled stringently in their expression by factors unrelated to the substrate supply from the diet and the resultant change in the metabolic environment. No available evidence supports the suggestion that an increased flux of carbon from glucose compensates for the decreased supply of carbon from a highly reduced ketone body pool. In fact, no change in the activity status of requisite enzymes was discovered. The present studies indicate that medium chain length fatty acids in milk fat and circulating ketone bodies, both normally available to the suckling rat in elevated amounts in development, are not mandatory substrates for growth and for the normal development of the brain. Finally, it is proposed that the differences we observed between artificially reared rat pups and their mother-reared controls, decreased brain weight, decreased desmosterol content of brain, and decreased vibrissal barrel fields of the somatosensory cortex, are a consequence of the artificial rearing condition. Further studies are necessary to determine if the effects are due to some aspects of the diet or the conditions of rearing unrelated to diet.

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