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ARTIFICIAL REARING OF RAT PUPS: Implications for Nutrition Research

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INTRODUCTION

The process of early metabolic adaptation in mammals is characterized by two critical periods of transition. The prenatal-postnatal transition occurs at birth

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and involves an abrupt change from a predominantly carbohydrate energy source from maternal circulation via the placenta to a high-fat, low-carbohydrate milk diet. The suckling-weaning transition is more gradual and occurs when the suckling animal begins to consume solid food, which is customarily high in carbohydrate and low in fat (e.g. a laboratory stock diet). In the rat, this latter transition begins after day 14 postnatal and continues until about day 28, when milk intake ends (25).

Each of the transitions described above is associated with enzymatic adaptations in key tissues. These adaptations enable the animal to metabolize the new diet presented at each transition. Clusters of enzymes that appear at the late fetal, neonatal, and late suckling stages of development have been described for the liver, brain, and kidney (reviewed in 18). The rapid changes in enzyme activity observed during these transitions suggest that the tissues are very active metabolically during this period and that a wealth of new information could be obtained by manipulating the diet during the interval between transitions. Such manipulation has been attempted using the technique of premature weaning of rat pups on postnatal day 15 onto a high-carbohydrate (HC) or a high-fat (HF) diet (21). In addition, premature weaning to a HC diet results in the reappearance of enzymes involved in lipogenesis and a decrease in those involved in gluconeogenesis. However, early weaning may have some adverse effects, including defects of CNS function (39).

A better approach to studying nutrient requirements of the neonate is to alter the composition or amount of milk fed. Nutritional studies during this early postnatal period are difficult to conduct because the experimental animal is dependent on maternal breast milk for nourishment at this time. Nevertheless, neonatal nutrient intake and composition can be altered by modifying litter size or the composition of the diet fed to the mother, or by rearing the suckling animals on an artificial milk-substitute diet. The latter technique, referred to as artificial rearing, has been used successfully to study various aspects of metabolism in the neonate during this critical period of early development.

Early research using the artificial rearing technique was carried out in small animals raised under germ-free conditions, including the rat, rabbit, hamster, mouse, chicken, turkey, and guinea pig (46). The milk formula diet was initially force-fed to rats, mice, and rabbits (45). Larger animals such as the monkey (47) and pig (35) are less troublesome to hand-rear than the smaller species, and the gastrointestinal physiology and body composition of the pig is similar to that of the human infant (51). However, despite the advantages of rearing larger animals artificially, the species most studied using the artificial rearing technique is the rat. In addition to the obvious cost advantages of feeding and housing a smaller animal, the rat has emerged as the species of choice for these studies because of its well-defined nutritional requirements and long history of use in metabolic studies. The relatively large number of rats in a litter also

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enables control and experimental animals to be chosen from the same litter. Finally, the small size of the rat makes its rearing apparatus easy to design and assemble.

This review describes the development of the artificial rearing technique, the preparation and composition of formulations fed using this technique, and examples of applications of this technique in nutrition research. Three broad areas of applications are discussed. The technique of artificial rearing has been used to study nutritional and hormonal influences on intestinal cell growth and expression of intestinal enzymes whose activities undergo rapid changes soon after birth. Numerous studies have also employed the technique to investigate the influence of nutrient status on brain development in neonatal rats. Using artificially reared (AR) rats, these studies have demonstrated the effects of specific dietary components and toxic substances such as alcohol on brain development. The artificial rearing technique has also been applied in the study of both the immediate (short-term) and long-term metabolic consequences of an early nutritional experience such as feeding a HC formula during the suckling period. A review article (40) and symposium (41) highlight some recent applications of artificial rearing. This review, although not comprehensive, gives an overview of the technique and presents several examples of its potential as a research tool in pediatric nutrition.

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ARTIFICIAL REARING TECHNIQUES

Hand Feeding

Over the past 50 years, several different approaches have been employed to rear rat pups artificially, with varying success (summarized in Table 1). The earliest attempts to rear rat pups in this manner were oriented toward the development of germ-free rats. The method of choice at the time was to hand-rear the rat pups (19). The milk formula was forced into the stomach gradually over a period of several minutes through a thin rubber tube inserted halfway down the esophagus. Refinements of the technique resulted in the use of a tapered nipple instead of rubber tubing for force feeding (45).

Surgical and Nonsurgical Implantation of Feeding Tubes

The hand-rearing technique is a laborious process and is difficult to use routinely in nutritional studies. Several attempts have been made in the last four decades to improve the rearing technique and to develop a formula with a composition similar to rat milk. In 1969, Messer et al described a semiautomatic technique for feeding newborn rats (33). First, a gastric cannula was surgically implanted in a newborn rat. The cannulated pup was then reared in an incubator containing a stainless steel basket, or nest, divided into several

Table 1 Artificial rearing techniques used in rat studies

Technique	Comments	Reference
Hand rearing with catheter	Few colonies of germ-free rats were raised. Induces stress on the animals. Esophagus subject to injury because of repeated insertion and removal of the rubber feeding tube.	Gustafsson, 1946 (19, 2
Gastric infusion technique—1	Semi-automated, surgical implantation of the cannula, mother surrogate used in the rearing system	Messer et al, 1969 (33
Gastric infusion technique—2	Semi-automated, nonsurgical implantation of the cannula, pups reared in styrofoam cups floating in a temperature-controlled water bath.	Hall, 1975 (22)
Intragastric tube feeding	Less invasive procedure, difficult to use after day 10.	Moore et al, 1986 (38)
Automatic feeder	Less stressful, fewer manipulations, and non-invasive technique.	Hoshiba, 1986 (28)
The palate cannula	Cannula implanted in the roof of the mouth through the hard palate, less stressful and can be used only from day 13.	Blake et al, 1988 (5)

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compartments. In each compartment was a section of warm, moist dialysis tubing that acted as a mother surrogate. The cannulas were connected to an infusion pump by polyethylene tubing, and the pups were fed continuously by infusion. The volume of milk was adjusted daily so that the pups would attain normal growth. Messer et al concluded that this method of feeding was more advantageous than the hand feeding method because it avoided the trauma that accompanied repeated oral passage of gastric feeding tubes. Furthermore, because this technique was semiautomatic, it eliminated the need for attendance during the night. However, the complexity of surgical procedures involved in implanting the cannula and of several other aspects of the rearing system have made the use of this technique less attractive.

In 1975, Hall described a unique and simple technique to implant the gastric cannula through the mouth and to rear rat pups in floating cups housed in a temperature-controlled waterbath (22). This technique (informally referred to as "pup in a cup") has been used in several studies. It has many advantages over the previously published methods and is described in detail in the following section.

Before the cannula is implanted, the pup is lightly anesthetized with ether. A length of sialistic tubing (~ 5 cm) lubricated with corn oil is then gently pushed through the mouth, into the esophagus, and into the stomach. Next, a length of piano wire (~ 8 cm) that is slightly curved at one end is passed

through the guide tube. Once it reaches the stomach, the wire is pushed through the stomach, peritoneal wall, and the skin until ~ 1 cm emerges. The wire is securely held with a forceps or by hand while the sialistic tube is withdrawn from the mouth. The end of the wire emerging from the mouth is friction fitted with the unflanged end of the cannula. The wire and cannula (which is lubricated outside with corn oil to prevent any damage to the esophagus) are then pulled down the esophagus and out of the abdomen. The cannula is pulled through until the disc is flush against the stomach wall. To secure the cannula, it is first threaded through a polyethylene sandwich bag disc and then through a polyethylene washer made of PE-50 tubing. Finally, the cannula is passed through a fold of skin at the back of neck to alleviate stress on the cannula.

The pups are housed individually in styrofoam cups, which are placed in a warm-water bath to maintain the inside environment in the cup at ~ 37° C. The cannula emerging from the hole in the plastic lid of the cup is friction fitted with a connecting polyethylene tube. This tube is in turn attached to a syringe containing milk formula, which is mounted on an infusion pump. Daily maintenance procedures include replacing the syringes with fresh milk formula, flushing the tubes, and renewing the bedding. The pups are stimulated to urinate and defecate twice daily by gentle stroking in the anal-genital region and are then wiped clean. The pups have been reared artificially using this protocol starting from day 1 and as late as day 12. The pups can be maintained on this regimen until day 21.

The main advantage of this technique over that employed by Messer et al (33) is that the implantation of the cannula is a nonsurgical procedure and requires only ~ 3 min to complete, whereas the surgical method takes ~ 12 min. The rearing system is simple to use and is less stressful to the pup. The milk is also fed intermittently rather than continuously.

Blake et al (5) reported a modification of the cannulation procedure that consists of implanting a cannula in the roof of the mouth through the hard palate. With this method, normal growth was maintained from postnatal day 13 to day 17. However, whether this procedure can be adapted to younger pups remains to be determined.

Moore et al (38) reported a less invasive method of artificially rearing rat pups less than 10 days old. In this approach, an orogastric feeding tube was prepared by inserting a 5.7 cm length of silicone rubber tubing [inner diameter (I.D.) 0.305 mm and outer diameter (O.D.) 0.635 mm] into a 35 cm length of silicone rubber tubing (I.D. 0.635 and O.D. 1.3 mm). The small tube portion of the cannula was gently inserted into the pup's mouth and then into the esophagus. Next, the cannula was attached to the pup's lip and to the top of the head and body with cyanoacrylate glue. It was then connected to a syringe filled with formula, which is then mounted on an infusion pump. The pups were reared in styrofoam cups as described by Hall. However, when the pups

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became more active around day 10, the cannulas tended to dislodge more frequently. Because of this difficulty, the method has very limited use in studies that continue beyond day 10.

Automatic Feeder Procedure

Hoshiba developed an elegant technique that uses an automatic feeder to feed infant rats (28) and that may overcome the limitations described in Hall's procedure. For this procedure, an automatic feeder for rat pups was developed that mimics the natural environment of the mother rat. The rectangular apparatus contains nursing tubes that pass through the front and back walls of the apparatus. Nursing bottles were inserted into the tubes from the back until the nipples projected 5 mm from the small holes in the rubber stoppers used to close the tubes in the apparatus. Around the nipples (made with silicone) in the front side, the box was covered with fur composed of nylon to mimic rat skin. A thermostat maintained the pups' environment at 35° C and the environment around the nipples at 38° C. Three day–old rats were trained a few times to find the nipples and to suckle milk dispensed from a reservoir to the nursing bottles by a peristaltic pump.

Pups reared artificially with this method had a normal growth rate compared with control rats, but no further studies using this approach have been reported. If the efficacy and adaptability of this technique can be documented, it may become the method of choice in future investigations since it requires fewer manipulations and is less stressful to the rat pups at the early stage of development.

PREPARATION OF MILK-SUBSTITUTE FORMULAS

As mentioned above, the artificial rearing system for rats was first used to raise germ-free animals. For this purpose, the ideal diet would be sterilized rat milk. Numerous attempts have been made to collect and sterilize rat milk, but without much success (reviewed in 48). If a milk-substitute formula homologous in composition to rat milk could be prepared, the difficulties and time involved in obtaining rat milk would be eliminated. Several combinations of ingredients, summarized in Table 2, were used in the preparation of milk-substitute formulas.

Milk-based Formulas

Commercially available milk products differ substantially in composition from rat milk. The major obstacle in preparing a milk-substitute formula for rats is the formula's high concentration of protein (~ 10 g/100 ml) and low concentration of carbohydrates (~ 3 g/100 ml). Most early artificial rat milk formulas were prepared with cow milk as a base. Gustafsson (20) used Aminosol, an

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Table 2 Composition of rat milk substitute formulas

Reference	Protein g/100 ml	Carbohydrate (% of calories)	Lipid	Calories/ 100 ml	Major ingredients
Rat milk (11)	9.36 (24)	3.16 (8)	12.03 (68)	159	
Gustafsson, 1946 (19)					
#204	10.1 (23)	3.8 (9)	13.5 (69)	177	Cream, aminosol
#105	5.5 (16)	5.1 (15)	10.5 (69)	137	Cream, aminosol, glucose
Pleasants, 1959 (45)					
#L449C	7.33 (21)	4.03 (11)	10.9 (68)	143	Whole milk, light cream, skim milk protein
Dymsza et al, 1964 (11)	5.33 (14)	9.29 (25)	10.2 (61)	150	Nonfat dry milk, corn oil, rat milk
Messer et al, 1969 (33)	5.5 (13)	8.9 (22)	12 (65)	166	Evaporated milk, corn oil
Austed et al, 1989 (3)	10.4 (25)	3.6 (9)	12.2 (66)	166	Evaporated milk, skim milk powder, whey protein, maize oil, soy oil, MCT oil
Moore et al, 1986 (38)	9.1 (23)	2.8 (7)	12.03 (70)	156	Modular feeding formula
Hiremagalur et al, 1992 (26)	9.36 (24)	3.16 (8)	11.9 (68)	157	Modular feeding formula, maize oil

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enzymatic digest of a casein preparation, as the principal source of protein in his experiments to raise germ-free rats. A number of formulas were prepared using combinations of Aminosol and cream (15% fat), whole milk powder, and precipitated casein to obtain protein concentrations of 5.5–11.1 g/100 ml. However, the growth and survival rates of the rat pups were not optimal compared with mother-fed (MF) rats (20). Bloating was common, and post-mortem examination revealed that the intestines were distended by undigested food. In addition, several nutrient deficiency symptoms were observed in the animals at various stages (20).

Pleasants developed a formula based on cow milk and light cream; to supplement protein, he added dried skim milk powder (45). This formula, together with a forced-feeding technique, made it possible to overcome some of the deficiencies observed previously in raising germ-free rats. However, the rats still differed considerably from MF controls in their growth pattern. Rising interest in newborn nutrition led some investigators to use the artificial rearing system of the rat to study the effect of various nutrients on growth and development during the suckling period. However, even with improvements in the artificial rearing technique, optimal growth was not achieved (37).

Dymsza et al supplemented a cow milk–based formula with rat milk in various proportions (11). Although the 5% rat milk–supplemented formula–fed group had body weight gains similar to those of the MF group, carcass analysis revealed that the artificially fed group had 35% more body fat than did MF controls. When

Miller & Czajka tried to improve these formulas in later studies by changing the protein sources (i.e. by substituting amino acid mixtures and protein hydrolysates for rat milk) they found that the stomachs of these animals failed to empty properly and developed a bloated appearance (36). By altering the osmolarity of the milk formula with glucose, Miller & Czajka showed that any increase in osmolarity above 624 mosmoles per liter resulted in decreased survival and reduced weight gain (36). Messer et al described a semiautomated gastric infusion technique that used a milk formula based on evaporated milk and corn oil (33). The composition of this formula differed from rat milk in its low protein and HC content. The growth rate achieved was improved compared with that of previous formulas, but some of the organs (cecum, liver, and kidneys) exhibited enlargements compared with MF controls. Further investigations showed that rats fed Messer's formula differed from control rats in carbohydrate and ketone body metabolism (54) and in abnormal organ growth (52). Smart et al used a modified formula prepared by dialyzing and concentrating evaporated cow milk and skimmed milk powder and then adding maize oil, medium-chain triglycerides, vitamins, minerals, and amino acids (53). Although this formula closely resembles rat milk in its macro- and micronutrient composition, the rats fed this formula had abnormal organ development (53).

Most of the above investigations using the artificial rearing technique resulted in abnormal growth of the stomach, intestine, and cecum and in deficiencies in other organs, such as the brain. Some investigators postulated that the abnormal organ growth was primarily the result of the artificial rearing system rather than of a deficiency in the milk composition. However, it was later shown that when rats were reared artificially on rat milk only, no such abnormalities occurred (56). These results implicate dietary factors rather than the artificial rearing technique per se in the excessive growth of the intestine and stomach. More recently, Auestad et al developed a rat milk–substitute formula from cow milk products (2). In this formula preparation, a premilk base was initially prepared from skim milk powder and evaporated milk by dialysis and concentration steps. Appropriate amounts of fat, carbohydrate, minerals, vitamins, and amino acids were mixed with the premilk base to obtain a composition similar to that of rat milk. The growth and development of various organs in rats fed this formula were satisfactory compared with the results obtained with previous milk substitute formulas with the exception of intestine, where abnormal growth still occurred (2). Amino acid profiles, ketone body metabolism, and carbohydrate metabolism were comparable to those of age-matched MF rats in this study (2).

Non-milk-based Formulas

Modular tube feeding formulas, in which the components are available separately and are mixed before use, have also been used as rat milk substitutes.

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These formulas allow more flexibility in manipulation of the macro- and micronutrient composition of the diet. Kris-Etherton et al used an infant formula base to prepare a cholesterol-free formula (31) that was fed to pups by intubation. However, although the formula was comparable to rat milk in macronutrient composition, the animals consuming this formula did not grow as well as MF rats. This result may be attributable to the feeding regimen, which was rather complicated and stressful to the rat. Moore et al used another modular tube feeding formula to study the effect of epidermal growth factor on the development of suckling rats fed by intragastric tubing (38). Due to the stress involved in intragastric feeding or to deficiencies in certain types of macronutrients such as long-chain fatty acids, the rats did not grow well compared with MF animals. We used the same modular tube feeding formula with some modifications, such as inclusion of long-chain fatty acids, but we relied on the semiautomated gastrostomy technique rather than on intragastric tubing to deliver the diet into the stomach (26, 27). The rats fed by gastrostomy had a normal growth rate, and no differences were observed between AR rats and MF controls in terms of body weight and lipid metabolism (27).

GROWTH AND METABOLIC ADAPTATIONS DURING THE PREWEANING PERIOD

Carbohydrate-Metabolizing Enzymes in the Intestine

At birth, the rat small intestine is well suited for the digestion and absorption of maternal milk (8, 32). Lactose is the principal source of carbohydrate, accounting for ~ 8% of total calories in rat milk. Lactase activity is very high in the intestinal epithelium at birth but decreases during the third week of the weaning process (66). The characteristics of intestinal maturation include an increase in maltase activity and the emergence of sucrase activity (49). The epithelial cell population simultaneously increases, resulting in an expansion of the lumen surface (64). These maturation changes occur in the rat during the period when the source of nutrients changes from a HF maternal milk to a HC weaning diet (laboratory chow). These changes may be influenced either directly by the composition of diets or by the influence of pituitary and growth hormones. Pituitary-adrenal and pituitary-thyroid hormonal systems can regulate intestinal growth and enzymatic maturation (7, 66, 67). The relative effect of dietary nutrients vs hormonal changes on these processes has been difficult to assess. The artificial rearing technique has enabled investigators to address these questions by independently varying hormone levels and the type of carbohydrate in a milk formula.

Yeh extensively studied the effect of milk-substitute formulas and caloric intake on development of the small intestine (62, 63). When AR rats were fed

Messer's formula (33), abnormal growth was observed in their intestine (~ 40% heavier than MF rats), even though the body weights were similar to those of MF control pups (63). Intestinal crypt depth and duodenal villus length increased in AR rats compared with MF rats (62). The small intestine of AR rats exhibited premature enzymatic differentiation. Initially, these changes were thought to result from differences between the macronutrient composition of Messer's formula and rat milk. However, when a modified formula that closely resembled the macronutrient composition of rat milk was fed to AR rats, similar differences in intestinal growth were observed (63). These results indicated that the artificial rearing technique itself or some other unidentified factor may influence the growth of the gastrointestinal tract. Tonkiss et al studied these aspects in some detail in groups of rats fed milk-substitute formulas as well as rat milk (55). Stomach and cecum weights and the length of small intestine were greater in AR rats fed milk-substitute formula than in MF control rats. However, these differences in gastrointestinal tract growth were not observed in AR rats fed rat milk, which indicates that the AR technique per se was not responsible for these growth abnormalities (55).

In the normal course of postnatal development, serum corticosterone concentrations are maintained at low levels until day 12, increase significantly by day 14, and continue to rise before peaking on day 24 (24). These changes in serum corticosterone were associated with the onset of intestinal sucrase activity (24). The implantation of an intragastric cannula on day 12 induced stress, resulting in a transient surge of serum corticosterone concentration that returned to normal after 48 h (65). This sudden increase in corticosterone levels led to a precocious induction of intestinal sucrase activity on day 16 (65).

In a recent study, Yeh & Yeh showed that hypophysectomy has no effect on intestinal growth in AR rats (68). However, in MF-hypophysectomized rats, a retardation in body and intestinal weight gains occurred compared with intact MF rats. These results suggest that the poor growth of MF-hypophysectomized rats may be due to a reduction in dietary intake (less suckling activity) or to the presence of some undefined trophic factors in the milk-substitute formula that promote intestinal growth in AR-hypophysectomized rats (68; Figure 1). Although no difference in body weight between AR rats and AR-hypophysectomized rats was observed, skeletal growth was retarded in AR-hypophysectomized rats, as indicated by a decrease in tail growth (68). This finding rules out the possible role of growth hormone and thyroid hormones on intestinal growth. Both MF- and AR-hypophysectomized rats had immature enzymatic profiles (higher lactase activity and lower maltase and sucrase activities; see Figure 1), which indicates that intestinal growth and differentiation are separate events and that trophic factors, if any, in the milk-substitute formula do not promote enzymic differentiation (68). Thus artificial rearing of rat pups has

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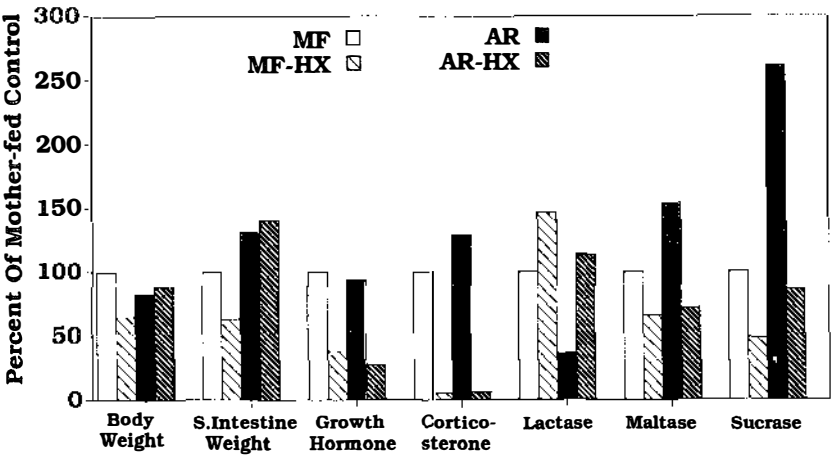

Figure 1 Effect of hormone and nutrition interactions on gastrointestinal development. Rat pups at 12 days of age were either normal or hypophysectomized (Hx) and were either MF or AR with a milk-substitute formula. The pups were killed on day 18 and examined for body weight, small intestine weight, jejunal lactase, maltase, and sucrase activities and for serum concentrations of growth hormone and corticosterone. Redrawn from the data of Yeh & Yeh (68).

allowed examination of the effect of dietary factors and endogenous hormones on intestinal development and maturation.

Brain Development

Fetal alcohol syndrome is a major consequence of socio-economic development in modern society. The most prominent clinical feature of fetal alcohol syndrome is dysfunction of the CNS, which is reflected by mental and psychomotor disabilities. Most of the initial studies were based on the feeding of ethanol to rats during pregnancy and lactation and the study of brain development in the offspring. These studies indicated that brain growth and behavioral development were impaired in rats exposed to alcohol during lactation. However, because these protocols have several limitations, it was not possible to ascertain whether the observed effects were due to alcohol exposure, to undernutrition during the neonatal period, or to a combination of both.

The artificial rearing technique has been used extensively in the last decade to study the effect of alcohol on brain development. For example, Diaz & Samson used it to examine the effect of ethanol consumption (from day 4–7) on brain development during the brain growth spurt in the rat (10). On day 18, the alcohol-fed group had significantly lower brain weights, although their body weights were similar to those of controls. The rats exhibited symptoms similar to those observed in fetal alcohol syndrome, including body tremors

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for 3–5 days after alcohol administration had been stopped and poor motor coordination (10). Sex differences also reportedly influenced the effect of alcohol feeding on brain growth retardation (30, 43, 60). Greater deficits in brain weight and higher blood alcohol concentrations (BACs) were observed in female rats than in male rats, although the alcohol dose was the same for both groups (43, 60). Similarly, impaired spatial navigation was observed in adult female rats but not in male rats exposed to alcohol during postnatal days 4–10 (30). BAC has proven a better predictor of the effect of alcohol feeding on brain development than has the amount of alcohol fed (6, 43, 44). The BAC threshold to produce microencephaly was 140–197 mg/dl (43). When the BAC reached 280 mg/dl, deficits in brain weight increased considerably, and BACs above 425 mg/dl were lethal to the animals (43). Another study showed that a dose of 4.5 g/kg of alcohol per day was sufficient to significantly decrease brain weight and cerebellar weight; however, a dose of 6.6 g/kg per day was required to substantially reduce brain stem weight (6).

Later studies revealed that even a single-day exposure to a dose of alcohol that resulted in a high BAC is sufficient to retard brain growth (15, 16). When rat pups were fed 7.5 g/kg on postnatal day 4, 5, or 6, brain weight was significantly reduced on postnatal day 10 (15). The cerebellum was more affected than the total brain, forebrain, or brain stem (15). With binge-like exposure to alcohol on postnatal day 4, cerebellar Purkinje cells were reduced significantly on postnatal day 10 compared with controls (16). When rat pups were fed various amounts of alcohol in different feeding patterns, pups fed a given amount of alcohol over a short time period had higher BACs and more severe deficits in brain weight and Purkinje cell loss than did those fed similar amounts over a longer time interval (59; Figure 2).

Retardation in brain growth due to alcohol exposure was also reflected in the decreased functional ability of rats to perform tasks at various stages of development. AR rats fed an ethanol-milk formula (2.15% wt/vol or 2.5% wt/vol) during the immediate postnatal period (days 4–12) performed poorly when traversing two parallel horizontal rods and in tests of hind limb and head elevation compared with controls (34). In another study, AR rats fed alcohol (4.5 g/kg per day) during the immediate postnatal period (days 4–9) performed poorly when tested on a rotarod at 405 days of age compared with controls (17). These studies demonstrate that alcohol feeding during brain development has long-lasting effects as a result of permanent structural and functional changes in the brain.

Hepatic Lipogenesis

In the rat, three distinct periods of metabolic adaptations are observed between fetal and adult life. Hepatic lipogenic capacity is high in the late fetal stage, declines to low levels during the suckling period, and increases again when

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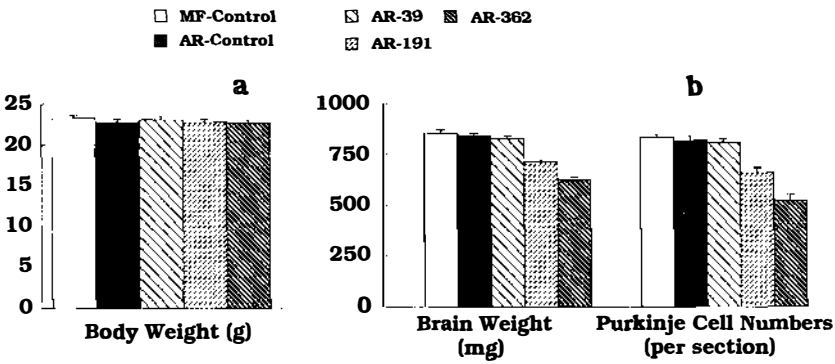

Figure 2 Effects on brain development of feeding alcohol to rat pups by gastrostomy during the preweaning period. Rat pups subjected to different patterns of alcohol exposure from postnatal days 4–9 were killed on day 10 and examined for body weight, brain weight, and Purkinje cell numbers in the cerebellum. MF-Control, MF rat pups without any alcohol exposure; AR-Control, AR by gastrostomy without any alcohol exposure; AR-39, AR pups fed 6.6 g/kg per day (in 12 equal doses) of alcohol, with a peak BAC of 39 mg/dl; AR-191, AR pups fed 4.5 g/kg per day (in 4 equal doses) of alcohol, with a peak BAC of 191 mg/dl; AR-362, AR pups fed 4.5 g/kg per day (in 2 equal doses) of alcohol, with a peak BAC of 362 mg/dl. Redrawn from the data of West (59).

the pups are weaned onto a laboratory stock diet (4). Similarly, plasma insulin levels are very high in the late fetal stage but decrease rapidly and remain low until the weaning process begins. The appearance of specific enzymes in the neonatal period is closely associated with changes in the availability of nutrients (14). For example, within the first few hours after birth (before glucose is available), phosphoenolpyruvate carboxykinase activity appears in the rat, coinciding with the development of gluconeogenic capacity (14, 41). Hepatic glucokinase also appears during the late suckling period, when the pups begin to consume the laboratory stock diet (HC diet). The developmental profiles of these enzymes are influenced by nutritional changes (18).

Haney et al reported the effect of a HC diet (starting on postnatal day 1) during the preweaning period on developmental profiles of hepatic glucokinase and malic enzyme (23). AR pups on a HC formula (56% of total calories from carbohydrate) became hyperinsulinimic and maintained higher levels of plasma insulin throughout the preweaning period than did MF controls (23; Figure 3a). Malic enzyme, which normally appears in the late suckling period, appeared by day 4 in the AR-HC pups and achieved 80% of the activity normally present in 31-day-old MF control rats (23; Figure 3d). Similarly, the activity of the lipogenic enzyme glucose-6-phosphate dehydrogenase, which normally decreases shortly after birth, did not decline in the AR-HC group and remained at higher levels throughout the suckling period than in MF control rats (23; Figure 3c). In another study, the hepatic synthesis of saponifiable lipids in-

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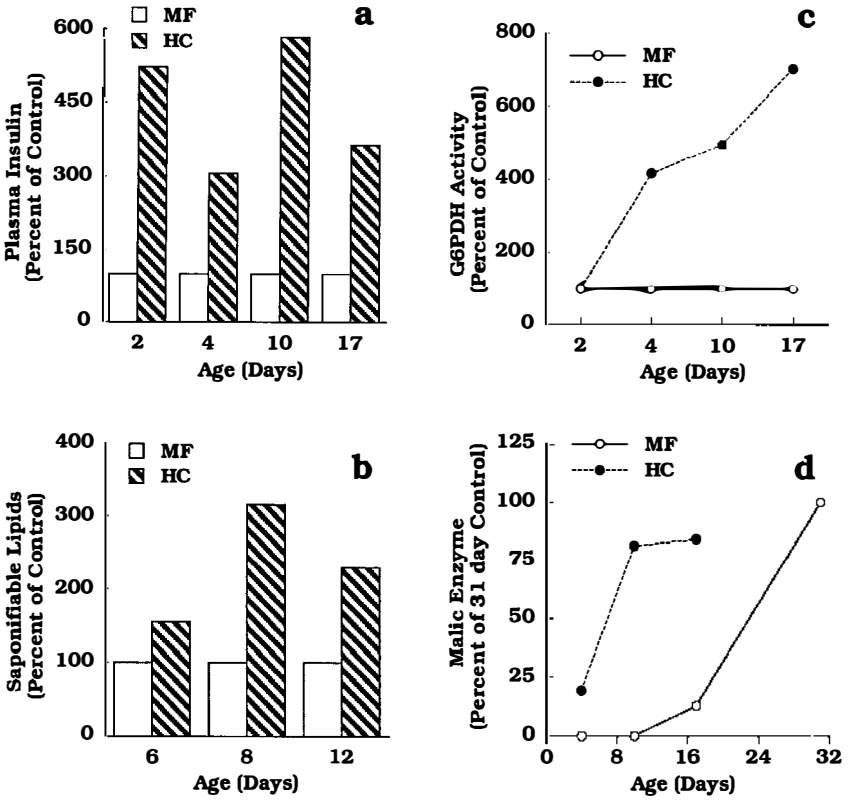

Figure 3 Effects of feeding a HC milk-substitute formula to rat pups by gastrostomy during the preweaning period from day 1 (a, c, d) or day 4 (b) to day 24 on (a) plasma insulin levels, (b) hepatic synthesis of saponifiable lipids, (c) hepatic glucose-6-phosphate dehydrogenase (G6PDH) activity, and (d) hepatic malic enzyme activity. Figures a, c, and d were redrawn from the data of Haney et al (23); figure b was redrawn from the data of Hiremagalur et al (26).

creased significantly in AR-HC rats compared with MF control rats (26; Figure 3b). These findings demonstrate that the feeding of a HC formula in the immediate postnatal period prevents the decline in hepatic lipogenic capacity seen in MR rats and precociously induces malic enzyme, which normally appears in the late suckling period. Another study examined the influence of the type of fat present in the diet on hepatic lipogenesis during the preweaning period (26). On days 4–12 of this study, rat pups were fed one of three formulas: (*a*) HC formula; (*b*) HF formula, which contained only medium-chain triglycerides (MCT) as the source of fat; or (*c*) HF formula containing both long- and medium-chain triglycerides (LCT + MCT) at levels similar to those found

in rat milk. Both HC and HF-MCT rats exhibited increased hepatic in vitro lipogenic capacity and higher lipogenic enzyme activities compared with age-matched MF control and HF(LCT + MCT) rats (26). Polyunsaturated fatty acids of dietary origin inhibit fatty acid synthesis (9, 50). Because no difference in plasma insulin levels was observed in the HF(LCT+MCT) and HF(MCT) groups, the increase in lipogenic capacity in the HF(MCT) group was attributed to the low levels of long-chain fatty acids in the diet (26).

Auestad et al studied the influence of dietary cholesterol on hepatic lipogenic potential in preweaning rats (2). Rat pups were fed a milk formula containing a low or normal concentration of cholesterol on postnatal days 5–17. The activities of cholesteroneogenic enzymes (3-hydroxy-3-methyl-glutaryl-CoA synthase and 3-hydroxy-3-methyl-glutaryl-CoA reductase) and of a hepatic lipogenic enzyme (fatty acid synthase) increased significantly in low cholesterol–fed AR rats compared with MF control and AR normal cholesterol–fed rats (2). Hepatic acetoacetyl-CoA ligase activity also appeared to be induced in both AR groups (low cholesterol and normal cholesterol), which indicates that factors other than cholesterol influence the activity of this enzyme (2). Plasma cholesterol levels decreased by ~ 20% in the low cholesterol–fed AR group compared with MF control rats. However, rats reared on a milk formula with normal cholesterol content exhibited increased plasma cholesterol levels (25–50%) compared with MF control rats (2).

LONG-TERM CONSEQUENCES OF EARLY NUTRITIONAL INTERVENTION

The influence of altered intake of nutrients in early childhood on the development of obesity and lipid metabolism in later life is not well understood. Most of the previous animal studies were accomplished by manipulating the litter size to produce either underfeeding with large litters or overfeeding with small litters (1, 12, 13, 29, 61). Subsequent to improvements in the artificial rearing technique, few attempts have been made to study the long-term consequences of manipulating nutrition in early postnatal life.

West et al used the artificial rearing technique to determine the long-term effects of overfeeding during the preweaning period on adiposity in later life (58). Rat pups were overfed by continuous gastric infusion from days 4–18, which resulted in an accelerated growth rate. These animals remained heavier as adults than did age-matched MF control rats. The overfed rats had significantly larger epididymal and retroperiotoneal fat depots, as well as an increased number and size of fat cells compared with MF control rats (58). However, AR rats that were not overfed also had significantly larger retroperitoneal fat depots, although their body weights were similar to those of MF control rats. This observation indicates that some unidentified nutritional factor(s) in the

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milk-substitute formula may have induced an increase in the number of fat cells in retroperitoneal fat depots (58). These findings suggest that the quantity of food consumed during early postnatal life and the quality of early nutrition affect adipose tissue development and that these effects persist into adult life.

Recently we found that changing the macronutrient composition of the milk-substitute formula without overfeeding also produces long-lasting effects on lipid metabolism in the rat (27, 57). Rat pups were reared artificially on either a HC formula (56% of calories from carbohydrate) or a HF formula that mimicked rat milk composition in macronutrients. The pups were then compared with MF control rats. Body weights of the three groups were the same when the pups in these groups were weaned onto a laboratory stock diet on day 24. The pups in all three groups were placed on a high sucrose diet on day 64 and were kept on this diet until they were killed on day 100. The HC formula–fed rats were hyperinsulinimic and became obese compared with MF control rats (27; Figure 4a,b). The lipogenic capacity of liver and epididymal

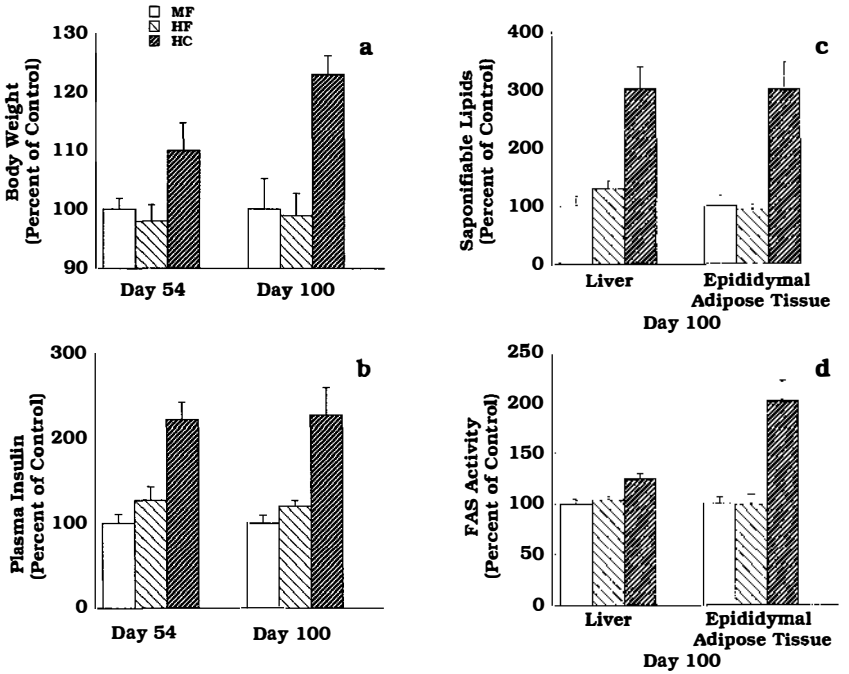

Figure 4 Long-term consequences of feeding a HC milk-substitute formula or a HF milk-substitute formula to rat pups during the preweaning period. Rat pups from both control (MF) and experimental groups (HC, HF) were weaned onto a laboratory chow diet on day 24 and subsequently fed a high sucrose diet from days 64–100 and examined for (a) body weight, (b) plasma insulin, (c) synthesis of saponifiable lipids in liver and epididymal adipose tissue, and (d) fatty acid synthase activity in liver and epididymal adipose tissue. Redrawn from the data of Hiremagalur et al (27).

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adipose tissue was higher in HC animals than in MF control rats (Figure 4c). Fatty acid synthase, glucose-6-phosphate dehydrogenase activities, and in vitro lipid synthesis increased significantly in HC rats compared with MF-control and HF rats on day 100 (27; Figure 4d). Epididymal adipose tissue weight also increased substantially in HC rats vs MF-control and HF rats, as did epididymal adipocyte cell size (27). Plasma and pancreatic insulin concentrations were also significantly elevated in HC rats (57). This hyperinsulinemia was associated with hypertrophy of β-cells in the pancreas in both 12-day- and 100-day-old HC rats. Insulin secretory response to an oral glucose load was impaired in HC rats compared with HF and MF rats (57). These findings indicate that a HC diet fed during the immediate postnatal period without overfeeding induces pancreatic changes that lead to chronic hyperinsulinemia and increased lipogenic capacity. These changes persist in later life, resulting in obesity.

CONCLUDING REMARKS

The artificial rearing technique has provided nutritionists with a powerful new research tool. Prior to the advent of this technique and to refinements over the past two decades, most nutritional investigations involving the early postnatal period were limited to undernutrition or overnutrition achieved by manipulating the litter size or maternal nutrition. Subsequent to the development of Hall's method (22), which greatly simplified the technique, the artificial rearing approach has been employed by many investigators in various fields of nutrition. The main advantage of the artificial rearing technique is that it allows precise control of the amount and composition of milk-substitute formula that each pup receives. As mentioned above, this experimental technique enables investigators to study the effect of overnourishment or undernutrition on the developmental pattern of various metabolic pathways as well as the effect of an individual micro- or macronutrient on metabolism and growth. The influence of drugs and toxic substances on various aspects of immediate postnatal life can also be examined, as exemplified by the effect of feeding alcohol on brain growth during the immediate postnatal period.

Although the artificial rearing technique has several advantages, some limitations do exist, which further refinements will nevertheless likely overcome. One such limitation of this technique is that it eliminates many of the maternal and sibling interactions experienced by naturally reared pups. Lack of these interactions may have profound effects on brain and behavioral development. Refinements in the preparation of milk-substitute formula allowing for easier preparation and variation of the nutrient composition are needed. Although investigators have had limited success preparing a milk-substitute formula that simulates natural rat milk in composition, the presence or absence of some unknown factor(s) in the milk-substitute formula may be responsible for ab-

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normal organ development, especially of the intestine and cecum. If the efficacy of the automatic feeder system developed by Hoshiba (28) is proved, it may eliminate some of the major limitations of the artificial rearing technique using gastrostomy. Further improvements in milk-substitute formula preparation and delivery may enable this procedure to be used in several previously unapproachable preweaning studies.

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