The stimulating effects of thyroid hormones on several enzymatic functions have been recognized (13). Whether they play a major role in the maturation of the enzymatic apparatus of lung surfactant remains to be demonstrated. Therefore, an etiologic role of thyroid deficiency in RDS cannot be considered and the association may be regarded as coincidental or possibly consequential. A deficiency of thyroid hormones, albeit relative and transient, may produce adverse consequences in a critical period of cerebral maturation. At present, however, the extent and the nature of such possibility remains conjectural. From the observations of T_3 changes in healthy preterm infants it appears that the birth-triggered increase in peripheral monodeiodination of T_4 to T_3 requires a gestational age-dependent maturational process. These limited observations emphasize the need for further evaluation of the role of thyroid hormones in RDS and in the early postnatal development of preterm infants.

CONCLUSION

A significant difference in the postnatal T_3 concentrations of healthy preterm infants compared to full term infants has been described. In preterm infants with RDS the immediate postnatal T_3 rise is lacking and abnormally low levels of T_3 persist up to 3 weeks of age. The difference in the postnatal T_3 concentration of healthy preterm infants from the pattern observed in healthy full term infants can be explained on the basis of thyroid immaturity but the mechanism of the prolonged hypotriiodothyroninemia in babies with RDS remains to be elucidated.

REFERENCES AND NOTES

- Abuid, J., Stinson, D. A., and Larsen, P. R.: Serum triiodothyronine and thyroxine in the neonate and the acute increase in these hormones following delivery. *J. Clin. Invest.*, **52**: 1195 (1973).
- Bermudez, F., Surks, M. I., and Oppenheimer, J. H.: High incidence of decreased serum triiodothyronine concentration in patients with nonthyroidal disease. *J. Clin. Endocrinol. Metab.*, **41**: 27 (1975).
- Chopra, I. J., Chopra, U., Smith, S. R., Reza, M., and Solomon, D. H.: Reciprocal changes in serum concentrations of 3,3',5'-triiodothyronine (reverse T_3) and 3,3',5-triiodothyronine (T_3) in systemic illnesses. *J. Clin. Endocrinol. Metab.*, **41**: 1043 (1975).
- Chopra, J., and Crandall, B. F.: Thyroid hormones and thyrotropin in amniotic fluid. *N. Engl. J. Med.*, **293**: 740 (1975).
- Cuevas, R. A., Lindall, A., and Engel, R. R.: Low thyroid hormones and respiratory-distress syndrome of the newborn: studies on cord blood. *N. Engl. J. Med.*, **295**: 297 (1976).
- Czernichow, P., Greenberg, A. H., Tyson, J., and Blizard, R. M.: Thyroid function studies is paired maternal cord sera and sequential observations of thyrotropic hormone release during the first 72 hours of life. *Pediatr. Res.*, **5**: 53 (1971).
- Dubowitz, L. M. S., Dubowitz, V., and Goldberg, C.: Clinical assessment of gestational age in the newborn infant. *J. Pediatr.*, **77**: 1 (1970).
- Erenberg, A., Phelps, D. L., Lam, R., and Fisher, D. A.: Total and free thyroid hormone concentrations in the neonatal period. *Pediatrics*, **53**: 211 (1974).
- Fisher, D. A., and Odell, W. D.: The acute release of thyrotropin in the newborn. *J. Clin. Invest.*, **48**: 1670 (1969).
- Fisher, D. A., Odell, W. D., and Hobel, C. J.: Thyroid function in the term fetus. *Pediatrics*, **44**: 526 (1969).
- Fisher, D. A., Dussault, J. H., Hobel, C. J., and Lam, R.: Serum and thyroid gland triiodothyronine in the human fetus. *J. Clin. Endocrinol. Metab.*, **36**: 397 (1973).
- Greenberg, A. H., Czernichow, P., Reba, R. C., et al.: Observations on the maturation of thyroid function in early fetal life. *J. Clin. Invest.*, **49**: 1790 (1970).
- Hoch, F.: Biochemical actions of thyroid hormones. *Physiol. Rev.*, **42**: 605 (1962).
- Larsen, P. R.: Direct immunoassay of triiodothyronine in human serum. *J. Clin. Invest.*, **51**: 1939 (1972).
- Man, E. B., Adelman, M., Jones, W. S., and Lord, R. M.: Development and B1 of full term and low birth weight infants through 18 months. *Amer. J. Dis. Child.*, **119**: 298 (1970).
- Murphy, B. E. P., Pattee, C. J., and Gold, A.: Clinical evaluation of a new method for the determination of serum thyroxine. *J. Clin. Endocrinol. Metab.*, **26**: 247 (1966).
- Patel, Y. C., Burger, H. G., and Hudson, B.: Radioimmunoassay of serum thyrotropin sensitivity and specificity. *J. Clin. Endocrinol. Metab.*, **33**: 768 (1971).
- Redding, R. A., and Pereira, C.: Thyroid function in respiratory distress syndrome of the newborn. *Pediatrics*, **54**: 423 (1974).
- The technical assistance of Ms. Carolyn Aldridge and secretarial assistance of Ms. Mary E. Holroyd is greatly appreciated.
- This research was presented at the annual meeting of the Southern Society of Pediatric Research, New Orleans, Louisiana, January 1976.
- This research was supported in part by Public Health Service Grant RR 5360-11, the Herman Goldman Foundation and National Institutes of Health Grant 5 MOI-RR-00060-13.
- Requests for reprints should be addressed to: V. Abbassi, M.D., Department of Pediatrics, Georgetown University Hospital, Washington, D.C. (USA).
- Received for publication May 21, 1976.
- Accepted for publication November 9, 1976.

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Printed in U.S.A.

Pediat. Res. **11**: 804-808 (1977)

Amino acids intestinal development
carbohydrates maternal nutrition
diet neonatal development

Effect of Maternal Protein Deprivation on the Development of Neonatal Intestinal Function in Rats

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Summary

The developmental pattern of *in vitro* intracellular accumulation (IA) was studied in the small intestine of the offspring of females fed a 27% protein diet (normal) (NPD animals) and the offspring of females fed an 8% protein diet (low) (LPD animals).

At 2 days of age, the IA of L-leucine (Leu) and D-galactose (Gal) was significantly reduced in the intestines of the LPD animals as compared to the NPD animals at initial velocities (75% and 81%) and under steady state conditions (82% and 84%). The IA of both compounds in the intestines of the LPD animals increased relative to the NPD animals until at weaning

the IA of Leu and Gal under steady state conditions was significantly greater in the LPD animals (139% and 133%). After weaning, the IA of Leu was statistically increased (137%) and the IA of Gal was the same in the LPD animals as compared to the NPD animals at initial velocities. The variations in the developmental patterns of the IA of Leu and Gal in the intestines of LPD animals as compared to NPD animals appear to be related to animal age, which may reflect an improved caloric intake at weaning.

Speculation

Developing rats from females fed an 8% protein diet demonstrated an increase in intestinal absorption during the weaning period as compared to rats from females fed a 27% protein diet. This may reflect accelerated gut maturation manifested by an increase in gut size, cell numbers, and transport sites relative to an improved caloric intake associated with the availability of solid food.

The association between poor maternal nutrition and retarded fetal and postnatal growth has been well established (15, 22, 25). Maternal malnutrition has been shown to delay the development and maturation of the gastrointestinal tract in the offspring (16, 24). The absorption of nutrients is known to be impaired in protein and protein-calorie malnourished children (9, 10, 17), and in diet-restricted human volunteers (1). In a study by Younoszai and Lynch (23), the total amount of hexose absorbed from the perfused gut of growth-retarded suckling rat pups was observed to be less than that of control animals. The authors felt that their data were biased by a reduction in the size of the intestine of the undernourished pups and that rates of absorption (milligrams per g dry wt per hr) were equal or even greater in the growth-retarded animals. Wapnir and Lifshitz (18) have demonstrated a selective alteration of amino acid transport in protein-restricted weaning rats when compared to control animals, *i.e.*, the transport rates of leucine and tyrosine were increased, whereas the transport rates of phenylalanine and glycine were unaltered and the transport rate of lysine decreased. These data tend to discount thickness of the intestinal wall or overall intestinal size as the sole factor in determining uptake rates in the perfused gut. Studies in our laboratories have shown the intracellular accumulation of L-leucine and D-galactose to be decreased in the 2-day-old progeny of females fed an 8% protein diet as compared to the progeny of females fed a 27% protein diet (6). As an extension of these studies, we have examined the functional development (from 2 days of age to 28–30 days of age) of the intestines of the offspring of females fed a low protein diet. The results are expressed as intracellular accumulation of L-leucine and D-galactose and compared to data obtained from the offspring of females fed a normal protein diet.

MATERIALS AND METHODS

White female and male Wistar-strain rats (26) were used in all experiments. A stock laboratory diet (27) was fed to the rats prior to breeding. The animals were mated and maintained in wire-bottomed cages and fed a 27% protein diet (normal) or an 8% protein diet (low) (28). The diets were isocaloric and were administered in pellet form. The composition and energy values of these diets are shown in Table 1. The pregnant females were placed in individual solid-bottomed breeding cages 2–3 days before parturition. Pups were left with dams until the time of death or until weaning at 20 days. After weaning, pups received the same diet as their dams. The number of pups per litter was reduced to eight shortly after birth in order to minimize variation due to litter size. The progeny of females fed the 27% protein diet (normal) are subsequently designated NPD rats and those of females fed the 8% protein diet (low) as LPD rats.

Animals were killed at 2, 8, 9, 14, 15, 16, 20, 21, 28, 29, and 30 days of age. Data from 8- and 9-, 14-, 15-, and 16-, 20-, and

Table 1. Composition (grams per kg) of diets used¹

	NPD ²	LPD ²
Casein ³	270.0	80.0
Corn starch	200.0	200.0
Sucrose	340.0	530.0
Vegetable oil, hydrogenated	140.0	140.0
Mineral mix ⁴	40.0	40.0
Vitamin mix ⁵	10.0	10.0

¹ Diets provide approximately 4.5 Kcal/g.

² NPD: 27% protein diet (normal control); LPD: 8% protein diet (low).

³ GBI, high protein.

⁴ From Bernhart, F. W., and Tomarelli, R. M.: *J. Nutr.*, 89: 495 (1966).

⁵ GBI #40060 (milligrams per kg diet): *p*-aminobenzoic acid, 110.1; ascorbic acid, 1016.6; biotin, 0.4; calcium pantothenate, 66.1; choline dihydrogen citrate, 3711.9; folic acid, 2.0; *D*-inositol, 110.1; menadione, 49.6; niacin, 99.1; pyridoxine hydrochloride, 22.1; riboflavin, 22.1; thiamine hydrochloride, 22.1; and vitamin B12 29.7; retinyl palmitate, 20.0 IU/g diet; ergocalciferol, 2.2 IU/g diet; and tocopheryl acetate, 0.1 IU/g diet.

21-, and 28-, 29-, and 30-day-old animals were combined. The animals were killed by decapitation and the small intestines quickly removed and placed in oxygenated saline. The intestines were split longitudinally while immersed in saline, yielding segments essentially free of luminal contents. The entire small intestine was used in the studies of 2-day-old animals. Four- to 5-cm segments from the older animals were used.

The segments were incubated in an oscillating water bath for 5, 15, or 30 min, in 5 ml of an oxygenated Krebs-Ringer-Tris buffer, pH 7.4, consisting of 118 mM NaCl, 25 mM Tris-HCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, and 1.2 mM KH₂PO₄ containing [*methyl*-³H]inulin (8,000–15,000 cpm/ml), and ¹⁴C-labeled radioactive (8,000–15,000 cpm/ml) and 1 mM nonradioactive L-leucine or D-galactose. The radioactive leucine and galactose were uniformly labeled and reported to be 90% pure by dilution analysis, paper chromatography, and paper electrophoresis (29). The specific activities of the radioactive compounds used in this study were: L-leucine (342 mCi/mmol) (29), D-galactose (43 mCi/mmol) (29), and [*methyl*-³H]inulin (372 mCi/mmol) (30). All common laboratory chemicals were of reagent grade quality (31).

After incubation the segments were removed from the flasks, drained, blotted, and weighed. The residual tissue was homogenized in four times its weight of 5% trichloroacetic acid to make a 20% (w/v) homogenate. The homogenate was centrifuged, and aliquots of the supernatant were counted in a liquid scintillation spectrometer (32) adjusted as previously described (6).

An extracellular fluid space was calculated from the distribution space of [*methyl*-³H]inulin and expressed as a percentage of tissue wet weight (6, 14). Amino acid and sugar transport values are expressed as intracellular accumulation, which is defined as the millimolar concentration of the amino acid or sugar in the cellular water after a given incubation period. This parameter was calculated on a basis of a formula by Crane and Mandelstam (3), modified as previously described (6). A paired-difference *t*-test was used to obtain the probability values, and a *P* of 0.05 or less was interpreted as significant (11).

RESULTS

Table 2 shows the ages and the body weights of the pups at the time of study. The offspring of females fed a 27% protein diet or an 8% protein diet are compared. The body weights were significantly lower in the LPD animals than in control pups at all ages studied. At 2 days of age the body weights of the LPD animals were 63% of those of the NPD animals. The disparity between the weights of the animal groups increased at each age studied

except the 28–30-day group. Interestingly, the LPD animals followed a growth pattern similar to that of the NPD animals, albeit, a severely retarded one. The weights of the 2-day-old animals in both groups compare well with a previous study in our laboratories (6). The NPD animal weights are also in agreement with published data regarding the offspring of animals fed a regular rat chow (19).

Table 3 demonstrates the effect of maternal diet on the intracellular accumulation of 1 mM L-leucine in the small intestine of the developing offspring. The data compare uptake in the NPD and LPD pups after 5-, 15-, and 30-min incubations at the ages shown. Intracellular accumulation was significantly lower in 2-day-old LPD animals at all three incubation times by approximately the same magnitude (18–25%). IA in the LPD animals in the 14–16-day-old period was also decreased 20% compared with the NPD animals after a 5-min incubation. At all other ages studied the IA in the LPD animals was statistically the same or significantly increased when compared to the NPD animals. The increase in the IA of L-leucine in the LPD animals was most dramatic in the 20–21-day-old and the 28–30-day-old animals. At 20–21 days the IA in the LPD animals was 137%, 144%, and 139% that in the NPD animals after incubations of 5, 15, and 30 min, respectively. At 28–30 days, the IA in the LPD animals was 159% that in the NPD animals after a 5-min incubation, 128% after a 15-min incubation, and 148% after a 30-min incubation.

The IA results obtained for L-leucine in the 2-day-old animals after a 5-min incubation correspond with an earlier study in our

laboratories using similar diets (6). The developmental pattern of IA of L-leucine in the NPD animals is in agreement with results obtained from a study utilizing the offspring of females fed a standard lab rat chow (7).

The effect of maternal diet on the intracellular accumulation of 1 mM D-galactose in the developing intestine of the offspring of females fed a 27% protein (NPD) or 8% protein (LPD) diet is demonstrated in Table 4. At 2 and 8–9 days of age, IA in the LPD animals was significantly lower than in the NPD animals at all incubation times. The decrease was approximately 20% in the 2-day-old animals and 11% in the 8–9-day-old animals at all incubation times studied. In both age groups the decrease was greatest after 5-min incubations. In the 14–16-day-old animals, IA was not statistically different in the diet groups. At 20–21 and 28–30 days of age, the IA of 1 mM D-galactose in the LPD animals was the same as that in the NPD animals after a 5-min incubation and increased an average of 35% with the longer incubation periods.

The IA of D-galactose in 2-day-old animal intestines after a 5-min incubation agrees with our earlier study (6). The developmental pattern for the NPD animals corresponds with previous studies (2, 7).

DISCUSSION

The purpose of this study was to examine the effect of the maternal diet on the development of intestinal transport in their offspring. Alterations in the developmental patterns of intestinal transport of L-leucine and D-galactose in the offspring of females fed a low protein diet as compared to the offspring of females fed a control protein diet were demonstrated. The *in vitro* method employed allowed us to compare the intracellular accumulation (IA) of Leu and Gal at various incubation times which include initial velocities (5 min), as well as steady state conditions (30 min) (13).

In this study the developmental patterns for the intracellular accumulation of Leu and Gal vary between diet groups at initial velocities (compare Tables 3 and 4, 5-min incubations). At 2 days postpartum, the IA of Leu and Gal after a 5-min incubation are significantly reduced in the intestines of the LPD animals as compared to the NPD animals. However, in the 8–9-day-old animals the IA of Leu is statistically the same for both animal groups while the IA of Gal is decreased significantly in the LPD animals as compared to the controls. At 14–16 days of age the IA of Leu is decreased in the LPD animals as compared to the

Table 2. Effect of maternal diet on weights of developing animals

Animal ages (postpartum)	Animal weights, g ¹	
	27% Protein diet ²	8% Protein diet ^{2,3}
2 Days	8.6 ± 0.1 ⁴	5.4 ± 0.4
8–9 Days	23.3 ± 1.2	10.4 ± 0.4
14–16 Days	28.5 ± 0.8	11.6 ± 0.2
20–21 Days	34.5 ± 0.5	11.1 ± 0.4
28–30 Days	61.3 ± 1.5	20.4 ± 0.8

¹ Each value represents at least six animals selected randomly from at least five litters.

² Female rats were fed diets throughout gestation and lactation. Offspring were fed diets at weaning.

³ Mean ± SEM.

⁴ All 8% values significantly different from 27% values at ages compared, $P < 0.001$.

Table 3. Effect of maternal diet on intracellular accumulation of 1 mM L-leucine in developing rat intestine

Animal ages (postpartum)	Maternal ² diet	Intracellular accumulation ¹					
		mM/5 min	% Control	mM/15 min	% Control	mM/30 min	% Control
2 Days	27%	4.36 ± 0.20 ³		6.07 ± 0.36		8.51 ± 0.28	
	8%	3.26 ± 0.14 ⁴	75	4.86 ± 0.34 ⁵	80	7.02 ± 0.09 ⁵	82
8–9 Days	27%	3.78 ± 0.27		6.44 ± 0.37		9.31 ± 0.44	
	8%	3.98 ± 0.25	105	7.79 ± 0.26 ⁵	122	10.04 ± 0.56	108
14–16 Days	27%	3.47 ± 0.14		5.70 ± 0.15		8.41 ± 0.33	
	8%	2.76 ± 0.08 ⁵	80	6.05 ± 0.30	106	8.69 ± 0.67	103
20–21 Days	27%	2.83 ± 0.29		4.60 ± 0.14		6.64 ± 0.57	
	8%	3.87 ± 0.21 ⁶	137	6.62 ± 0.41 ⁴	144	9.24 ± 0.40 ⁴	139
28–30 Days	27%	1.69 ± 0.14		4.05 ± 0.17		4.17 ± 0.29	
	8%	2.69 ± 0.17 ⁴	159	5.17 ± 0.30 ⁶	128	6.18 ± 0.36 ⁶	148

¹ Each value represents at least six experiments each using one intestinal segment from one rat. Animals were selected randomly from at least five different litters in each diet group.

² Female rats were fed diets throughout gestation and lactation. Offspring were fed diets at weaning. 27%: 27% protein diet (normal control); 8%: 8% protein diet (low).

³ Mean ± SEM.

⁴ Significantly different from 27% diet group, $P < 0.01$.

⁵ Significantly different from 27% diet group, $P < 0.001$.

⁶ Significantly different from 27% diet group, $P < 0.02$.

Table 4. Effect of maternal diet on intracellular accumulation of 1 mM D-galactose in developing rat intestine

Animal ages (postpartum)	Maternal ² diet	Intracellular accumulation ¹					
		mM/5 min	% Control	mM/15 min	% Control	mM/30 min	% Control
2 Days	27%	1.88 ± 0.08 ^a		3.41 ± 0.08		4.81 ± 0.26	
	8%	1.52 ± 0.11 ⁴	81	2.80 ± 0.11 ⁴	82	4.04 ± 0.47 ⁴	84
8-9 Days	27%	2.61 ± 0.13		4.84 ± 0.37		6.30 ± 0.21	
	8%	2.16 ± 0.12 ⁴	83	4.44 ± 0.26 ⁴	92	5.74 ± 0.31 ⁴	91
14-16 Days	27%	1.49 ± 0.08		3.34 ± 0.11		4.53 ± 0.19	
	8%	1.48 ± 0.07	99	3.41 ± 0.22	102	4.92 ± 0.23	109
20-21 Days	27%	1.68 ± 0.24		3.26 ± 0.24		3.63 ± 0.18	
	8%	1.68 ± 0.13	100	4.04 ± 0.12 ⁴	124	4.84 ± 0.40 ⁴	133
28-30 Days	27%	1.59 ± 0.05		2.44 ± 0.08		3.37 ± 0.21	
	8%	1.65 ± 0.15	104	3.52 ± 0.18 ⁴	144	4.75 ± 0.24 ⁴	141

¹ Each value represents at least six experiments each using one intestinal segment from one rat. Animals were selected randomly from at least five different litters in each diet group.

² Female rats were fed diets throughout gestation and lactation. Offspring were fed diets at weaning. 27%: 27% protein diet (normal control); 8%: 8% control diet (low).

^a Mean ± SEM.

⁴ Significantly different from 27% diet group, $P < 0.05$.

NPD controls, whereas the IA of Gal is the same in both groups at initial velocities. At 20-21 and 28-30 days of age, the percentage IA of Leu is significantly increased in the LPD animals as compared to the NPD animals. These percentages also reflect a decrease in the IA of Leu in the control animals. At 20-21 days the IA of Gal after a 5-min incubation was the same in both diet groups.

After 15-min and 30-min incubations (compare Tables 3 and 4) the IA of Leu was decreased in the 2-day-old LPD animals as compared to controls. At all other age periods, the IA values for Leu after 15-min and 30-min incubations were similar in both diet groups or showed a percentage increase in the LPD animals. At steady state conditions (30-min incubation) this increase was significant at 20-21 and 28-30 days of age. The IA of Gal was decreased in the LPD animals as compared to the controls in the 2- and 8-9-day-old groups after 15- and 30-min incubations. In 14-16-day-old animals, the IA of Gal was similar in both diet groups after 30-min incubations. However, the IA of Gal in the LPD animals was significantly increased at 20-21 and 28-30 days of age at steady state conditions.

The selective variations in the developmental patterns of the IA of Leu and Gal in the intestines of LPD animals as compared to NPD animals observed in these studies appear to be related to animal age and incubation time. At 20-21 and 28-30 days of age, the relative IA of Leu is increased in the LPD animals as compared to the NPD animals at initial velocities and steady state conditions; the IA of Gal in the LPD animals is maintained at the same level as the controls at initial velocities, and increased relative to the NPD animals at steady state conditions. An increased transport of carbohydrates in semistarved animals (5) and growth-retarded suckling rat pups (23) has been attributed to thinner gut walls as a consequence of dietary deficiency. Hindmarsh *et al.* (8), using everted intestinal sacs from semistarved animals, demonstrated that changes in intestinal wall thickness were not sufficient to account for the observed increase in the transport of glucose and histidine. Further, Wapnir and Lifshitz (18) have shown selective alterations in amino acid transport in malnourished animals. In the present study, a retarded weight curve was evident in the suckling young of malnourished females (see Table 2). It is interesting, however, that relative increases in intracellular accumulation occur after weaning (20-21 to 28-30 days) in the LPD animals when a doubling of body weight is demonstrated. The natural food of mammals during the suckling period is milk. Maternal malnutrition has been shown to negatively affect lactation and thereby reduce the amount of milk available to the offspring (4, 12). It is possible

that the availability of solid food *ad libitum* from the weaning period (18-21 days) through the rest of our study (28-30 days) has influenced the results and that the observed improvement in transport reflects an improved caloric intake even though the diet for the LPD animals continued to be relatively protein deficient. Previous studies have described a decrease in gut size and intestinal cell numbers in malnourished animals (16, 23, 24). Recent studies of catch-up growth in malnourished animals have demonstrated a partial rehabilitation in body size and weight parameters when the animals were fed normal diets at weaning (20, 21). Thus the values obtained in these studies may reflect an increase in gut maturation with more sites available for transport. Our data do not preclude the possibility that the LPD animals have an increased affinity for the substrate between 20-21 and 28-30 days of age as compared to the NPD animals. Indeed, multiple factors including gut size, cell numbers, available transport sites, and affinity must contribute to the overall developmental pattern of intracellular accumulation in malnourished animals and must be considered in drawing correlations between the malnourished animal model and malnourished infant.

CONCLUSION

Pregnant rats were fed a 27% protein diet or an 8% protein diet throughout gestation and lactation. The *in vitro* absorptive capacity of the offspring was studied by measuring the intracellular accumulation of L-leucine and D-galactose. The absorption of both compounds was decreased at 2 days of age in the offspring of the females fed the 8% protein diet as compared to the offspring of the females fed the 27% protein diet. However, by the weaning period the low protein diet animals exhibited an increased ability to absorb L-leucine and D-galactose which was equal to or greater than that of the control animals.

REFERENCES AND NOTES

- Adibi, S., and Allen, E. R.: Impaired jejunal absorption rates of essential amino acids induced by either dietary calorie or protein deprivation in man. *Gastroenterology*, 59: 404 (1970).
- Bamford, D. R., and Ingham, P. A.: Sugar absorption by foetal and neonatal rat intestine *in vitro*. *J. Physiol.*, 248: 335 (1975).
- Crane, R. K., and Mandelstam, P.: The active transport of sugars by various preparations of hamster intestine. *Biochim. Biophys. Acta*, 45: 460 (1960).
- Enwonwu, C. O., and Glover, V.: Effect of maternal malnutrition during pregnancy and lactation on hepatic protein metabolism in the infant rat: biochemical and ultrastructural studies. *Amer. J. Clin. Nutr.*, 26: 3 (1973).
- Esposito, G.: Intestinal absorption of sugars in semi-starved rats. *Proc. Soc.*

- Exp. Biol. Med., 125: 452 (1967).
6. Fitzgerald, J. F., and Ferdinandus, L. D.: Effect of maternal protein deprivation on in vitro neonatal intestinal function in rats. *J. Nutr.*, 105: 1476 (1975).
 7. Fitzgerald, J. F., Reiser, S., and Christiansen, P. A.: Developmental pattern of sugar and amino acid transport in the postnatal rat small intestine. *Pediat. Res.*, 5: 698 (1971).
 8. Hindmarsh, J. T., Kilby, D., Ross, B., and Wiseman, G.: Further studies on intestinal active transport during semistarvation. *J. Physiol.*, 188: 207 (1967).
 9. James, W. P. T.: Intestinal absorption in protein-calorie malnutrition. *Lancet*, 1: 333 (1968).
 10. James, W. P. T.: Sugar absorption and intestinal mobility in children when malnourished and after treatment. *Clin. Sci.*, 39: 305 (1970).
 11. Natrella, M. G.: Experimental statistics. In: Bureau of Standards, Handbook 91, p. 3 (Washington, D.C., 1963).
 12. Perisse, J., and Salmon-Legagneur, E.: Influence of the nutrition level in the course of pregnancy and lactation on milk production of the rat. *Arch. Sci. Physiol.*, 14: 105 (1960).
 13. Reiser, S., Fitzgerald, J. F., and Christiansen, P. A.: Kinetics of the accelerated intestinal valine transport in 2 day old rats. *Biochim. Biophys. Acta*, 203: 351 (1970).
 14. Rosenberg, L. E., Downing, S. G., and Segal, S.: Extracellular space estimation in rat kidney slices using C^{14} saccharides and phlorizin. *Amer. J. Physiol.*, 202: 800 (1962).
 15. Sachs, B.: Maternal nutrition—what price? *New Engl. J. Med.*, 292: 208 (1975).
 16. Shrader, R. E., and Zeman, F. J.: Effect of maternal protein deprivation on morphological and enzymatic development of neonatal rat tissue. *J. Nutr.*, 99: 401 (1969).
 17. Torres-Pinedo, R., Rivera, C., and Rodriguez, H.: Intestinal absorptive defects associated with enteric infections in infants. *Ann. N. Y. Acad. Sci.*, 176: 284 (1971).
 18. Wapnir, R. A., and Lifshitz, F.: Absorption of amino acids in malnourished rats. *J. Nutr.*, 104: 843 (1974).
 19. Weber, G., Queener, S. F., and Ferdinandus, J. A.: Control of gene expression in carbohydrate, pyrimidine and DNA metabolism. *Advan. Enzyme Regul.*, 9: 63 (1971).
 20. Williams, J. P. G., Tanner, J. M., and Hughes, P. C. R.: Catch-up growth in male rats after growth retardation during the suckling period. *Pediat. Res.*, 8: 149 (1974).
 21. Williams, J. P. G., Tanner, J. M., and Hughes, P. C. R.: Catch-up growth in female rats after growth retardation during the suckling period: comparison with males. *Pediat. Res.*, 8: 157 (1974).
 22. Winick, M.: Biological correlations. *Amer. J. Dis. Child.*, 120: 416 (1970).
 23. Younoszai, M. K., and Lynch, A.: In vivo intestinal absorption of hexose in growth-retarded suckling rat pups. *J. Nutr.*, 104: 671 (1974).
 24. Younoszai, M. K., and Ranshaw, J.: Gastrointestinal growth in the fetus and suckling rat pups: Effect of maternal dietary protein. *J. Nutr.*, 103: 454 (1973).
 25. Zeman, F. J.: Effect on the young rat of maternal protein restriction. *J. Nutr.*, 93: 167 (1967).
 26. Harlan Industries, Inc., Indianapolis, Ind.
 27. Lab Blocks, Wayne Feeds, Castleton, Ind.
 28. Tek-Lad Test Diets, Madison, Wis.
 29. Amersham/Searle Corporation, Arlington Heights, Ill.
 30. New England Nuclear, Boston, Mass.
 31. Sigma Chemical Co., St. Louis, Mo.
 32. Tri-Carb liquid scintillation spectrometer model 3320, Packard Instrument, Downers Grove, Ill.
 33. The authors would like to thank the Laboratory Animal Medicine Program for their assistance in the maintenance and care of the animals used in this study. The authors are deeply indebted to Vicki Haviland for assistance in the preparation of this manuscript.
 34. This research was supported by Riley Memorial Association, Project 75-7.
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 36. Received for publication June 25, 1976.
 37. Accepted for publication November 23, 1976.

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Printed in U.S.A.

Pediat. Res. 11: 808-811 (1977)

Bilirubin electrophoresis
bilirubin binding paper chromatoelectrophoresis

A Paper Chromatoelectrophoresis Method for the Determination of Bilirubin Binding to Serum Protein

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Summary

A technique is described in which paper chromatoelectrophoresis is used to measure the bilirubin binding capacity of albumin in serum. It is equivalent to the Sephadex G-25 method and its results are highly reproducible. From a single pool of serum, multiple determinations indicated the mean bilirubin binding capacity to be 23.7 ± 0.76 (SD) mg/dl and the greatest difference between any two values was 2 mg/dl. This new technique can determine the bilirubin binding capacity of a serum sample in 15 min. Highly skilled technicians are not needed and very small quantities of serum are required, which gives it a definite advantage over the Sephadex G-25 method.

Speculation

Paper chromatoelectrophoresis is a simple, rapid, and inexpensive technique which can be used to measure the bilirubin

binding capacity in very small volumes of serum. It appears to have sufficient accuracy and reliability to be applied as a clinical guide to the severity of unconjugated (indirect acting) hyperbilirubinemia in the newborn infant. If further experience with the method supports this report, decisions regarding phototherapy and exchange transfusion to treat infants with unconjugated hyperbilirubinemia might be made on a more rational basis.

The technique could be made much more sensitive by using radioactive bilirubin. In this way, it could become a useful research tool in the study of bilirubin metabolism.

Serum unconjugated (indirect acting) bilirubin that is not bound to albumin is a cytotoxin (6, 16). It has been assumed that unbound bilirubin is responsible for bilirubin encephalopathy (14). There are several common problems of newborn infants that result in marked unconjugated hyperbilirubinemia and because there are therapeutic measures available to prevent bilirubin