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THE EFFECTS OF MATERNAL PROTEIN DEPRIVATION ON RENAL DEVELOPMENT AND FUNCTION

By

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ABSTRACT

THE EFFECTS OF MATERNAL PROTEIN DEPRIVATION ON RENAL DEVELOPMENT AND FUNCTION

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The present study was undertaken to investigate the effects of pre- and/or postnatal maternal protein deprivation on renal functional development in the rat. While the effects of prenatal protein deprivation on body and kidney growth were negligible, malnutrition during the nursing period produced a persistent deficit in body and kidney weight. Renal transport functions, quantified in vitro, were differentially affected by these dietary manipulations. Renal transport capacity for organic acids and bases was depressed in animals nursed by protein deprived dams. Maturation of the renal organic acid transport system was delayed in animals malnourished during the nursing period. Renal amino acid accumulation was significantly enhanced in 10 day old animals stressed by pre- and postnatal protein deprivation. Renal gluconeogenic and ammoniagenic capacity was not impaired by pre- and/or postnatal protein deprivation. No differences in protein or water content of renal cortical slices were observed.

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INTRODUCTION

The relationship between maternal nutritional status and the subsequent growth and development of the offspring has been the subject of many investigative studies (Chow and Lee, 1964; Brasel and Winick, 1972; Barnes et al., 1973; Lau and Ritchey, 1977). Specifically, the restriction of dietary protein during the pre- and/or post-natal period appears to have long-lasting and profound effects on the morphological and biochemical characteristics of organ tissues (Zamenhof et al., 1971; Brasel and Winick, 1972; Barnes et al., 1973; Koshy et al., 1975). The developing kidney is one of the organs most severely retarded in growth as a consequence of prenatal protein deprivation (Zeman, 1968). Zeman (1968) reported that in newborn rats exposed to prenatal protein deprivation, the kidneys were disproportionately small and morphologically immature when compared to controls. Even though these offspring were fostered and nursed by control dams, kidney development was comprised at least until 90 days of age as reflected by a reduced kidney weight, kidney cell number and nephron number (Allen and Zeman, 1973).

While the effects of maternal protein deprivation on the morphological development of the kidney have been well documented, relatively little information is available concerning the functional integrity of these developing kidneys. To date, it has not been determined whether the functional demands imposed by extrauterine life can be met

by kidneys of offspring born and/or nursed by mothers receiving low protein diets. The present study was therefore undertaken to elucidate the effects of maternal protein deprivation during gestation and/or lactation on renal functional development of the offspring.

Kidney Maturation

Renal function in the newborn differs in a number of ways from that of the adult. Glomerular filtration rate (GFR) is markedly low in the newborn even when corrected for body weight or body surface area (Weil, 1955). In addition, kidneys of infants have a decreased ability to excrete a water or solute load (Edelmann and Spitzer, 1969), an inability to concentrate urine and to conserve water (Falk, 1955) and an impaired capacity to excrete titratable acid and ammonia (Goldstein, 1970) when compared to adult standards. While various explanations for this low functional capacity have been offered, much of the evidence has been inconclusive.

One of the main contributing factors to the functional immaturity of the neonatal kidney may, in part, be related to the striking anatomic and cellular underdevelopment of this organ. Anatomical studies of the human infant kidney have revealed the presence of immature proximal tubules; that is, these tubules are appreciably small relative to the size of their corresponding glomeruli (Edelmann and Spitzer, 1969). In addition, the newborn kidney is characterized by nephron heterogeneity which is far in excess of that observed in later life. This heterogeneity in nephron population is related to the centrifugal pattern of kidney development; that is, the deeper structures of the

medullary and juxtamedullary regions are formed first whereas the outer nephrons of the cortical region develop later (Baxter and Yoffey, 1948; Bogomolova, 1966).

In the human, the anatomic development of the kidney is largely confined to intrauterine life (Edelmann and Spitzer, 1969). Formation of new glomeruli ceases in utero when the fetus achieves a weight of 2.1-2.5 kg (Edelmann and Spitzer, 1969) and thus, the full complement of glomeruli and tubules is present in full term infants at birth. In contrast, the rat kidney undergoes considerable cellular differentiation and nephrogenesis after birth (Baxter and Yoffey, 1948). The number of nephrons doubles within the first two weeks and triples within the first four (Falk, 1955). In addition, the tubule length of the rat kidney has been reported to increase approximately twenty-fold after birth. Cellular hyperplasia, then, begins in utero and continues until 20-30 days of postnatal life in the rat kidney; growth thereafter is largely a function of cellular hypertrophy (Baxter and Yoffey, 1948).

Renal Tubular Secretion of Organic Acids and Bases

Numerous substances are added to the urine by tubular secretion; that is, they are transported from the peritubular fluid into the tubular lumen. Marshall and Vickers (1923) first demonstrated the renal tubular secretion of an exogenous compound using the dye, phenolsulfonphthalein (PSP). Since then, an exhaustive amount of work has been conducted to identify several independent secretory mechanisms. The renal secretory mechanisms may be either active or passive transport processes. Of those compounds that are actively secreted, at least three separate

systems have been identified: one responsible for the secretion of a variety of weak organic acids (p-aminohippurate (PAH), diodrast, phenol red), another responsible for the secretion of several organic bases (N-methylnicotinamide (NMN), tetraethylammonium (TEA), quinidine) and another responsible for the secretion of ethylenediaminetetra-acetate (EDTA) (Pitts, 1963). All three transport systems are functionally distinct and thus, no cross-competition occurs between substances handled by these different secretory mechanisms (Pitts, 1963).

Of the active secretory systems, the organic anion transport system has been studied most extensively. The utility of renal cortical slices for the study of this system was first demonstrated by Cross and Taggart (1950) who reported that rabbit renal cortical slices accumulated the organic acid, PAH, from a saline medium against a concentration gradient. In addition, Cross and Taggart (1950) concluded that the *in vitro* intracellular accumulation of PAH, expressed as a slice/medium (S/M) ratio, is closely related to the tubular excretion of this compound in the intact animal. In the intact animal, PAH is actively secreted from the peritubular fluid into the proximal tubule and then diffuses down a concentration gradient into the tubular lumen or urine (Tune *et al.*, 1969). Similarly, the active transport component of the *in vitro* slice preparation is the accumulation of PAH by the proximal tubular cell. However, the major difference between the *in vivo* and *in vitro* systems is the absence of a continuous filtration process in the slice preparation. While this difference may exert an effect on certain quantitative aspects of PAH transport, positive correlations have repeatedly been established between properties of PAH transport *in*

vivo and in vitro (Cross and Taggart, 1950; Mudge and Taggart, 1950; Forster and Copenhaver, 1956). In addition, the slice system has several advantages in that the chemical composition of the bathing medium can be rigidly controlled and that as an isolated system, it excludes the influences of extrarenal factors.

Accordingly, the in vitro slice preparation has been extensively used to delineate the functional characteristics of the organic ion transport systems in the developing kidney. Studies employing this technique have produced a characteristic pattern of development in tissue from rabbits (Hirsch and Hook, 1970), rats (Kim et al., 1972) and dogs (Rennick et al., 1961; Hook et al., 1970). Generally, PAH S/M ratios are low immediately following birth and progressively increase with age. Peak values are obtained between 1-4 weeks of age, depending on the species. The PAH S/M ratio then declines with age until adult levels are obtained. In contrast, the development of the organic base (NMN) accumulation process progressively increases with age and appears to be more closely allied with organ growth than that of PAH (Kim et al., 1972; Rennick et al., 1961).

Renal Tubular Transport of Amino Acids

Our current knowledge of the processes involved in amino acid transport by the kidney has been greatly facilitated by micropuncture and microperfusion techniques, clearance studies and stop-flow analyses. Generally speaking, amino acids are actively reabsorbed in the proximal tubular region of the nephron in the adult kidney; that is, they are transported from the tubular lumen to the peritubular fluid against a concentration gradient (Pitts, 1963). Under normal circumstances,

only negligible quantities of amino acids are excreted in the urine; nearly all that is filtered at the glomerulus is reabsorbed (Silbernagl *et al.*, 1975).

More recently, the *in vitro* slice preparation devised by Cross and Taggart (1950) has been adapted for the study of the renal cortical accumulation of amino acids. Rosenberg *et al.* (1961) reported that kidney cortical slices accumulated the non-metabolizable amino acid, α-aminoisobutyric acid (αAIB), against a concentration gradient. The relative simplicity of the slice technique compared to the *in vivo* preparations has facilitated much investigative work in the developmental patterns of renal amino acid transport. It is fairly well established that infants tend to excrete a relatively larger amount of amino acids in the urine than do adults (Christensen, 1973). Paradoxically, newborn renal cortical tissue has a greater capacity to accumulate amino acids than adult tissue (Webber and Cairns, 1968; Reynolds and Segal, 1976), suggesting that the newborn kidney may have a greater reabsorptive capacity for amino acids than the adult. Explanations for this apparent discrepancy are few and conjectural. One attractive hypothesis is based on the observations of Segal *et al.* (1971) who reported that the influx of amino acids in newborn cortical tissue is comparable to that of the adult; however, the efflux is markedly reduced in newborns. It was suggested that if the *in vitro* efflux is equated with the *in vivo* movement of amino acids out of the tubular cells into the peritubular capillaries, a deceleration of this process would cause a higher intracellular concentration of amino acids and at the same time, account for a slower removal of amino acids from the tubular urine

(Segal et al., 1971). Alternatively, it may be that the renal reabsorptive process is too complex to be quantified by the slice system.

More recently, Reynolds and Segal (1976) reported that the renal cortical tissue accumulation of αAIB in newborns was significantly enhanced by a preincubation period while adult tissue was comparatively unresponsive. In addition, the stimulatory effect of preincubation was abolished by anaerobiosis, dinitrophenol, cyclohexamide and puromycin. It was suggested that the increased accumulation of αAIB by newborn tissue after preincubation may be the consequence of an induction of protein carrier synthesis which is absent or "turned off" in the more mature kidney.

Renal Ammoniagenesis and Gluconeogenesis

It is well documented that during periods of chronic metabolic acidosis, the kidney plays a major compensatory role by increasing the urinary excretion of hydrogen ions in the form of ammonium (Pitts, 1963). Ammonia is produced from glutamine in sequential reactions involving the deamidation by glutaminase I to form glutamate. Glutamate, then, undergoes oxidative deamination to form α-ketoglutarate (αKG) which, in turn, may be oxidized to form carbon dioxide or converted to glucose (Pitts, 1963).

The control mechanisms for renal ammonia production have been the subject of much investigative work. Factors proposed to account for the increase in ammonia production during period of metabolic acidosis include an increase in the renal content of glutaminase (Davies and Yudkin, 1952), enhanced activity of glutaminase (Rector et al., 1955), a decreased rate of glutamine synthetase activity (Damian and Pitts,

1970), an increased NAD^+/NADH ratio (Preuss et al., 1968), a decreased concentration of intracellular glutamate (Goldstein, 1966) and an increased activity of phosphoenolpyruvate carboxykinase (PEPCK) (Iynedjian and Peters, 1974). However, according to these authors, none of these factors alone appear to be rate limiting in the production of ammonia during acidosis. Thus, the regulatory mechanisms for this response remain speculative.

Renal ammonia excretion has been observed to be low in developing animals (Goldstein, 1970). In addition, the response of nursing rats to an acid load is significantly less than the adult response (Goldstein, 1970). The inability of immature kidneys to respond to acidosis is accompanied by a correspondingly low level of renal glutaminase activity during the first two weeks of postnatal life in the rat (Wacker et al., 1961). In addition, renal glutamine concentrations are low in 9 day old rats but increase to near adult levels by 12 days of age (Goldstein, 1971). Benyajati and Goldstein (1975) observed that repeated administrations of ammonium chloride to nursing rats increased ammonia excretion approximately 2-3 fold within two days. This adaptive response was associated with a concomitant rise in renal phosphate-dependent glutaminase activity, suggesting that glutaminase activity may play a direct role in the adaptation of ammonia excretion to acidosis in infant rats.

Among the factors suggested as a regulatory mechanism for ammonia excretion is gluconeogenic activity (Goodman et al., 1966; Pagliara and Goodman, 1970). According to this theory, the key rate limiting enzyme of the gluconeogenic pathway, PEPCK, is induced during metabolic

acidosis. This results in a decreased concentration of citric acid cycle intermediates, including αKG and in turn, accelerates the flux of glutamate to αKG. Therefore, it has been proposed that the increase in gluconeogenic activity could theoretically stimulate ammonia production by lowering the concentration of glutaminase inhibitors, such as glutamate.

Similar to renal ammoniagenesis, low levels of gluconeogenic capacity have been observed in nursing rats during the first two weeks of postnatal life (Goldstein and Harley-DeWitt, 1973). Gluconeogenic capacity matures to adult levels by 12 days of age and the response to acidosis matures by 14 days (Goldstein and Harley-DeWitt, 1973).

Effects of Dietary Protein on Kidney Function

In adults, alterations in dietary protein levels have been shown to substantially affect kidney function. Significant changes in renal concentrating ability (McCance et al., 1969); urea reabsorption (Schmidt-Nielsen et al., 1958), glomerular filtration rate (Dicker, 1949; Pullman et al., 1954), renal plasma flow (Dicker, 1949; Pullman et al., 1954) and the maximal rate of tubular secretion of PAH (Pullman et al., 1954) have been reported. The mechanisms underlying these responses are speculative for these changes may reflect a direct adverse effect of malnutrition on the kidney or they may simply represent an adaptation by the kidney to a nutritional insult.

Protein-calorie malnourished (PCM) children exhibit a decrease in both renal plasma flow (RPF) and glomerular filtration rate (GFR). Arroyave et al. (1961) reported a mean GFR of 14 ml/min/m^2 in 9

malnourished children when compared to a mean of 45 ml/min/m^2 in 17 well nourished controls. Jamaican children with severe malnutrition had a mean inulin clearance of 45 ml/min/m^3 and a mean RPF of 214 ml/min/m^3. After recovery, these values averaged 92 ml/min/m^3 and 321 ml/min/m^3, respectively (Alleyne, 1967). Although there is considerable variation between studies, there is general agreement among investigators that infantile malnutrition severely compromises kidney function. This may be related to a reduced cardiac output in these children and/or a diminished glomerular capillary filtering surface (Klahr and Alleyne, 1973).

In addition to diminished GFR and RPF, malnourished Jamaican children demonstrated impaired renal concentrating ability which was improved upon nutritional rehabilitation (Alleyne, 1967). Similarly, Edelmann (1973) reported that infants fed a low protein diet had reduced concentrating ability following water deprivation when compared to infants fed a high protein diet. Significant improvements in concentrating ability have been observed in infants fed either urea or a high protein diet (Klahr et al., 1967; McCance et al., 1969). It was hypothesized by Klahr (1967) that decreased urea concentration in the renal medulla is, in part, responsible for the concentrating defect observed in malnutrition.

In addition, balance studies have indicated that protein deficient children have impaired ability to handle sodium loads compared to their ability following protein repletion (Klahr and Alleyne, 1973). This may contribute to the water and electrolyte anomalies observed in children suffering from kwashiorkor.

Maternal Dietary Protein and Kidney Development

More recently, the effects of maternal protein deprivation on the kidneys of the young have been examined. Kidneys of newborn rats exposed to prenatal protein deprivation were disproportionately small and morphologically immature when compared to newborns of control dams (Zeman, 1968). Even though these offspring were fostered and nursed by control dams, retarded kidney development as reflected by a reduced kidney weight, kidney cell number and nephron number persisted until 90 days of age (Allen and Zeman, 1973). Kidneys from these pups had fewer and less well differentiated glomeruli, a greater proportion of connective tissue and relatively fewer collecting tubules (Zeman, 1968). Histological studies indicated that the proximal convoluted tubules of these kidneys were shorter and had fewer convolutions. While there were no differences in the activities of ATPase, nonspecific esterase and leucine aminopeptidase, there were significant decreases in alkaline and acid phosphatase activity, indicative of a reduced differentiation of the proximal tubules and reduced lytic activities, respectively (Zeman, 1968). Improved postnatal nutrition, achieved by reducing litter size from ten to four pups per nursing dam, resulted in a transient increase in kidney cell number in prenatally protein deprived pups when compared to those nursing in normal sized litters (Allen and Zeman, 1973).

To determine whether these effects were accompanied by functional alterations, Hall and Zeman (1968) conducted a battery of renal functional tests in offspring of pregnant rats fed either a 24% or 6% casein diet. Depressed urine excretion during both water diuresis

and osmotic diuresis was observed in pups exposed to prenatal protein deprivation (Hall and Zeman, 1968). In addition, GFR was markedly reduced in these pups at 6 days of age (Hall and Zeman, 1968), an effect which persisted at least until 22 days (Allen and Zeman, 1973). Improved postnatal nutrition, achieved by reducing litter size, only partially compensated for the deficit in GFR (Allen and Zeman, 1973).

From these studies, it was concluded that prenatal protein deprivation severely compromised kidney development, an effect which could not be reversed or corrected by compensatory postnatal feeding (Allen and Zeman, 1973). In contrast, Winick et al. (1968) reported that growth retardation of the rat kidney consequent to a nutritional deprivation can be overcome if an adequate diet is instituted during the nursing period, before the normal period of hyperplasia ends. Thus, recovery of kidney cellular and organ growth has been observed when an adequate diet is implemented during the nursing period (Winick et al., 1968). In this regard, it is noteworthy that Zeman (1967) reported a high mortality rate in young of prenatally protein deprived dams even though these offspring were nursed by controls. These animals may have been too weak to suckle adequately and this may, secondarily, have resulted in an insufficient nutrient intake during the early postnatal period. Thus, the growth retardation observed in these pups may be the sum of effects observed in the newborn plus the consequences of postnatal malnutrition. In addition, Allen and Zeman (1973) suggested that the impaired renal function in prenatally protein deprived pups was related to the morphological immaturity of these kidneys. It cannot be dismissed that the effects on renal

function may have been attributable to extrarenal influences; i.e., cardiovascular and/or endocrine factors.

Therefore, the present study was undertaken to: (1) determine the effects of maternal protein deprivation on renal functional development in such a way as to eliminate extrarenal factors, (2) isolate the effects of a low protein diet given during gestation from one given during lactation, and (3) assess the reversibility or persistence of any observed effects.

MATERIALS AND METHODS

Animals and Diets

Timed pregnant Sprague-Dawley rats were obtained from a local supplier (Spartan Research Farms) on day 1 of gestation. Animals were individually caged and fed a purified diet containing either 24% or 8% protein (Table 1). Diets were isocaloric and complete with respect to fat, vitamin and mineral content. Since no significant differences in food consumption were detected in preliminary experiments, food intake was measured only during selected periods of gestation: days 3-5, 10-12 and 17-19. Animals were fed the diet ad libitum beginning on day 1 of gestation and continued on this feeding regimen until litters were weaned. The protein content of the experimental diet was increased to 10% at birth to ensure adequate lactation (Venkatachalam and Ramanathan, 1964; Barnes et al., 1973).

At birth, litters were weighed and adjusted in size so that each dam nursed 10 pups. In order to isolate the effects of a low protein diet during gestation from those of lactation, each litter was cross-fostered at birth, yielding four experimental models:

	Gestation diet/Lactation diet	Group
(1)	24% protein/24% protein	Control/Control (C/C)
(2)	24% protein/10% protein	Control/Deficient (C/D)
(3)	8% protein/24% protein	Deficient/Control (D/C)
(4)	8% protein/10% protein	Deficient/Deficient (D/D)

Litters of the C/C and D/D groups were also fostered at birth to eliminate

TABLE 1

Diet Composition

Diet	24% Protein	10% Protein	8% Protein
Ingredient		g/100 g diet	
Casein (90% protein)	26.70	11.10	8.90
Methionine	0.40	0.17	0.13
Vitamin Mix[a]	0.40	0.40	0.40
Mineral Mix[b]	4.00	4.00	4.00
Choline Chloride	0.20	0.20	0.20
Corn Oil	5.00	5.00	5.00
Fiber	5.00	5.00	5.00
Dextrose	58.30	74.13	76.37

[a] In mg/100 g diet: thiamin HCl, 2.2; pyridoxine, 2.2; riboflavin, 2.2; Ca pantothenate, 6.6; p-aminobenzoic acid 11.0; menadione, 5.0; inositol 10.0; ascorbic acid, 20.0; niacin, 10.0; vitamin B_{12}, 0.003; biotin, 0.06; folic acid, 0.4. In IU/100 g diet: Vitamin A, 2,000; α-tocopherol acetate, 10; vitamin D_3, 220.

[b] In mg/100 g diet: calcium, 600; phosphorous, 500; sodium, 50; potassium, 180; chlorine, 50; magnesium, 40; manganese, 5; iron, 2.5; zinc, 1.2; copper, 0.5; iodine, 0.015; sulfate, 100.

cross-fostering as a variable. Litters of the C/C and D/D groups remained with their foster mothers until 28 days of age at which time they were weaned to a control diet. Body weight, brain weight, kidney weight and kidney function were determined at 1, 5, 10, 28 and 42 days of age.

Accumulation of Organic Ions by Renal Cortical Slices

Animals were killed by cervical dislocation, kidneys were immediately removed, decapsulated, weighed and placed in cold isotonic (0.9%) saline. Renal cortical slices were prepared free hand, kept briefly in cold saline, and then incubated in 2.7 ml of Ringer's phosphate medium containing 7.4×10^{-5}M PAH (Eastman Kodak) and 6.0×10^{-6}M (2.5×10^{-2} μCi/ml) ^{14}C-NMN (New England Nuclear). Incubations were carried out in a Dubnoff metabolic shaker at 25° under 100% oxygen for 90 minutes as described by Cross and Taggart (1950). Following incubation, the slices were quickly removed from the beakers, blotted and weighed. Slices were homogenized in 3.0 ml of 10% trichloracetic acid (TCA) and the final volume adjusted to 10.0 ml with distilled water. Similarly, a 2.0 ml aliquot of the incubation medium was added to 3.0 ml of TCA and appropriately diluted to 10.0 ml with distilled water. After centrifugation at 1400 rpm for 10 minutes, a 1.0 ml aliquot of the supernatant of both the slice and the medium was used to determine the concentration of PAH as described by Smith et al. (1945). In addition, one ml aliquots of slice and medium acid homogenates were added to scintillation vials containing 10 ml of modified Bray's solution (6 gm of 2,5-diphenyloxazole and 100 gm of naphthalene per liter of dioxane). Concentrations of ^{14}C-NMN in the slice and in the medium were determined using a Beckman LS-250 liquid scintillation

counter and employing internal standards. By determining the concentration of PAH or NMN in the slice (mg/g or dpm/gm) and in the medium (mg/ml or dpm/ml), a slice/medium (S/M) ratio was determined and used to represent the intrinsic transport capacity for organic acids and bases, respectively.

Accumulation of Amino Acids By Renal Cortical Slices

Renal cortical slices were prepared as previously described. For preliminary incubation, slices were placed in flasks containing 2.0 ml of Krebs-Ringers buffer (0.154 M NaCl, 0.154 M KCl, 0.11 M $CaCl_2$, 0.154 $MgSO_4$, 0.154 M KH_2PO_4 and 0.154 M $NaHCO_3$) and pH was adjusted to 7.4. Incubation flasks containing the slices were gassed briefly with 95% O_2:5% CO_2, stoppered and then incubated for 30 minutes at 37° in a Dubnoff metabolic shaker (Reynolds and Segal, 1976). Following this period, slices were transferred to another flask containing 3.0 ml of Krebs-Ringer bicarbonate buffer containing 0.07 mM α-aminoisobutyric acid (αAIB) (Sigma Chemical Company) and ^{14}C-αAIB (.025 μCi/ml) (New England Nuclear). Incubation flasks were gassed briefly with 95% O_2:5% CO_2, stoppered and incubated for one hour at 37°. After incubation, slices were quickly removed from the flasks, blotted and weighed. Both slice and medium were treated and analyzed as described for NMN. Net accumulation of αAIB was expressed as a slice/medium (S/M) ratio.

Renal Ammoniagenic and Gluconeogenic Capacity

Glucose and ammonia production by renal cortical slices were measured by the technique described by Krebs et al. (1963). Approximately 50 mg renal cortical tissue were incubated in 5.0 ml Krebs-Ringer

bicarbonate containing either 10 mM sodium glutamate or diluted appropriately with distilled water. In each case, pH was adjusted to 7.4. Incubation flasks containing the slices were flushed with 95% O_2:5% CO_2, stoppered and incubated for 90 minutes at 37° in a Dubnoff metabolic shaker. At the end of incubation, slices were removed from the flasks, blotted and dried in preweighed crucibles at 100° overnight to determine dry weight. Immediately after removal of tissue from the flasks, the incubation medium was added to 0.5 ml of 10% (v/v) $HClO_4$ and centrifuged to remove precipitated protein. The supernatant was assayed for ammonia and glucose. Glucose was determined using a glucose oxidase reagent containing glucose oxidase, peroxidase and chromagen (Glucostat; Worthington Biochemical Corp.). Ammonia was determined according to the method described by Kaplan (1965). Net production of glucose or ammonia was calculated as the difference between glucose or ammonia content of the incubation medium with and without the substrate, glutamate. Results were expressed as micromoles of glucose or ammonia produced/hour/gram, dry weight.

Protein and Water Composition of Renal Cortical Slices

Slices were prepared as previously described and kept in cold saline until blotted, weighed and dessicated as described above. Total water content of cortical slices was determined as the difference between the wet and dry weight. Results were expressed as percent of the wet weight. After drying, slices were solubilized in 3 N KOH and diluted with distilled water. A one ml aliquot was used for the determination of protein as described by Lowry et al. (1951). Results were expressed as percent protein per mg wet weight.

Statistical Analyses

All data were reported as means ± S.E. Differences between means were analyzed statistically using Student's "t" test or the Least Significant Difference (LSD) test following analysis of variance. Data in Figure 10 were subjected to the Student-Newman Keuls test. The 0.05 level of probability was used as the criterion for significance (Sokal and Rohlf, 1969).

RESULTS

Food Intake

No significant differences in food consumption between the control and the low protein fed groups were observed during early, mid- and late gestation (Table 2).

Body Weight

Pups born of dams receiving an 8% casein diet during pregnancy weighed 5.65±0.59 g at birth. This was significantly different from the birth weights of pups born of control dams (5.99±0.55 g). At one day of age, there were no significant differences in body weights among the four experimental groups examined (Figure 1). At all ages examined, there were no significant differences in body weights of the C/C group compared to the D/C group. Similarly, body weights of the C/D group did not differ from those of the D/D group (Figure 1). However, by 5 days of age, animals of C/D (9.41±0.31 g) and D/D (9.15±0.23 g) litters weighed significantly less than animals of the C/C (12.23±0.32 g) or the D/C (11.21±0.43 g) litters. This deficit in body weight of animals nursed by protein deprived dams (C/D and D/D) persisted at 10, 28 and 42 days of age (Figure 1).

Brain Weight

No differences in brain weight were observed among the four groups at 1 and 5 days of age (Figure 2). However, by 10 days of age, the

TABLE 2

Food Intake of Dams During Gestation

Group	Period (Day of Gestation)		
	3-5	10-12	17-19
Control	18.40±0.58 (67)[a,b]	20.62±0.48 (35)	21.55±0.80 (25)
Low Protein	17.51±0.69 (73)	20.74±0.20 (41)	19.13±0.11 (26)

[a]Values represent means ± S.E. (n).

[b]Grams of food consumed per day during the 3 day period.

Figure 1. Effect of maternal protein deprivation on body weight of offspring. Each value represents mean ± S.E. obtained from at least 4 litters. Absence of a vertical bar indicates that S.E. is within the radius of the point. Asterisks denote a statistical difference from animals nursed by protein deprived dams (C/D, D/D) and animals nursed by controls (C/C, D/C).

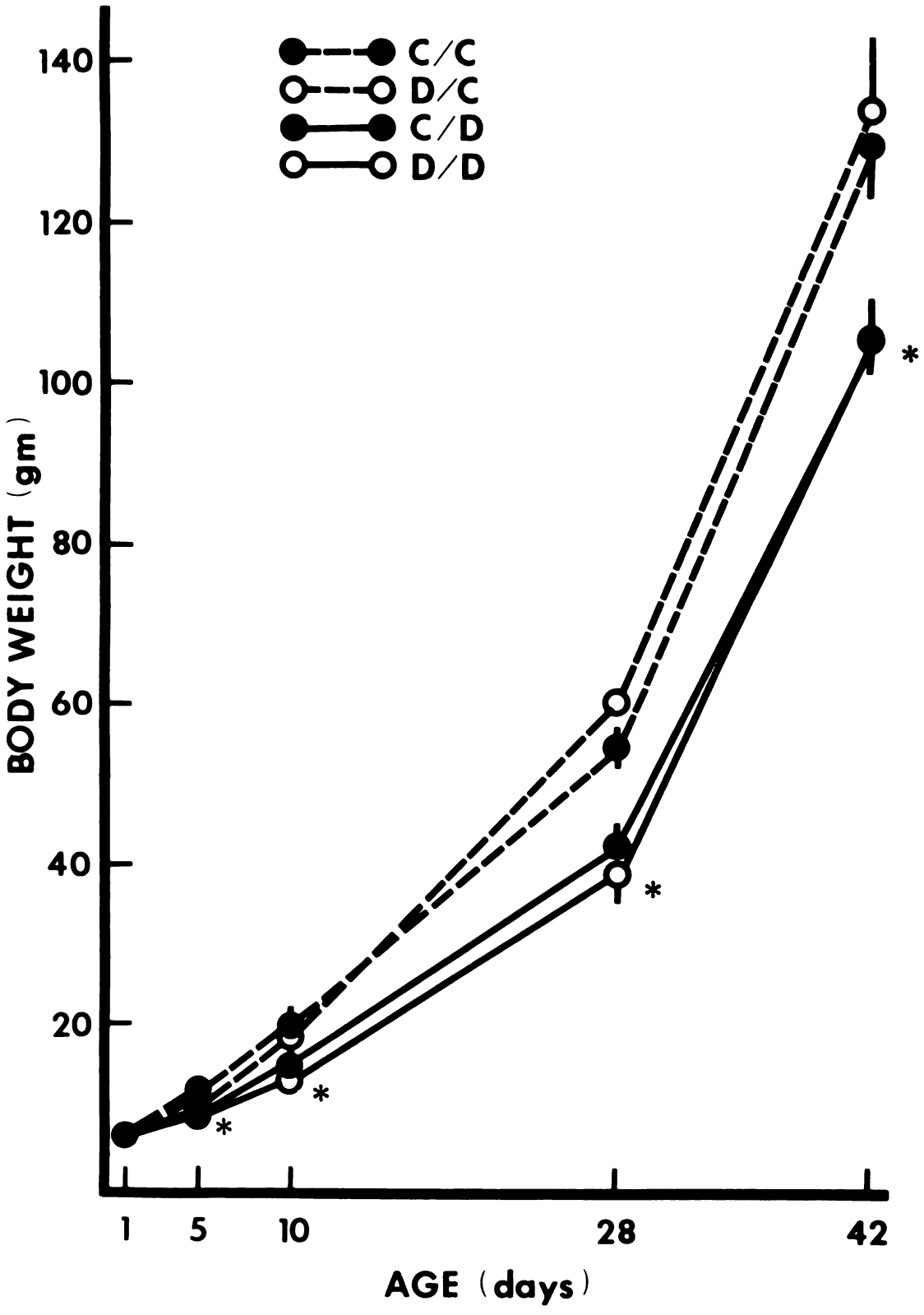
C/C
D/C
C/D
D/D
140
120
100
80
60
40
20
BODY WEIGHT (gm)
1
5
10
28
42
AGE (days)
*
*
*
*

Figure 1

Figure 2. Effect of maternal protein deprivation on brain weight of offspring. Each value represents mean ± S.E. obtained from at least 4 litters. Absence of a vertical bar indicates that S.E. is within the radius of the point. Asterisks denote a statistical difference from C/C values ($p<.05$).

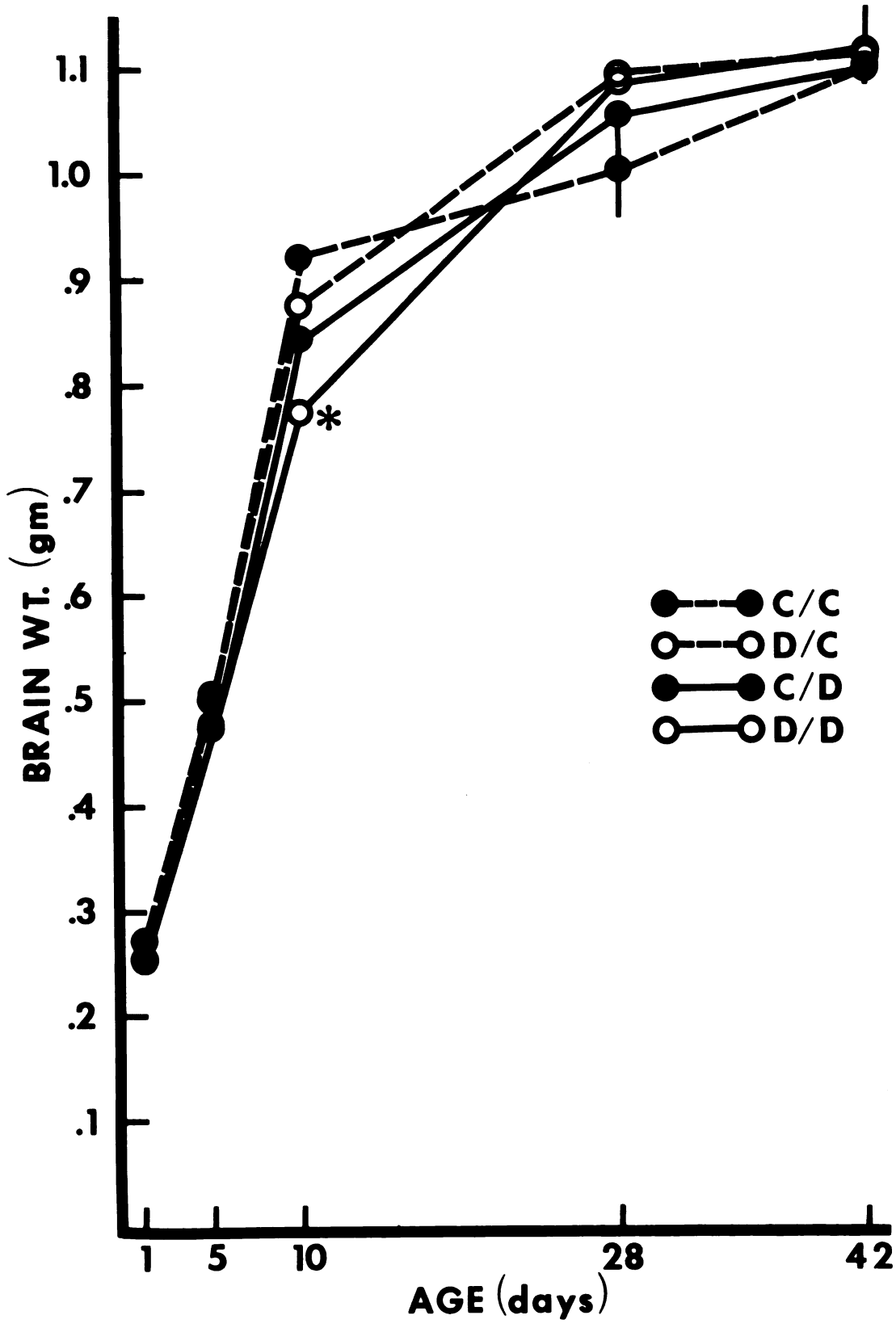

Figure 2

brain weight of animals of the D/D group (0.7745±.019 g) was significantly less than that of the C/C group (0.9218±.011 g) (Figure 2). No further differences were observed among the groups at 28 and 42 days of age (Figure 2).

Ratio of Brain Weight to Body Weight

Brain weight, relative to body weight, was not different among the groups at 1 day of age (Figure 3). However, by 5 days of age, the brain weight/body weight ratio of animals of the C/D (5.05±0.25) and D/D (5.29±0.20) groups was significantly greater than that of the C/C (4.29±0.13) or the D/C groups (4.49±0.15). The disproportionately large brains of animals nursed by protein deprived dams (C/D and D/D) was also observed at 10, 28 and 42 days of age (Figure 3). In addition, the ratio of brain weight to body weight of animals of D/C litters was significantly different from C/C values at 10 days of age (Figure 3).

Kidney Weight (KW)

Kidney weights of D/C (47.2±1.4 mg) and D/D (47.9±2.6 mg) animals were significantly different than those of the C/C (55.9±1.7 mg) and C/D (52.1±1.7 mg) animals at 1 day of age (Table 3). The deficit in KW of animals exposed to prenatal protein deprivation (D/C, D/D) persisted at 5 and 10 days of age compared to C/C values (Table 3). However, by 28 days of age, kidney weights of D/C pups (590.6±12.5 mg) were comparable to those of C/C (527.2±27.9 mg) (Table 3). Kidneys of animals exposed to postnatal malnutrition alone (C/D) weighed significantly less than those of the C/C group by 5 days of age (Table 3). The deficit in KW of animals nursed by protein deprived dams (C/D, D/D) persisted at 10, 28 and 42 days of age when compared to the C/C group (Table 3).

Figure 3. Brain weight/body weight ratio of animals born and/or nursed by protein deprived dams. Each value represents mean ± S.E. obtained from at least 4 litters. Absence of a vertical bar indicates that S.E. is within the radius of the point. Asterisks denote a statistical difference from C/C values ($p<.05$).

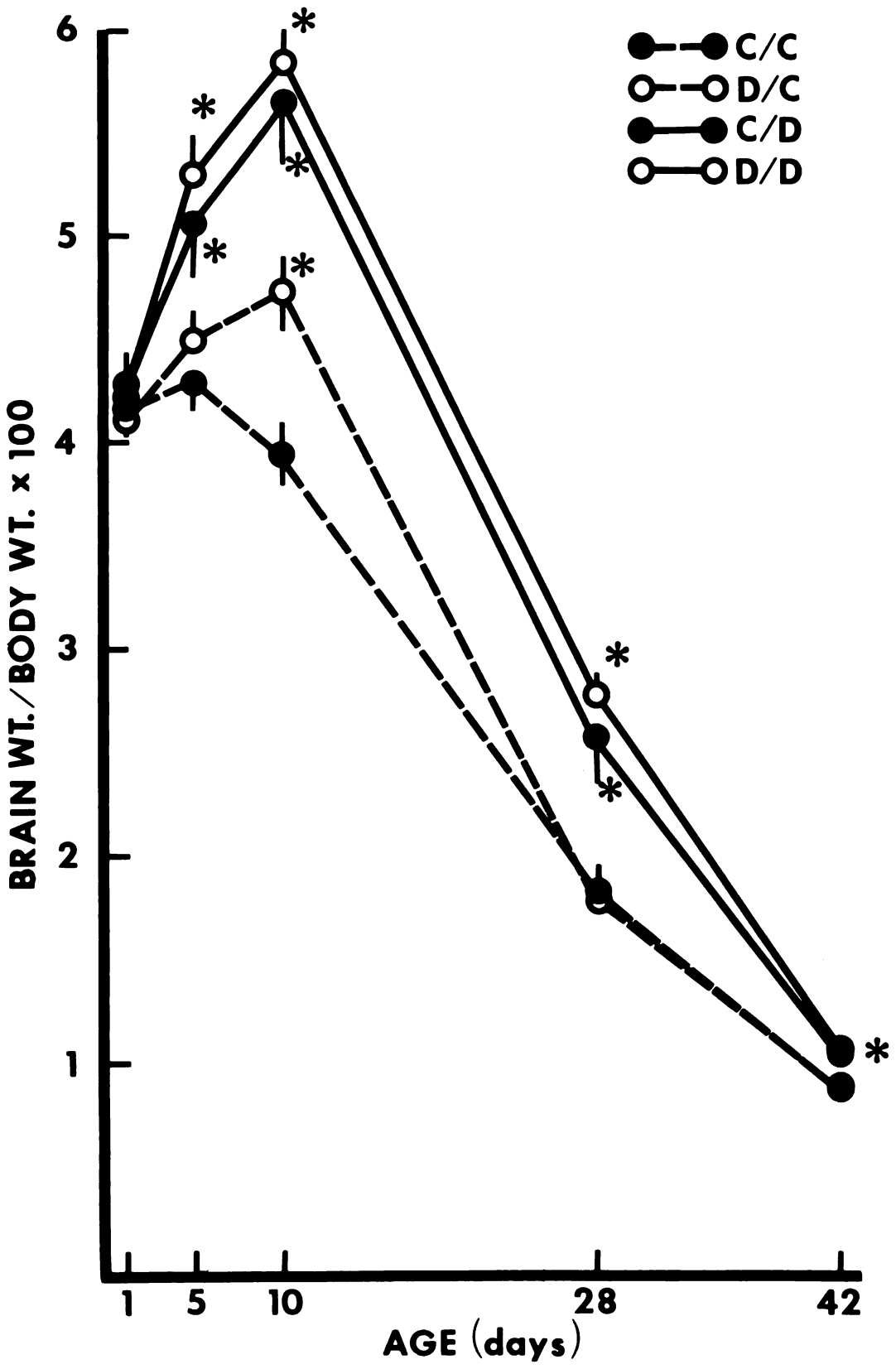

Figure 3

TABLE 3

Effect of Maternal Protein Deprivation During Gestation and/or Lactation on Kidney Weight (KW) of Offspring

Group	Kidney Weight (mg)				
	Age (days)				
	1	5	10	28	42
C/C	55.9±1.7 (15)[a]	131.9±3.1 (14)	220.8±13.4 (7)	527.2±27.9 (5)	1056.0± 89.8 (5)
D/C	47.2±1.4 (14)[b,c]	111.1±3.7 (14)[b]	184.3± 5.0 (9)[b]	590.6±12.5 (5)	1147.9±111.3 (5)
C/D	52.1±1.7 (13)	100.7±4.1 (9)[b]	152.6± 7.5 (7)[b,d]	353.6±15.3 (5)[b,d]	895.3± 66.2 (5)[b,d]
D/D	47.9±2.6 (15)[b]	93.9±3.1 (14)[b,d]	132.9± 4.5 (10)[b,c,d]	338.6±16.9 (5)[b,d]	880.5± 37.8 (5)[b,d]

[a] Values represent means ± S.E. (n).

[b] Significantly different from C/C ($p<.05$).

[c] Significantly different from C/D ($p<.05$).

[d] Significantly different from D/C ($p<.05$).

Ratio of Kidney Weight to Body Weight (KW/BW)

Kidney weight, relative to body weight, was significantly different in animals of the D/C (0.76±.01) and D/D (0.77±.02) groups when compared to those of the C/C (0.86±.01) or the C/D (0.86±.02) groups at 1 day of age (Table 4). This difference was also observed at 5 days of age (Table 4). By 10 days of age, however, no differences in KW/BW ratios were observed among the four groups (Table 4). KW/BW ratios were significantly reduced in the C/D (0.85±.02) group at 28 days of age compared to those of the C/C (0.96±.04) or D/C group (0.97±.02) (Table 4). By 42 days of age, no differences among the groups were observed (Table 4).

Water and Protein Content of Kidney Cortical Slices

No differences in water (Figure 4) or protein content (Figure 5) of renal cortical slices were observed among the four groups at any age examined.

Accumulation of Organic Acid (PAH) by Renal Cortical Slices

Accumulation of PAH by renal cortical slices increased gradually from 1 day of age to 28 days in D/C and C/C litters (Figures 6 and 7). By 28 days of age, PAH S/M ratios of C/C (12.03±1.82) and D/C (12.43± 0.98) groups reached maximum values and thereafter declined to 11.12± 0.51 and 10.69±0.43, respectively by 42 days of age (Figure 6). On the other hand, PAH S/M ratios of the C/D and D/D groups were greater at 42 days of age than at 28 days (Figures 6 and 7).

No differences in accumulation of PAH by renal cortical slices were observed among the groups at 1 day of age (Figure 6). However, by 5 days of age, kidneys of animals exposed to pre- and postnatal

TABLE 4

Effect of Maternal Protein Deprivation During Gestation and/or Lactation on Kidney Weight/Body Weight (KW/BW) Ratio

Group	KW/BW x 100				
	Age (days)				
	1	5	10	28	42
C/C	.86±.01 (11)[a]	1.09±.01 (13)	1.10±.07 (7)	.96±0.4 (4)	.81±.03 (4)
D/C	.76±.01 (13)[b,c]	.99±.01 (10)[b,c]	.99±.02 (10)	.97±.02 (4)	.83±.03 (4)
C/D	.86±.02 (12)	1.08±.02 (7)	1.02±.03 (5)	.85±.02 (4)[b,d]	.86±.02 (4)
D/D	.77±.02 (13)[b,c]	1.03±.02 (14)[b,c]	1.01±.04 (9)	.89±.02 (4)	.84±.02 (4)

[a] Values represent means ± S.E. (n).

[b] Significantly different from C/C ($p<.05$).

[c] Significantly different from C/D ($p<.05$).

[d] Significantly different from D/C ($p<.05$).

Figure 4. Effect of maternal protein deprivation on water composition of renal cortical slices. Each bar represents mean ± S.E. of 4 experiments.

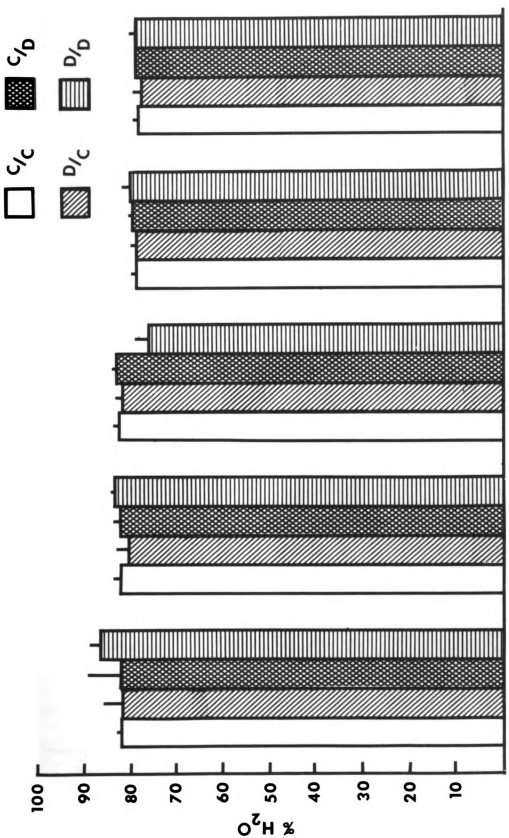

Figure 4

Figure 5. Effect of maternal protein deprivation on protein composition of renal cortical slices. Each bar represents mean ± S.E. of 4 experiments.

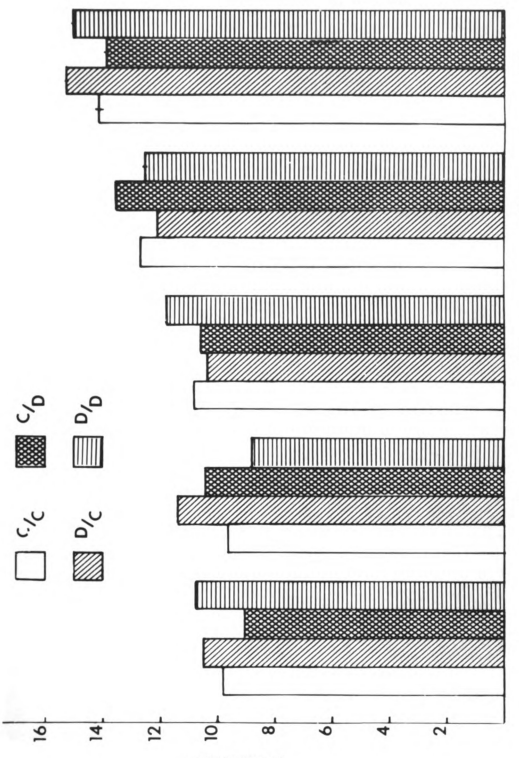

Figure 5

Figure 6. Effect of maternal protein deprivation on renal cortical slice accumulation of PAH (S/M ratio) from kidneys of offspring. Each bar represents the mean ± S.E. of 4 experiments. Asterisks denote a statistical difference from C/C values ($p<.05$). Crosses denote a statistical difference from D/C values ($p<.05$).

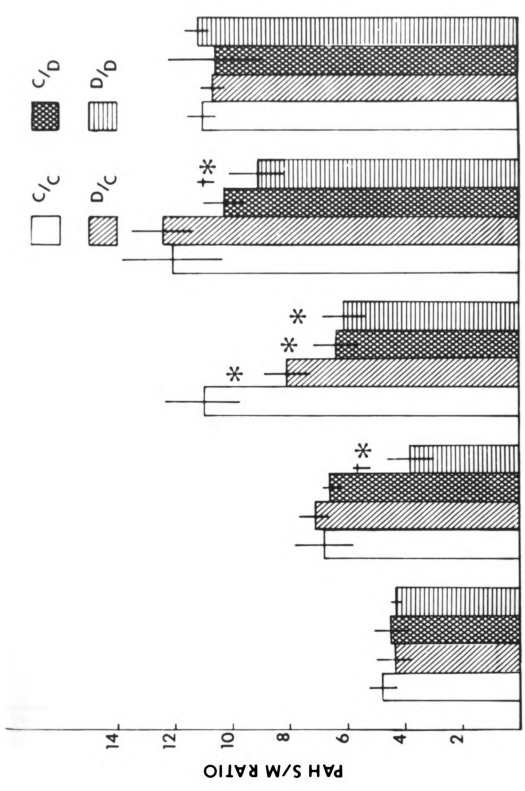

Figure 6

Figure 7. Comparison of prenatal and postnatal protein deprivation on PAH accumulation by renal cortical slices from kidneys of offspring. Each bar represents the mean ± S.E. of data pooled from C/C and D/C groups and from C/D and D/D groups plotted in Figure 6.

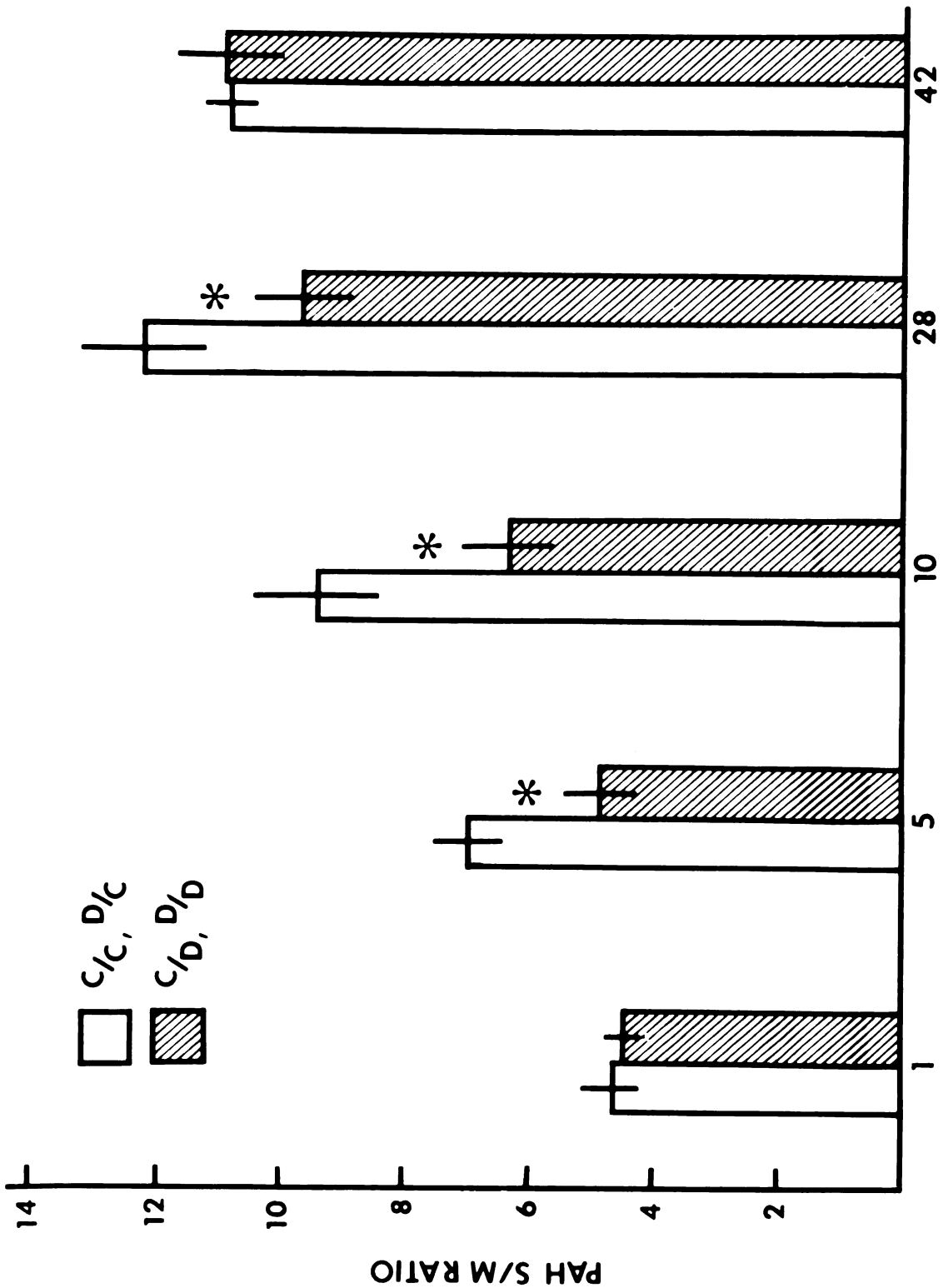

Figure 7

malnutrition (D/D) manifested a reduced capacity to accumulate PAH (S/M: 3.86±0.77) compared to C/C (S/M: 6.94±1.04) or D/C pups (S/M: 7.21±0.37) (Figure 6). The depression in PAH S/M ratios of the D/D group was also observed at 10 and 28 days. PAH accumulation by kidneys of animals exposed to postnatal malnutrition alone (C/D) was significantly reduced at 10 and 28 days of age compared to C/C values (Figure 6). Since there were negligible differences in PAH S/M ratios of litters receiving an adequate postnatal diet (C/C and D/C), data from these groups were pooled (Figure 7). Data obtained from litters nursed by protein deprived dams (C/D and D/D) were similarly treated (Figure 7). PAH S/M ratios were significantly reduced in animals of litters subjected to postnatal malnutrition at 5 (4.03±0.54), 10 (6.39±0.49) and 28 days (9.78±0.61) compared to those receiving an adequate postnatal diet (7.07±0.52, 9.59±0.89 and 12.23±0.96, respectively) (Figure 7). However, by 42 days of age, no differences in PAH accumulation among the groups were observed (Figure 7).

Accumulation of Organic Base (NMN) by Renal Cortical Slices

No developmental differences in accumulation of NMN by renal cortical slices were observed between 1 and 5 days of age among the groups (Figures 8 and 9). However, there was a significant increase in NMN accumulation between 5 and 10 days and this continued to rise until 28 days of age in all groups (Figures 8 and 9). Maximum values for NMN uptake were observed at 28 days for all groups (Figures 8 and 9). By 42 days, NMN S/M ratios began to decline (Figures 8 and 9).

Figure 8. Effect of maternal protein deprivation on renal cortical slice accumulation of NMN (S/M ratio) from kidneys of offspring. Each bar represents mean ± S.E. of 4 experiments. Asterisks denote a statistical difference from C/C values ($p<.05$). Crosses denote a statistical difference from D/C values ($p<.05$).

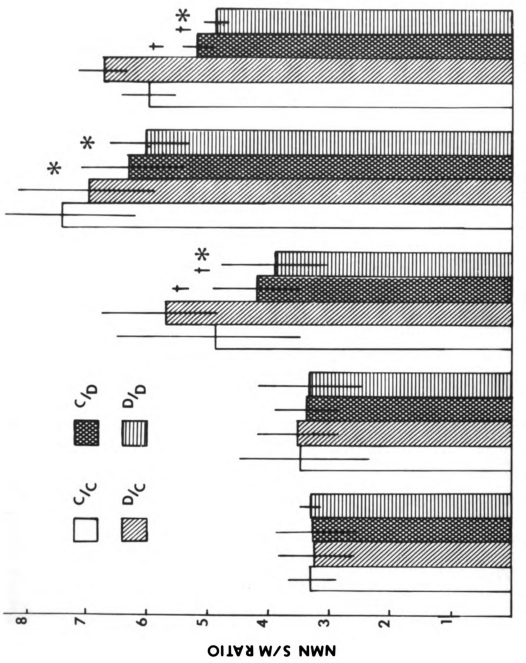

Figure 8

Figure 9. Comparison of prenatal and postnatal protein deprivation on NMN accumulation by renal cortical slices from kidneys of offspring. Each bar represents the mean ± S.E. of data pooled from C/C and D/C groups and from C/D and D/D groups plotted in Figure 8.

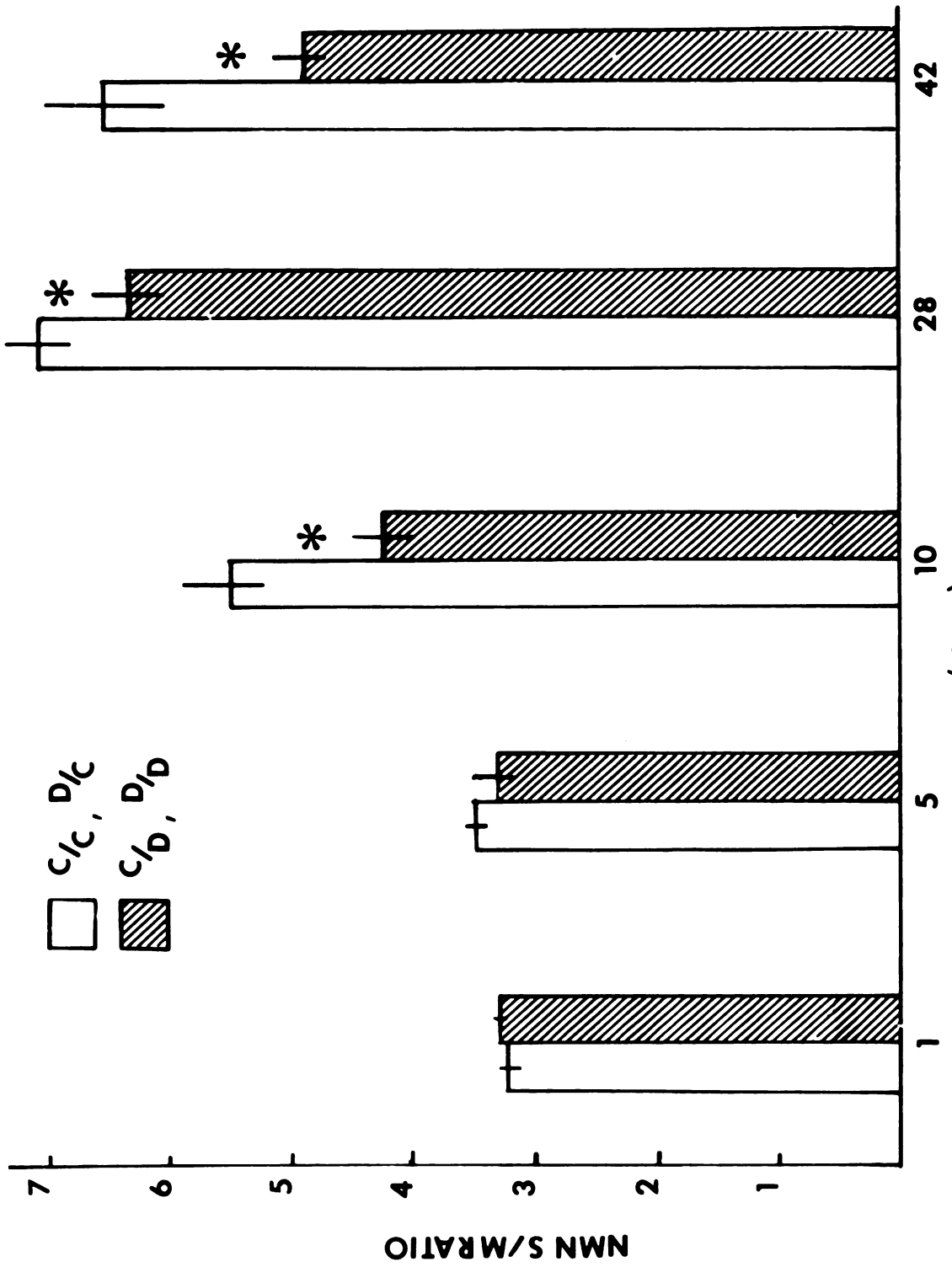

Figure 9

No differences among the groups in NMN accumulation were observed at 1 and 5 days of age (Figure 8). However, by 10 days, NMN S/M ratios of the C/D (4.39±0.56) and D/D (4.15±0.80) were significantly different than those of the C/C (5.14±0.65) and/or the D/C groups (5.90±0.67) (Figure 8). The depression of NMN accumulation by kidneys of animals nursed by protein deprived dams persisted until 28 and 42 days of age (Figures 8 and 9). Since there were minimal differences in NMN S/M ratios between C/C and D/C groups, these data were pooled. NMN S/M ratios of C/D and D/D groups were similarly treated. While no differences were observed at 1 and 5 days of age, the NMN S/M ratios were significantly reduced in kidneys of animals nursed by protein deprived dams compared to those nursed by controls at 10, 28 and 42 days of age (Figure 9).

Accumulation of α-Aminoisobutyric Acid (αAIB) by Renal Cortical Slices

Accumulation of αAIB by renal cortical slices was greatest at 1 day of age in all four groups (Figure 10). Thereafter, αAIB S/M ratios progressively decreased with age, reaching steady values between 28 and 42 days of age (Figure 10).

Even though there were no statistical differences among the groups at 1 and 5 days of age, αAIB S/M ratios of D/D pups tended to be higher at these ages (Figure 10). These differences were magnified by 10 days of age in which αAIB S/M ratios of the D/D group averaged 11.21±1.91 while those of the C/C group averaged 5.79±1.00 (Figure 10). By 28 and 42 days of age, these differences were no longer apparent (Figure 10).

Figure 10. Accumulation of αAIB by renal cortical slices from kidneys of animals exposed to pre- and/or postnatal protein deprivation. Each bar represents the mean ± S.E. of 4 experiments. Asterisk denotes a statistical difference from C/C values ($p<.05$).

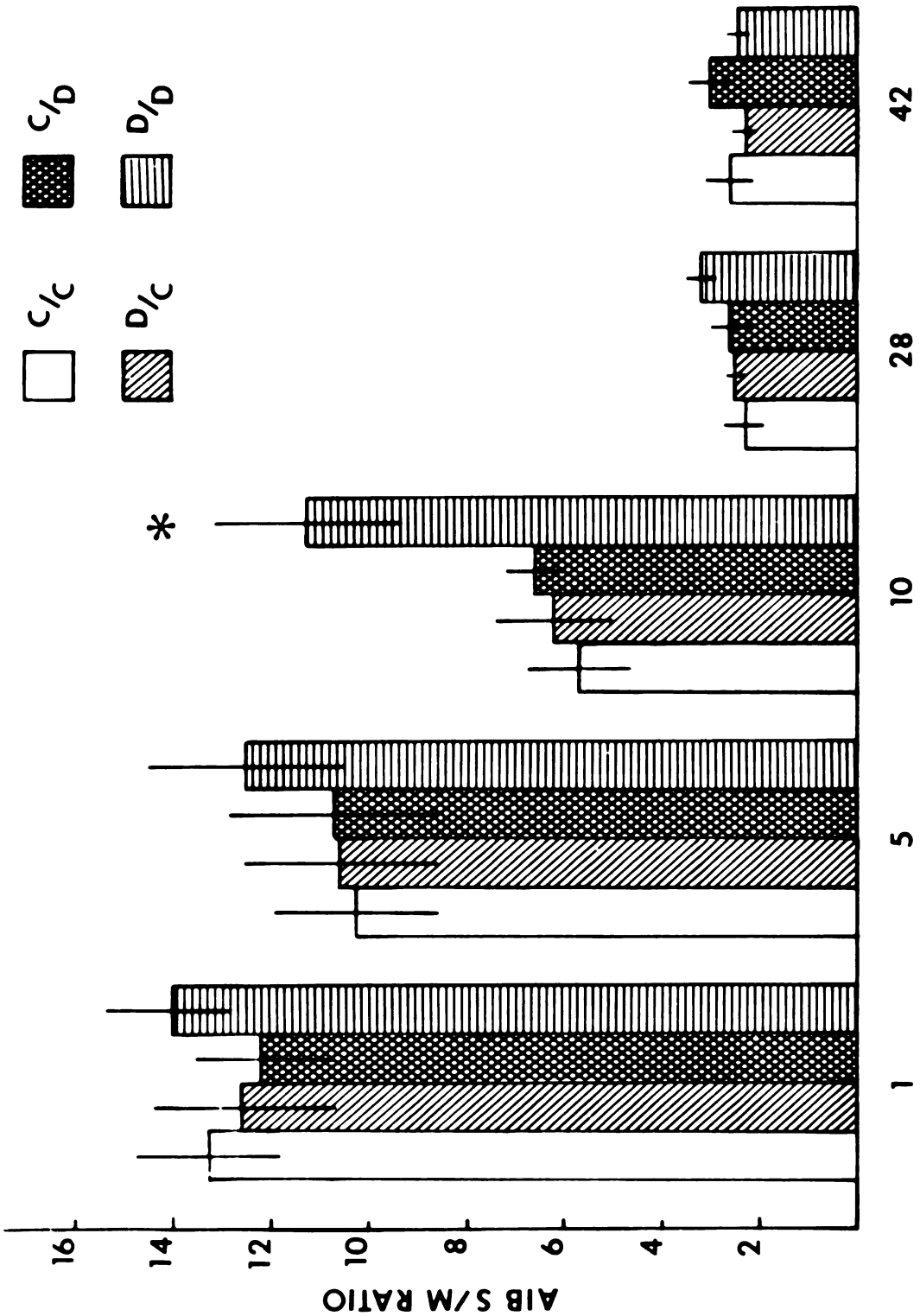

Figure 10

Renal Ammoniagenic Capacity

Renal ammoniagenic capacity was low for all groups at 1, 5 and 10 days of age (Figure 11). Between 10 and 28 days, a three to four-fold increase in ammonia production was observed in all groups (Figure 11). No further increases were noted by 42 days (Figure 11).

No differences in renal ammoniagenic capacity were observed at 1, 5 and 10 days of age in litters born and/or nursed by protein deprived dams (Figure 11). However, by 28 days a significant increase in ammoniagenic capacity was observed in C/D and D/D groups when compared to C/C or D/C groups (Figure 11). By 42 days of age, no differences were observed (Figure 11).

Renal Gluconeogenic Capacity

Renal gluconeogenic capacity was low in all groups at 1, 5 and 10 days of age (Figure 12). However, between 10 and 28 days, a significant increase in glucose production was observed in all four groups (Figure 12). No further increases were noted at 42 days of age (Figure 12). No differences in gluconeogenic capacity were observed among the four groups at any age studied (Figure 12).

Figure 11. Effect of maternal protein deprivation on ammoniagenic capacity of kidneys of offspring. Each bar represents mean ± S.E. of 4 experiments. Asterisks denote a statistical difference from C/C and D/C values ($p<.05$).

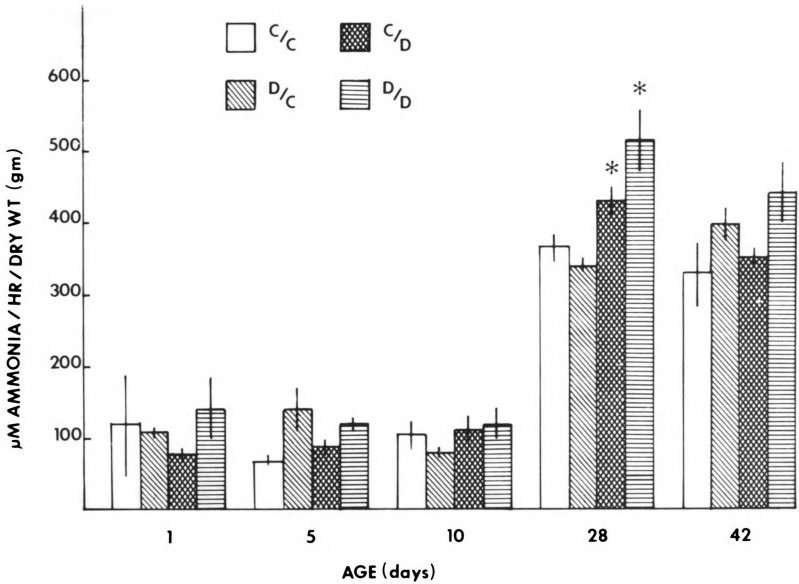
C/C
C/D
D/C
D/D
600
500
400
300
200
100
μM AMMONIA / HR / DRY WT (gm)
*
*
1
5
10
28
42
AGE (days)

Figure 11

Figure 12. Effect of maternal protein deprivation on gluconeogenic capacity of kidneys of offspring. Each bar represents mean ± S.E. of 4 experiments.

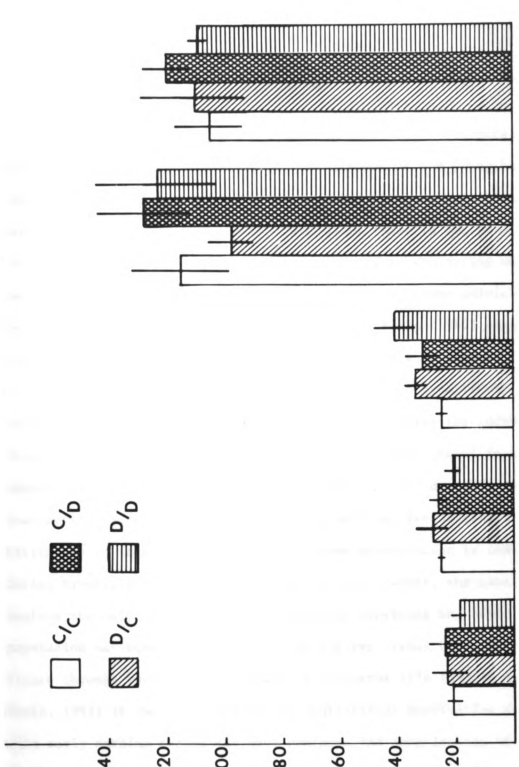
μM GLUCOSE/HR/DRY WT (gm)
140
120
100
80
60
40
20
C/C
C/D
D/C
D/D
1
5
10
28
42
AGE (days)

Figure 12

DISCUSSION

The study of the relationship between pre- and/or postnatal malnutrition on the subsequent growth and development of the young has been approached from a variety of directions and consequently, several general principles have emerged. Chief among these is the principle that mammalian organs undergo a period of rapid growth during which a nutritional insult may bring about permanent alterations (Winick and Noble, 1966). Studies conducted by Winick and Noble (1965) suggest that normal organ growth is partitioned into three distinct phases: hyperplasia, hyperplasia accompanied by hypertrophy, and hypertrophy. Although the chronological period of these phases varies for individual organs, Winick and Noble (1968) observed that a nutritional insult imposed during the hyperplastic phase, when cellular division is the most active, permanently retards organ growth despite nutrition rehabilitation in later life. Conversely, when malnutrition is induced during hypertrophy, cell size is compromised; however, the capacity to replete the cell to normal size is retained providing the adult cell population has been reached. Since in the rat, renal hyperplasia continues through the first four weeks of postnatal life (Winick and Noble, 1965) it was hypothesized that nutritional deprivation during this early nursing period may have profound and long-lasting effects on kidney development, both morphologically and functionally.

In this study, the food consumption by dams fed the control and low protein diet during gestation was similar (Table 2); thus, any effects observed in the progeny were solely ascribed to maternal protein deprivation, not protein-calorie restriction.

While a statistical difference in birth weights was detected in animals exposed to prenatal protein deprivation, the physiological significance of this finding is questionable. Pups born of a low protein fed dam were visually indistinguishable from those born of controls at birth. This observation is consistent with other studies reporting no striking differences in newborn rats exposed to prenatal protein deprivation compared to controls (Venkatachalam and Ramanathan, 1964; Barnes et al., 1973).

Even though the effects of prenatal protein deprivation may not be reflected in lowered birth weights, it has been suggested that such a nutritional stress may impair the subsequent growth of offspring (Venkatachalam and Ramanathan, 1964). However, the data reported here suggest that body weights of D/C animals were comparable to those of C/C animals at all ages studied (Figures 1), indicating that any potential growth-retarding effects of prenatal protein deprivation alone (D/C) were compensated by an adequate diet during the nursing period. Similarly, the body weights of C/D animals did not differ from those of the D/D group at any age studied (Figure 1). This suggests that an adequate diet in utero alone (C/D) is unable to compensate for the effects induced by postnatal malnutrition. However, animals nursed by protein deprived dams (C/D, D/D) weighed significantly less at 5, 10, 28 and 42 days of age than animals nursed by controls (C/C,

D/C) (Figure 1). Furthermore, despite an adequate diet subsequent to weaning, this deficit in body weight persisted until 42 days of age (Figure 1). These data are in agreement with others (Venkatachalam and Ramanathan, 1964; Barnes et al., 1973) reporting a persistent stunting of body growth in rats malnourished during the first three weeks of postnatal life. This growth retardation may be related to a quantitative decrease in milk availability to the young. Milk yield is severely compromised when lactating dams receive a low protein diet while the quality of milk with respect to total solids, protein, fat and ash is unaffected (Mueller and Cox, 1946; Venkatachalam and Ramanathan, 1964). Thus, it appears that the stress of protein deprivation on the lactating dam is manifested as a quantitative reduction in milk available to the young, which secondarily results in a generalized undernutrition during the nursing period. Alternatively, the growth retardation observed in animals nursed by protein deprived dams may be due to an inability of these pups to utilize the consumed milk for body growth.

Similar to the results reported by Allen and Zeman (1973), kidney weights were significantly reduced in animals exposed to prenatal protein deprivation at 1, 5 and 10 days of age (Table 3). However, contrary to the data reported here, Allen and Zeman (1973) observed that improved postnatal nutrition, achieved by reducing litter size and thus, increasing the milk supply available per pup, did not correct the deficit in kidney weight. In the present study, kidney weights of animals exposed to prenatal protein deprivation alone (D/C) were comparable to the kidney weights of the C/C group by 28 days (Table 3). Thus, the data reported in this study suggest that the deficit in

kidney weight induced by prenatal protein deprivation can be reversed by an adequate diet during the nursing period. These results are consistent with those of Winick et al. (1968) who reported that the reduced kidney weights of litters malnourished from birth until 9 days of age can be completely compensated by an adequate diet beginning at 10 days of age. It was thus suggested that growth retardation consequent to a restricted food intake can be overcome if compensatory feeding is instituted before the normal period of hyperplasia ends.

While the effects of prenatal protein deprivation on kidney weight are reversible, the effects of maternal postnatal protein deprivation (C/D, D/D) on kidney growth appear to be long-lasting (Table 3). The deficit in kidney weight induced by a postnatal nutritional insult persisted until 42 days of age (Table 3), despite an adequate post-weaning diet. It has been reported that a nutritional deficiency applied during the period of maximum hyperplasia may permanently retard organ growth even though nutrition rehabilitation is instituted later in life (Winick et al., 1968). This principle coupled with the data reported in this study suggest that malnutrition in the rat during the hyperplastic phase of kidney development, or the first four weeks of postnatal life, severely compromises renal growth. Furthermore, this growth retardation may not be corrected by compensatory feeding subsequent to weaning.

To determine the tissue component responsible for the differences observed in kidney weight, renal cortical slices were analyzed for water and protein content. However, since there were no differences in water (Figure 4) or protein content (Figure 5) of kidney slices

among the groups, the differences in kidney weight cannot be attributable to changes in these tissue constituents.

The kidneys as a percentage of body weight were disproportionately small in animals exposed to prenatal protein deprivation (D/C, D/C) at 1 and 5 days of age; however, no differences were observed by 10 days (Table 4). By 28 days, the KW/BW ratios of D/C animals were comparable to those of the C/C group (Table 4), indicating that the disproportionately small kidneys of pups exposed to prenatal protein deprivation alone (D/C) were corrected by an adequate diet during the nursing period. In addition, KW/BW ratios of animals nursed by protein deprived dams (C/D, D/D) were less when compared to those nursed by controls (C/C, D/C) at 28 days (Table 4). This further reinforces the importance of the early postnatal diet in relation to the growth and development of the rat kidney. Since the KW/BW ratios of all four groups were comparable by 42 days of age (Table 4), it appears that by this age both kidney and body weights are equally affected by the dietary manipulations employed.

While postnatal malnutrition in the rat impaired somatic and kidney growth, brain weight was comparatively unaffected (Figure 2). Similarly, Forbes et al. (1977) observed no differences between brain weights of rats born and nursed by protein deprived dams from those of controls. These results suggest that in the face of pre- and/or postnatal protein deprivation, the rat brain is spared from the growth retarding effects observed on body and kidney. However, since body growth of animals nursed by protein deprived dams was severely compromised (Figure 1), brain weight/body weight ratios were elevated

in these animals compared to those nursed by controls (Figure 3). These data are in agreement with other studies reporting disproportionately large brains of rats malnourished during the nursing period (Dobbing and Sands, 1971; Forbes et al., 1977).

Since kidney growth was compromised in offspring born and/or nursed by dams receiving a low protein diet, experiments were conducted to determine the effect of these dietary manipulations on renal function. In these studies ability of rat renal cortical slices to accumulate PAH (Figures 6 and 7) revealed a characteristic pattern of development similar to those reported in other species (Rennick et al., 1961; Hirsch and Hook, 1970; Hook et al., 1970). PAH S/M ratios in control litters (C/C) were low at one day of age, progressively increased to maximum values by 28 days and subsequently declined by 42 days of age (Figures 6 and 7). The peak in PAH accumulation at four weeks of age has been described in several species (Rennick et al., 1961; Hirsch and Hook, 1970; Ecker and Hook, 1974) and several explanations for this developmental pattern have been offered. Since the S/M ratio is calculated using the slice wet weight, the increase in PAH S/M ratios between 1 and 28 days of age may be due, in part, to decreased water content of the cortical slices. However, the data presented in this study indicate no significant differences in water content of slices obtained from 28 day old rat kidneys compared to 1 day olds (Figure 4). In addition, the progressive decrease in water content of slices with age would not account for the decline in S/M ratios observed between 28 and 42 days of age. It has also been suggested that since PAH S/M ratios represent intracellular accumulation and

the respective influx and efflux of the anion, it is possible that the mechanisms responsible for PAH entry into the proximal tubular cell develop at an earlier age than those responsible for efflux (Ecker and Hook, 1971). It has also been postulated that endogenous inhibitors may contribute to the decline of S/M ratios normally seen after 4 weeks of age (Ecker and Hook, 1974).

The dietary manipulations employed in this study provide another tool to further elucidate the developmental patterns of PAH accumulation. PAH S/M ratios of litters nursed by control dams (C/C, D/C) reached maximum values by 28 days and subsequently decreased by 42 days (Figure 8). In contrast, S/M ratios of litters nursed by protein deprived dams (C/D, D/D) continued to rise at 28 and 42 days of age (Figure 8). Thus, malnutrition during the nursing period may delay the maturation of this transport system, shifting the developmental curve towards the right. If this indeed were the case, the decline in PAH S/M ratios occurring between 28 and 42 days in animals nursed by controls would be expected to occur in C/D and D/D animals later in life.

An important factor contributing to the maturation of the PAH transport system is that of substrate availability (Hirsch and Hook, 1970). The enhanced activity of the organic acid transport system to substrates such as PAH and penicillin demonstrates that this system is capable of responding to alterations in organic acid concentration (Hirsch and Hook, 1970). Conversely, dietary restriction in the form of a low protein diet reduces the ability of rabbit kidneys to accumulate PAH (Brockelbank et al., 1976). Thus, dietary restriction may

affect the functional capacity of the PAH transport system by altering substrate concentrations. In this manner, litters nursed by protein deprived dams were receiving quantitatively less milk (Mueller and Cox, 1946; Venkatachalam and Ramanathan, 1964), an effect which may have altered the substrate concentrations needed to induce functional maturation. These data are consistent with the observations of Edelmann and Wolfish (1969) who noted that the magnitude of the working load imposed upon the kidney by the diet is a major contributing factor to the maturation of the kidney. The present study, therefore, suggests that malnutrition during the nursing period retards the development of the renal organic acid transport system of the rat and that the well-described maturational pattern for PAH accumulation is dependent upon an adequate diet.

Unlike the maturational pattern of the PAH transport system, there were no developmental changes in organic base (NMN) transport between 1 and 5 days of age (Figures 8 and 9). However, between 5 and 10 days, increase in NMN S/M ratios was observed in all groups studied. Similar to PAH development, the increase in NMN S/M ratios continued until 28 days of age, at which time maximum values were attained (Figures 8 and 9). Kim et al. (1972) reported that NMN S/M ratios of rat kidneys are low during the first 6 days of postnatal life, increase progressively until 14 days, stabilize between 14 and 20 days of age and thereafter increase to adult values. While Kim et al. (1972) reported no changes in NMN S/M ratios bewteen 14 and 20 days of age, it appears from the present study that significant developmental changes do occur between 10 and 28 days of age (Figures 8 and 9). It

may be that the increase in NMN uptake observed at 28 days is a consequence of developmental changes occurring between 21-28 days. In addition, since these investigators (Kim et al., 1972) did not measure NMN transport in kidneys of rats 21-42 days of age, they were unable to observe the decline in NMN S/M ratios occurring between 10 and 28 days as reported in the present study. Thus, it appears that the maturation of NMN accumulation is not necessarily a function of kidney growth.

Unlike the developmental patterns observed in this study, Rennick et al. (1961) reported a progressive increase in organic base accumulation by dog renal cortical slices from one to eight weeks of age. However, species specificity in the maturation of NMN accumulation is not an uncommon finding. Hirsch and Hook (1970) demonstrated that the developmental patterns for NMN uptake are markedly different in rats and in rabbits. Therefore, it is not surprising to observed developmental differences in NMN accumulation between rats and dogs.

The depression in NMN S/M ratios in litters nursed by protein deprived dams persisted until 42 days of age, despite an adequate diet subsequent to weaning (Figures 8 and 9). In addition, the delayed maturational rate of PAH transport induced by postnatal malnutrition was not apparent for NMN accumulation. Rather, maximum NMN S/M ratios were reached by 28 days and subsequently decreased by 42 days in all four experimental groups (Figure 8). Thus, it appears that pre- and/or postnatal protein deprivation had little effect on the developmental pattern for NMN transport. However, dietary restriction during the nursing period did depress the overall functional capacity of this transport system. It may be that, like the PAH transport system, the optimal functioning of NMN transport is

substrate dependent. Since an adequate post-weaning diet did not compensate for the depression in NMN S/M ratios in animals nursed by protein deprived dams (Figures 8 and 9), it is possible that the deficient nutrient or substrate availability during the nursing period may have prevented the synthesis of some critical component of this transport system.

The developmental pattern observed for αAIB accumulation is quite distinct from that of PAH or NMN uptake (Figure 10). Tha ability of kidney cortical slices to concentrate αAIB is greatest at one day of age and progressively decreases with age until 28 days (Figure 10). This is consistent with other studies reporting a higher distribution ratio for αAIB in kidneys of newborns when compared to adults (Webber and Cairns, 1968; Reynolds and Segal, 1976).

While malnutrition during the nursing period affected both PAH and NMN transport capacity, this nutritional stress alone (C/D) did not affect αAIB concentrating ability at any given age (Figure 10). Similarly, prenatal protein deprivation alone (D/C) did not alter αAIB S/M ratios at any age (Figure 10). However, the combination of both pre- and postnatal protein deprivation (D/D) did induce a sufficient stress to affect the ability of kidney slices to accumulate αAIB (Figure 10). The αAIB S/M ratios of D/D litters at 10 days of age were comparable to S/M ratios of the C/C litters at 1 day of age (Figure 10), suggesting that the amino acid concentrating ability of litters exposed to pre- and postnatal malnutrition is relatively immature. Since no differences in αAIB concentrating ability were

observed among the groups at 28 days (Figure 10), it appears that these effects are not permanent.

The pattern of development for renal ammoniagenic capacity is different than the patterns observed for PAH, NMN or αAIB accumulation. While no differences were observed between 1 and 10 days of age, there was a striking increase in ammonia production by 28 days of age (Figure 11). These results are in agreement with other studies reporting low rates of ammonia excretion during the first two weeks of postnatal life in the rat followed by a rapid increase between 14 and 21 days of age (Goldstein, 1970; Goldstein, 1971). The relatively low level of glutaminase activity in rat kidneys at one and two weeks of age could contribute to this level of ammonia production (Goldstein, 1971). Similarly, the progressive increase in glutaminase activity between 9 and 21 days of age could account for the dramatic rise in ammonia production by 21 days (Goldstein, 1971).

It has been observed that malnourished rats respond appropriately to an acid load by increasing urinary ammonia (Fraser and Alleyne, 1974). This suggests that the intrinsic capacity of the malnourished kidney to produce ammonia is not impaired. The results reported in this study strongly support this speculation since the kidneys of animals exposed to pre- and/or postnatal malnutrition were equally capable of producing ammonia as the controls (Figure 11). In fact, by 28 days of age, the kidneys of animals nursed by protein deprived dams (C/D, D/D) manifested a greater capacity to produce ammonia when compared to those nursed by controls (C/C, D/C) (Figure 11). Adults on low protein diets have an increase in plasma glutamine (Swenseid,

1966), which is the major source of renal ammonia. Thus, it may be that animals nursed by protein deprived dams are ingesting the low protein diet during the third or fourth week of postnatal life, producing an elevation in plasma glutamine levels and secondarily, increasing the substrate availability for renal ammoniagenesis. This, then may account for the increase in renal ammoniagenic capacity which is observed in C/D and D/D litters only at 28 days (Figure 11). Alternatively, animals nursed by protein deprived dams may have been undergoing a mild metabolic acidosis and this, in turn, may have induced an increased ammoniagenic capacity which was not manifested until enzyme development fully matured.

The developmental pattern for renal gluconeogenic capacity closely paralleled that of ammonia production (Figures 11 and 12). Zorzoli et al. (1969) reported that glucose formation from glutamate or pyruvate and the activity of glucose-6-phosphatase in the kidney cortex increases rapidly during the first two to three weeks of postnatal life in the rat. Activities of fructose 1,6-diphosphatase and extramitochondrial PEPCK reach adult values by 30 days of age (Hauser and Barley, 1975). In this manner, the maturation of gluconeogenic enzyme activity closely parallels the developmental patterns observed in glucose production. While postnatal malnutrition during the nursing period enhanced renal ammoniagenic capacity by 28 days, renal gluconeogenic capacity was unaffected. Even though it has been suggested that changes in renal ammonia production are usually accompanied by parallel changes in renal glucose production, this does not seem to be the case in this study.

Inasmuch as maternal protein deprivation compromised kidney growth, the results presented in this study suggest that these dietary manipulations differentially affected various parameters of renal function. Since the kidney provides a major excretory route of foreign compounds, it would be particularly relevant to determine whether pre- and/or postnatal protein deprivation alters the response and sensitivity of the kidney to drug therapy and/or nephrotoxin exposure.

SUMMARY

The present study was undertaken: 1) to investigate the developmental patterns of renal function relative to maternal dietary protein intake; 2) to isolate the effects of a low protein diet during gestation and lactation on renal function of offspring; and 3) to assess the reversibility or persistence of these effects. Pregnant rats were fed either a control or low protein diet ad libitum from day 1 of gestation until litters were weaned. At birth, each litter was cross-fostered, yielding four experimental models: pups born of a control and nursed by a control (C/C) or protein deprived (C/D) dam and pups born of a protein deprived dam and nursed by a control (D/C) or protein deprived (D/D) dam. Litters remained with the foster dams until 28 days of age at which time animals were weaned to a control diet. Body, brain and kidney weight and renal function were assessed at 1, 5, 10, 28 and 42 days of age.

Animals born of protein deprived dams weighed significantly less at birth than controls. Maternal protein deprivation during the postnatal period produced a persistent deficit in body weight of progeny. Lactating dams receiving low protein diets produce quantitatively less milk (Mueller and Cox, 1946; Venkatachalam and Ramanathan, 1964); thus, the growth retardation observed in offspring

nursed by protein deprived dams may be related to a decreased milk availability which secondarily results in a generalized undernutrition.

While kidneys of animals exposed to prenatal protein deprivation weighed less than those of C/C at 1, 5 and 10 days of age, an adequate diet during the nursing period compensated for this deficit in kidney weight by 28 days. On the other hand, kidneys of animals nursed by protein deprived dams weighed significantly less than those of the C/C group at 5, 10, 28 and 42 days. Thus, despite an adequate diet subsequent to weaning the deficit in kidney weight of offspring nursed by protein deprived dams persisted until 42 days of age.

In contrast to the deficit in body and kidney weights induced by postnatal malnutrition, the brain weight of animals stressed by pre- and/or postnatal protein deprivation was not affected. The brain, therefore, appears to be comparatively resistant to this nutritional deprivation.

Kidney function was quantified in vitro utilizing the renal cortical slice technique as described by Cross and Taggart (1950). The accumulation of a prototype organic acid (PAH), organic base (NMN) and amino acid (αAIB) by renal cortical slices was expressed as a slice/medium (S/M) ratio.

Accumulation of PAH by renal cortical slices of kidneys of animals nursed by control dams (C/C, D/C) increased progressively with age from 1 to 28 days; thereafter, PAH accumulation declined. This maturational pattern of PAH accumulation is in agreementwith results obtained in other studies. However, PAH S/M ratios of kidneys of offspring nursed by protein deprived dams (C/D, D/D) continued to

increase to 42 days of age. Thus, it appears that postnatal malnutrition during the nursing period may retard the development of this transport system. In addition, the capacity to accumulate PAH was significantly reduced in kidneys of animals stressed by postnatal malnutrition. This may be related to a reduced substrate availability in pups receiving quantitatively less milk during the nursing period.

Accumulation of NMN by renal cortical slices was maximal at 28 days in all groups. Unlike the effects of these dietary manipulations on PAH accumulation, the developmental curve for NMN accumulation was unaffected. However, the capacity to accumulate NMN was significantly compromised in kidneys of animals nursed by protein deprived dams (C/D, D/D). This was observed at 10, 28 and 42 days of age. It may be that nutritional deprivation during the nursing period prevented the synthesis of some critical component of the NMN transport system.

A progressive decrease in αAIB accumulation by renal cortical slices was observed between 1 and 28 days of age in all groups. Neither prenatal nor postnatal protein deprivation alone altered αAIB accumulation by renal cortical slices. However, these stresses combined (D/D) did significantly affect αAIB S/M ratios by 10 days of age. It is suggested that pre- and postnatal protein deprivation may affect the development of this transport system. However, these differences were no longer apparent by 28 and 42 days of age.

Renal ammoniagenic and gluconeogenic capacity were low at 1, 5 and 10 days but significantly increased by 28 days. Kidneys of animals exposed to pre- and/or postnatal protein deprivation were as

capable of producing ammonia and glucose as controls (C/C) at all ages studied. In fact, by 28 days, kidneys of animals nursed by protein deprived dams manifested a greater capacity to produce ammonia than litters nursed by controls. This may be related to the consumption of a low protein diet during the third and fourth week of life which would secondarily result in increased plasma glutamine concentrations; thus, increasing substrate availability for renal ammoniagenesis.

Since no differences were observed in the water or protein content of renal cortical slices, the effects observed on renal function cannot be ascribed to quantitative changes in these components. Rather, these data indicate that the various renal functional parameters measured are differentially affected by these dietary manipulations.

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